Effects of spatial heterogeneity in soil contamination - an ecological modelling approach

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Preface

My PhD project is part of the EU-funded CREAM Marie Curie initial training network.

The motivation behind the CREAM (Chemical Risk Effects Assessment Model) project lies in the recognition that, so far, regulators and industry have to a large extent lacked understanding of what benefits mechanistic models can deliver. These benefits include the ability to implement both exposure and toxicity, and important ecological characteristics of the species of concern and the landscape under consideration. All factors that are virtually impossible to fully address empirically and upon which population-level effects of chemicals depend. This is in part due to the lack of consistency in the modelling approaches applied and the incompleteness of model descriptions, which have lead to widespread scepticism about ecological models, preventing their use in risk assessments. Therefore, there is a pressing need for examples that clearly demonstrate the power of mechanistic effect models for risk assessment. There is also a European-wide need for researchers as well as employees for industry and regulatory authorities that are well-trained in both mechanistic effect modelling and regulatory risk assessment.

The aim of CREAM is to fulfil both these needs; its two main objectives are (from CREAM grant agreement Annex 1):

1. Develop a suite of well-tested and validated mechanistic ecological effect models, such as population models and toxicokinetic-toxicodynamic models, for an array of organisms and ecosystems relevant for chemical risk assessments.
2. Provide world class training for the next generation of ecological modellers, emphasizing transparency and rigorous model evaluation as core elements of models for decision support.

In order to achieve these objectives CREAM involves all relevant sectors (industry, academia, regulatory authorities) as active partners, as well as modelling experts that cover a wide range of organisms, chemicals, and model types. Within the network, guidance regarding Good Modelling Practice (GMP) is also being formulated. This ensures that model development and evaluation of all the individual projects are scientifically sound and yet coherent and efficient; and provide a comprehensive and unique network training in ecological modelling, risk assessment, and complementary skills.

CREAM fellows are expected to disseminate the framework and approaches of CREAM to

different sectors all over Europe, improving relationships between them, and help to develop concrete guidance on GMP for ecological risk assessments.

CREAM is divided into five work packages (Fig. 1), of which the first three group individual projects by the type of ecosystem and organisms addressed:

- Work package 1: Aquatic Invertebrates
- Work package 2: Terrestrial Invertebrates
- Work package 3: Vertebrates

The fourth work package organizes the formulation, testing, and refinement of the Good Modelling Practice:

- Work package 4: Good Modelling Practice

The last work package is responsible of collecting and organizing the original data sets produced within CREAM, which can be used for future tests and validations of mechanistic effect models:

- Work package 5: Validation Data Sets

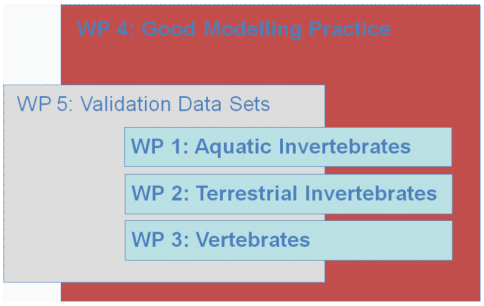

Fig. 1. Representation of the work packages structure of the CREAM ITN.

The research described in this thesis is part of work package 2 "terrestrial invertebrates", specifically the SOIL-1 project. WP 2 is comprised of four PhD projects, which include both experimental and modelling approaches to investigate the effects of different types of heterogeneity, both spatial and temporal, and to link them to population-level risks for collembolans:

- SOIL-1: Impact of spatial heterogeneity in soil contamination on collembolan populations.
- SOIL-2: Disturbance interactions: the combined effects of toxicants and environmental stochasticity on collembolans.
- SOIL-3: Disturbance interactions: modelling environmental and demographic stochasticity for populations exposed to toxicants.
- MATRIX: Life-table experiments and elasticity analyses for linking toxicity to ecological risk.

In the following I am going to present and discuss the results of the research I conducted during the three years of my PhD project within CREAM. Hope you enjoy!

Chapter 1
Introduction

The use of chemical products has benefits upon which modern society depends, for example, in food production, medicines, cosmetics, etc. Chemicals also make an important contribution to the economic and social wellbeing of citizens in terms of trade and employment. There are around 100,000 different substances registered in the EU market; the chemical industry is Europe's third largest manufacturing industry, generating large economic profits and employing millions of people directly or in jobs dependent on it (European Commission, 2001). On the other hand, in the recent past some chemicals have caused serious damage to human health and the environment. The most infamous example is probably the abundant use of DDT, which Rachel Carson in her book *Silent Spring*, published in 1962, claimed causes reproductive disorders in birds and cancer in humans. Other well-known examples are asbestos, which causes lung cancer and mesothelioma or benzene, which leads to leukaemia. Knowledge about the hazards related to the use of these substances only became available after they were produced in large quantities: they have thus been banned or subjected to other controls only after the damage was done.

EU chemicals policy "must ensure a high level of protection of human health and the environment as enshrined in the Treaty both for the present generation and future generations while also ensuring the efficient functioning of the internal market and the competitiveness of the chemical industry" (European Commission, 2001). This policy is based upon the Precautionary Principle: "Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (United Nations, 1992).

Specific legislation exists for certain sectors and areas, and others are under development or in the process of being updated. For example industrial chemicals are regulated under the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation on chemicals and their safe use (EC 1907/2006). The REACH regulation places the burden of proof on industry, which has to collect or generate data on the risks related to the use of both existing and new chemicals. These data are expected to help to close the current information gap on existing chemicals (European Commission, 2001). Other sectorial chemicals legislation includes the Framework Directive on the Sustainable Use of Pesticides (2009/128/EC), the Regulation concerning the Placing on the Market and Use of Biocidal Products (Regulation (EU) No 528/2012; it will repeal and replace Directive 98/8/EC) and the Regulation on Authorization of Plant Protection Products (EC 1107/2009, which has replaced council directives 79/117/EEC and 91/414/EEC).

1.1 Risk assessment of agrochemical products

Specifically, the purpose of the Regulation EC 1107/2009 is to "ensure a high level of protection of both human and animal health and the environment, and at the same time to safeguard the competitiveness of Community agriculture" (European Commission, 2009). For this reason, the regulation should ensure that industry demonstrates that substances or products produced or placed on the market do not have any harmful effects on human or animal health or any unacceptable effects on the environment.

The risk assessment process, in relation to both human health and the environment, is comprised of an assessment of both effects and exposure. According to the Regulation EC 1488/94 (European Commission, 1994), effects assessment comprises the identification of "the adverse effects which a substance has an inherent capacity to cause" (hazard identification), and the assessment of the dose-response relationship. Exposure assessment is instead defined as the estimation of the concentrations to which human populations or environmental compartments are or may be exposed. The risk is then characterised by quantifying the likelihood that adverse effects occur due to actual or predicted exposure to the substance of concern. Characterization of risk is based on the comparison between exposure and toxicological parameters. The concentration to which organisms are exposed can be estimated with predictive models (PEC: Predicted Environmental Concentration), or derived from monitoring data. Concentration of a chemical that does not cause negative effects on ecosystems is usually estimated through extrapolation from laboratory data of acute or chronic exposure, obtained by applying standard methodologies.

Risk assessment procedures commonly follow a tiered testing strategy (Fig. 1.1). Initial risk assessments represent worst-case scenarios: both the ecotoxicological tests and the exposure assessments on which they are based are very simple, and very conservative assumptions are made in the assessment factors used. If low risk is indicated at the first tier, usually no further testing is necessary. However, if a chemical fails the initial risk assessment, additional refinements of effects and exposure are often required: the aim of this tiered approach is in fact to focus testing efforts on chemicals that are more likely to cause adverse impacts on human health or the environment. However, there are substantial uncertainties in translating the test responses used in risk assessment to effects of concern in complex ecological systems (Calow and Forbes, 2003).

Two approaches are traditionally followed to extrapolate these estimated measures of toxicity to the "real world". The first one consists in taking the lowest concentration that caused an effect in the tests conducted and dividing it by a fixed factor (so-called assessment or safety factor) to obtain a predicted no-effect concentration (PNEC). The alternative is to use the Species Sensitivity Distribution (SSD) method, which requires calculation of a distribution of the sensitivity of species from laboratory toxicity data on a few representative test species and estimating from

this distribution the maximum toxicant concentration that is protective for most (usually 95%) of the species (Calow et al., 1997).

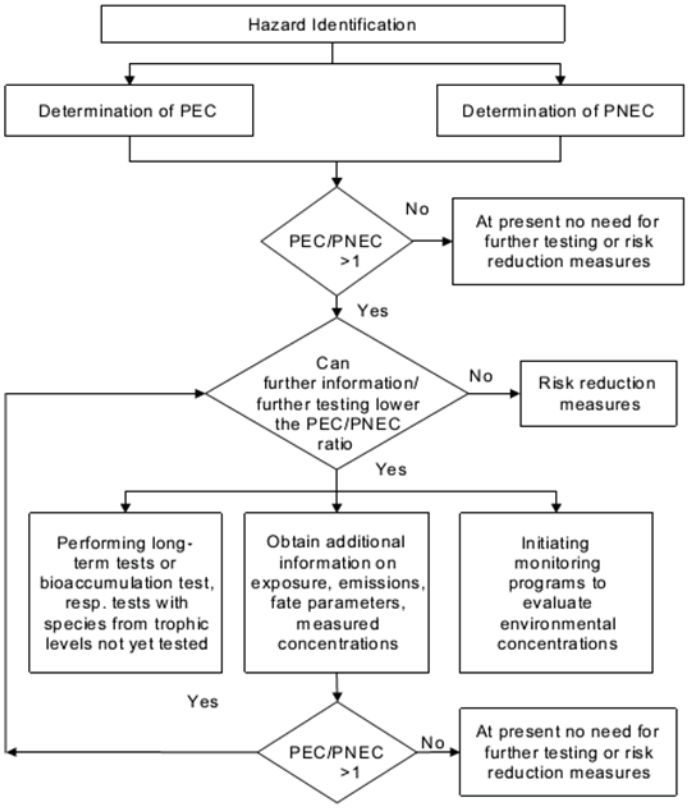

Fig. 1.1. General procedure for environmental risk assessment (Adapted from TGD part 2, chapter 3, page 174).

Terrestrial risk assessment: current practice

The principles of risk assessment described in the previous section are operationalized into different schemes aimed at characterizing the risk for all the groups of non-target organisms that might be affected by the chemical of concern. For practical purposes, each of these schemes, such as for birds, aquatic organisms, bees and other arthropods, etc., is described in a guidance document, which is applied every time a risk assessment is carried out. As the focus of this thesis is on soil invertebrates, in the following a short description of the pertaining risk assessment scheme currently applied is given.

The guidance document for terrestrial risk assessment (Sante´ des Consommateurs, 2002) states

that "General adverse effects on the terrestrial environment" include, among others "effects on soil, above-ground and foliar invertebrates, which represent food for other organisms, and cover essential roles as pollinators, detritivores, saprophages, pest controller, etc". The risk assessment scheme for soil invertebrates is, however, rather minimal. Where contamination of the soil is possible, an acute effects test on earthworms is required, while the requirement for a test on sublethal effects (e.g. reproduction) on earthworms depends on the exposure pattern to the active substance (continued or repeated exposure). This test is only required when specified triggers for persistence of the active substance and the number of applications are exceeded. Where the assessment of chronic risk for earthworms gives a TER_{lt} (long-term toxicity – exposure ratio: ratio between the NOEC (No-Observed Effect Concentration) from the reproduction test and the PEC (Predicted Environmental Concentration)) of less than 5, an earthworm field study is required.

A collembolan reproduction test or a test on gamasid mites is required only "where contamination of soil is possible" and $DT90_f$ (time it takes until 90 % of the initial amount or concentration has disappeared, estimated in a field study) is between 100 and 365 days and the standard hazard quotient for arthropods (*Typhlodromus* and *Aphidius* sp.) is higher than 2 (Sante´ des Consommateurs, 2002). A hazard quotient is defined as the ratio between exposure and toxicity: the higher the figure the greater the risk. The collembolan test is used as a potential waiver (Sante´ des Consommateurs, 2002) for the litter-bag test, a study used to assess effects, especially of persistent compounds, on the breakdown of litter material by the soil organism community (Organisation for Economic Co-operation and Development, 2006). According to the guidance document (Sante´ des Consommateurs, 2002), "if the litter-bag test is triggered anyway by other criteria (effect on soil micro-organisms > 25 % or TER_{lt} for earthworms < 5) then this test could be omitted".

The problem of extrapolating effects from the individual to the population level

A widespread concern among stakeholders involved in ecological risk assessment (ERA) is that most of the testing procedures used to characterize the risk posed by plant protection products focus on toxic effects at the level of individuals, while the protection goals of the EU regulation are, with the exception of birds and mammals, at the population level. The pesticide legislation states that plant protection products may not" have any long-term repercussions for the abundance and diversity of non-target species" (European Commission, 2011). Therefore, extrapolating from the individual to the population level is often seen as one of the major challenges in ERA (Forbes et al., 2001; Forbes and Calow, 2002).

Consensus is growing among stakeholders over the fact that ecological modelling is a useful tool for ERA. Mechanistic effect models could help to improve extrapolation of toxic effect from the individual to the population or community level (Forbes et al., 2008). They also represent

a means to incorporate ecological complexities that are disregarded in current risk assessment schemes and that could influence estimates of risk under realistic field conditions: for instance, extrapolation of effects between different exposure profiles (Hommen et al., 2010).

There is increasing evidence that the assessment factor method is not consistent in the level of protection ensured and can lead to both over- and under-protective risk assessments (Forbes et al., 2008). For instance, Forbes and Calow (2002) determined that the assessment factor intended to extrapolate from acute to chronic toxicity (ACR) is equal to 10. Re-analyzing previously published data (Roex et al., 2000), the authors found that on average, the ACR was 9.1, but the range was between 0.79 and 5,495, which means that in many cases the standard safety factor was either under-protective or over-protective. Furthermore, Hanson and Stark (2012) found that the uncertainty of risk estimates derived from simple matrix models was reduced by more than 88% and by 76% when compared to acute and chronic individual-level data, respectively. Based on this growing evidence, a number of initiatives have been taken in recent years to discuss the inclusion of ecological models as a refining option for the risk assessment of chemicals. Some of these initiatives include:

2003 Pellston Workshop

The workshop dealt with issues of population level ERA. Outcomes of this workshop highlight how current risk assessments lack genuine estimates of effects of chemicals at the population level, which could lead to bad environmental management decisions (Barnthouse et al., 2008). Both empirical and modelling methods were discussed, as well as how the standard ecological risk assessment framework can be adapted to specifically address populations.

LEMTOX (2007)

The workshop brought together stakeholders from academia, regulatory authorities and industry to discuss the role of ecological modelling in ERA of pesticides. Participants agreed on the benefits of using mechanistic effect models in ERA of pesticides, in terms of exploring the importance of ecological complexities that cannot be tested empirically. They also stressed the need for guidance on Good Modelling Practice, as well as for case studies that explore the added value of ecological models for risk assessment (Forbes et al., 2009).

RUC09 (2009)

The workshop focused on addressing the issue of which actions should be taken to implement population modelling into ERA, after pointing out several reasons why population modelling should play an important role in bridging the gap between the protection goals and what is actually measured (Forbes et al., 2011). Unlike the other two initiatives mentioned above, this

workshop did not focus on pesticides per se, but included other groups of substances, such as industrial chemicals.

MeMoRisk (2008 – ongoing)

A SETAC-Europe Advisory Group on "Mechanistic effect models for ecological risk assessment of chemicals". The advisory group was established as a platform to bring together all stakeholders involved in the regulatory process of ERA (Preuss et al., 2009): the purpose is to take concerted actions towards standardisation of ecological modelling approaches for ERA of chemicals, after recent reviews (Pastorok et al. 2003; Grimm et al. 2009) have strongly emphasized this need. In order to achieve this goal, a number of actions have been promoted by the advisory group (see e.g. CREAM and MODELINK).

CREAM (2009-2013)

A European project on mechanistic effect models for ecological risk assessment of chemicals. CREAM is a EU-funded Marie Curie Initial Training Network, outcome of the LEMTOX workshop, where both specific models and general guidance for good modelling practice are being developed (Grimm et al., 2009).

MODELINK (2012 – 2013)

A series of two SETAC Europe workshops initiated by the MeMoRisk advisory group, focused on the issue of linking ecotoxicological tests to protection goals. The purpose of the workshops is to provide recommendations on how to use mechanistic effect models to create this link, as well as to define criteria for deciding when the use of ecological models in ERA schemes and for the choice of model types.

1.2 Brief review of ecological models for ERA

Exposure models are routinely used in risk assessment to predict the fate of the compound of concern in different environmental compartments, and therefore estimate the concentrations to which organisms in nature may be exposed. On the effect side of risk assessment, models are much less utilised. Aside from statistical models to derive, for instance, dose-response curves and determine concentrations that do not cause adverse effects on the exposed individuals, no other models are regularly used. However, as stated in the previous section, the potential of ecological models for improving risk assessment of chemicals, in particular for plant protection products, is increasingly recognized.

Mechanistic effect models are ecological models that represent key processes necessary to link toxic effects at different levels of biological organization, for instance, from sub-individual to individual and population levels. Mechanistic effect models have been successfully used in a number of ecological applications. Some examples are predictions of recovery time (Van et al., 2007), effects of multiple stressors (Ashauer et al., 2007a), interaction of toxicant effects with life history (Stark and Banken, 1999; Stark et al., 2004), density dependence (Forbes et al., 2001; Forbes et al., 2003) and landscape structure (Topping et al., 2003; Thorbek and Topping, 2005; Topping et al., 2005).

Within the broad spectrum of existing ecological models, three major types can be identified in the context of chemical risk assessment: differential and difference equations, matrix models, and individual- or agent-based simulation models.

Differential and difference equations models

Two main categories of ecological models for risk assessment of chemicals lie within this type: Toxicokinetic-toxicodynamic models (TKTD), and Dynamic Energy Budget (DEB) models.

TKTD models simulate "the time-course of processes leading to toxic effects on organisms" (Jager et al., 2011). Toxicokinetics convert an external concentration of a toxicant to an internal concentration over time through the processes of uptake and elimination, while toxicodynamics quantitatively link the internal concentration to the effect at the level of the individual organism over time (Jager et al., 2011). TKTD models have been used successfully to extrapolate toxic effects between different exposure scenarios (Ashauer et al., 2007b), and to explain effects of mixtures over time (Jager et al., 2010).

The dynamic energy budget (DEB) theory for metabolic organisation specifies quantitatively the processes of uptake of food by organisms and its use for the purposes of maintenance, growth, maturation and reproduction (Kooijman, 2000). In the standard DEB model, individuals are considered equal, feed on a single food source and have three life-stages: embryo, juvenile and adult (Kooijman, 2000). The basic DEB theory has been extended to also include effects of chemical compounds (DEBtox). Effects at the individual level are expressed in terms of uptake, elimination and (metabolic) transformation of the compounds (Kooijman et al., 2009), and are linked to the energy budget through toxicokinetics relationships. Effects at the population level are instead evaluated from those at the individual level, by considering populations as a set of interacting individuals (Kooijman et al., 2009): effects of a toxicant on the energy allocation of single average individuals are linked to the consequences for the populations.

Demographic models

Demographic models describe individuals in terms of their contribution to recruitment and

their survivorship. A convenient and widely used mathematical formulation of age- or stage-structured demographic models is based on linear algebra: the use of matrices in fact provides the advantage of a relatively simple representation of underlying biological phenomena, and an equally simple analysis of the model (Charles et al., 2009).

The complexity of matrix population models varies widely. Such models can incorporate, if necessary, density-dependence and demographic and environmental stochasticity (Caswell, 2001).

They can also incorporate a spatial dimension, which is useful to model spatially fragmented populations, in what are called metapopulation models (Hanski and Gilpin, 1991). Metapopulations are systems of local populations connected by dispersing individuals. Most individuals are born and die within a local population (Hanski and Gilpin, 1991); individual variability within local populations is generally ignored in metapopulation models.

Projection matrix models can incorporate effects of toxicants on all vital rates, allowing an integrated assessment of toxicant impacts on population dynamics (Forbes and Calow, 2002), and therefore can be a relevant tool for ecotoxicology and environmental risk assessment.

Individual- and agent-based models

According to the definition given by Grimm (1999), individual-based models (IBMs) are "simulation models that treat individuals as unique and discrete entities which have at least one property in addition to age that changes during their life cycle, e.g. weight, rank in a social hierarchy, etc.".

IBMs are particularly well-suited to study systems that are heterogeneous both in space and time, as they model single individuals – which can therefore be characterised by different state variable values. Individuals interact with, and can adapt to, their surrounding environments, and with IBMs it is possible to investigate how different conditions affect individual life history and behaviour. IBMs allow researchers to study how system level properties emerge from the adaptive behaviour of individuals (Railsback, 2001; Strand et al., 2002) as well as how, on the other hand, the system affects individuals.

Current use of IBMs for ecological risk assessment is limited: in their literature review, Schmolke et al. (2010b) found that only 13% of the models reviewed were IBMs, but their potential is increasingly recognized (Topping et al., 2009).

1.3 Good modelling practice and guidance documents on ecological models

While the potential benefits of using ecological models in risk assessment are clearly recognized, their actual use is not yet established. One of the main reasons for this is that current modelling practices are lacking in transparency and consistency (Grimm et al., 2010a). Ecological models are

developed for different purposes, which often leads to a great variety of model types and modelling styles (Schmolke et al., 2010a). This in turn generates confusion and distrust in users that are not familiar with the modelling process, and discourages them from using the models in a legal context.

I. Model development

Problem formulation: *Context* in which the model will be used, and the type of audience addressed; *specification of the question(s)* that should be answered with the model; statement of the *domain of applicability* of the model, including the extent of acceptable extrapolations; assessment of the *availability of knowledge and data*; specification of necessary *model outputs*.

Design and formulation: Description of the *conceptual model*; description and justification of the *modeling approach* used and of the *complexity*; *entities and processes represented* in the model; most important, the applied *assumptions* about the system.

Model description: Detailed *description of the actual model* and how it has been *implemented* (programs, software platforms, scripts).

Parameterization: *List of all parameter values* used in the model, the *data sources*, and how the parameter values were obtained or calculated; *uncertainties* associated with each parameter.

Calibration: Documentation of the *data sets used* for calibration; *which parameters* were calibrated; what *optimization method* was used.

II. Model testing and analysis

Verification: Assessment of whether the model is working according to its specifications; documentation of what tests have been conducted.

Sensitivity analysis: Exploration of the model behavior for *varying parameters*; documentation of which *parameter combinations* have been tested; *justification* of used parameter ranges and combinations.

Validation: Comparison of model or submodel outputs with *empirical data that were not used for parameterization or calibration*; documentation of *data sources*; what *parts (submodels) have been validated*; what *validation methods* were applied.

III. Model application

Results: Outputs that are used to *inform decisions*; description of *simulation experiments (scenarios)* conducted; *statistics applied* to analyze model outputs.

Uncertainty analysis: *Uncertainties in model outputs* used for recommendations; description of *variance, noise, and bias in empirical data*; determination of *stochasticity* in the model; description of *model uncertainty* which can be assessed through application of different models or submodels; best- and worst-case scenarios.

Recommendation: Description of how *initial question(s) could be answered*; summary of *conclusions* drawn from model; clarification of *extrapolations* used (in time and space).

Fig 1.2. Structure of a TRACE document (adapted from Schmolke et al. (2010a)).

In their review, Schmolke et al. (2010b) identified the main areas of concern about current modelling practice to be unknown sensitivities and uncertainties of model predictions, unclear sources of parameterization and lack of thorough model analysis (Schmolke et al., 2010b).

Therefore, to ensure the suitability of ecological models for the risk assessment of chemicals, "good modelling practice" is needed. The elements to address are nothing new, as they have been already described (e.g., Jakeman et al., 2006): they can be summarised as model development, analysis, evaluation, documentation, and communication. What is really necessary to establish GMP is sufficient involvement of decision makers and stakeholders in the modelling process and some incentives for modellers to follow it (Schmolke et al., 2010a; Thorbek et al., 2009).

Instead of inventing a completely new format, Schmolke et al. (2010a) propose looking at other initiatives which have proven to be well-functioning. The example they suggest is a bottom-up process which has been tried recently for documenting individual- and agent based models: the ODD protocol proposed by Grimm et al. (2006), which provides overview, design concepts and details about the model.

This approach is becoming more and more popular among individual- and agent-based modellers: ODD has been already used in more than 50 publications (Grimm et al., 2010b). Reviewing the uses to date of ODD, authors observed that using a standard structure to describe models increased understanding of model descriptions, because readers knew what information about a model was provided where and in what order. Furthermore, ODD has promoted rigorous model formulation, as modellers started using it as a hierarchical checklist for formulating models (Grimm et al., 2010b).

Schmolke et al. (2010a) therefore suggest to follow the same kind of bottom-up process to establish good modelling practice through a more or less self-organizing process: for this purpose they introduce a standardized documentation of ecological models, the so-called framework for transparent and comprehensive ecological modelling (TRACE). Fig. 1.2 summarizes the TRACE documentation structure: the sequence of the elements corresponds to the sequence of tasks in the iterative modelling cycle (Schmolke et al., 2010a).

1.4 Aim of the thesis

The aim of the present thesis is to, following the principles of Good Modelling Practice, develop, test and use a combination of metapopulation modelling and individual-based modelling to predict the impacts of spatial heterogeneity in soil contaminant levels for the population dynamics of the collembolan, *Folsomia candida*. In order to develop models that better suit the needs of environmental risk assessment, I also participated in a study that aimed at clarifying how ecological models are perceived by stakeholders involved in ERA of chemicals and what should be done in order to get them accepted in ERA procedures.

In **Chapter 2** I contributed to study perspectives of three stakeholder groups on population

modelling in ERA of pesticides, by analysing the responses of 43 in-depth, semi-structured interviews that were conducted with stakeholders from regulatory authorities, industry, and academia all over Europe. Participants for this study were recruited using the key informant approach: they were first identified as key stakeholders in the field and then sampled by means of a purposive sampling, where each stakeholder identified as important by others was interviewed and asked to suggest another potential participant for the study.

In **Chapter 3** I present the spatially explicit individual-based population model I developed to investigate the effects of heterogeneous soil contamination on *F. candida*. In the model, individuals are assumed to sense and avoid contaminated habitat with a certain probability that depends on contamination level. Avoidance of contaminated areas thus influences the individuals' movement and feeding, their exposure, and in turn all other biological processes underlying population dynamics. A large part of the chapter is dedicated to describing how the model has been developed, parameterized, tested and evaluated according to the pattern-oriented modelling theory.

The same model has been used in **Chapter 4** to explore how the interaction of different patterns of microscale fragmentation caused by the presence of a persistent pollutant in soil, combined with disturbance events, which can be both natural (e.g. drought) and anthropogenic (e.g. pesticide applications), affects the population dynamics of *F. candida*. To simulate loss and fragmentation of habitat caused by a persistent contaminant, copper sulphate was used. A midpoint displacement algorithm has been implemented in the IBM to generate fractal landscapes with varying degree of spatial autocorrelation and percentage of contaminated habitat. Other submodels introduced in the IBM to conduct this study include procedures for simulating effects on survival and/or reproduction of a drought period and of disturbance events.

In **Chapter 5** I have taken the individual-based model described in the previous chapters, and contrasted it with a relatively simpler, more standardized approach, based on the generic metapopulation matrix model RAMAS. With the two models I have then explored consequences of model aggregation in terms of assessing population-level effects for different spatial distributions of a toxic chemical. With this comparison I tried to shed light on the factors that should drive the choice of model type to be used in ERA of chemicals.

Finally, in **Chapter 6** I discuss the findings of my thesis, especially simulation results of both models, both in a specific and wider perspective, and how the models and the results can be used to inform risk assessment.

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Chapter 2

Stakeholders' Perspectives on Ecological Modeling in Environmental Risk Assessment of Pesticides: Challenges and Opportunities

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Abstract

The article closely examines the role of mechanistic effect models (e.g., population models) in the European environmental risk assessment (ERA) of pesticides. We studied perspectives of three stakeholder groups on population modeling in ERA of pesticides. Forty-three in-depth, semi-structured interviews were conducted with stakeholders from regulatory authorities, industry, and academia all over Europe. The key informant approach was employed in recruiting our participants. They were first identified as key stakeholders in the field and then sampled by means of a purposive sampling, where each stakeholder identified as important by others was interviewed and asked to suggest another potential participant for our study. Our results show that participants, although having different institutional backgrounds often presented similar perspectives and concerns about modeling. Analysis of repeating ideas and keywords revealed that all stakeholders had very high and often contradicting expectations from models. Still, all three groups expected effect models to become integrated in future ERA of pesticides. Main hopes associated with effect models were to reduce the amount of expensive and complex testing and field monitoring, both at the product development stage, and as an aid to develop mitigation measures. Our analysis suggests that, although the needs of stakeholders often overlapped, subtle differences and lack of trust hinder the process of introducing mechanistic effect models into ERA.

2.1 Introduction

The registration and use of pesticides in Europe requires that substances undergo a thorough risk assessment in which the environmental fate, human health effects and ecological effects of the pesticides are estimated. The overall process is carried out using a tiered approach that starts with worst-case assessments of exposure and effects at lower tiers, and proceeds to more realistic assessments at higher tiers for those substances that fail to pass critical thresholds for exposure and/or effects. For environmental risk assessment (ERA), environmental fate and exposure are typically estimated using models (Boesten, 2004; Boesten et al., 1995), whereas effects are usually estimated from the results of laboratory toxicity tests or (occasionally) from mesocosm or field studies. For most taxonomic groups, ERA aims to prevent unacceptable effects of pesticides at the population level (European Commission, 2009; EFSA Panel on Plant Protection Products and their Residues, 2010), with the exception of birds and mammals for which effects on individuals are of concern.

In recognition of the difficulties in extrapolating effects of standard toxicity tests and mesocosms to likely impacts of pesticides on populations in the field, mechanistic effect models (e.g., ecosystem or population models) are seen as a way to bridge the gap between test endpoints and the ecological entities that the current risk assessment schemes aim to protect (EFSA Panel on Plant Protection Products and their Residues, 2010; Forbes et al., 2008).

In our article we defined mechanistic effect models after Grimm et al. (2009) as *"models that mechanistically represent key ecological processes (...) and individual-level models quantifying adverse effects of chemicals on organisms based on mechanistic understanding."* (p. 615)

The number of suitable models grows every year, as do initiatives to come up with a unified and standardized approach to models and modeling documentation (Grimm et al., 2006; Schmolke et al., 2010b; Schmolke et al., 2010a). Schmolke et al. (2010b) conducted a comprehensive literature search for models that deal with the effects of pesticides on populations or communities. From this review, it is clearly evident that a broad range of effect models have been applied to address ecotoxicological problems. Schmolke et al. grouped the models used to assess chemical risk into three broad classes (see also Forbes et al., 2008; Bartell et al., 2003; Pastorok et al., 2003): differential equations, matrix- and individual-based models. Their review points out that the first two, more traditional modeling approaches, are the most commonly used, whereas relatively new tools, represented by individual-based models, are still not widespread, although their popularity is slowly in-creasing. Still, stakeholder groups involved in ERA of pesticides have different views on the applicability of effect models in real-life decision-making processes. Whereas predictive modeling is used widely in many areas in our daily life, such as weather fore-casting, and in other fields of natural science (e.g., conservation ecology: Starfield, 1997), models have not been as widely used to predict effects of pesticides or other toxic chemicals.

At the same time, fate models are used in ERA of pesticides for predicting concentrations of active substances in ground-and surface water (FOCUS Work Group, 2011). The majority of national regulatory bodies employ FOCUS models for that purpose (EFSA Panel on Plant Protection Products and their Residues, 2004). The FOCUS group, established as an initiative of the European Commission in 1993 was based on cooperation of scientists and modelers from industry, academia, and regulatory authorities. For both ground water and surface water the group developed several different scenarios representing typical agricultural conditions in the EU, and a set of models simulating the distribution of pesticides in surface water and ground water. There are detailed regulatory guidelines about how to apply FOCUS modeling outputs to risk assessments. The legal status for use of effect models is very different, however. For instance, European Food Safety Authority (EFSA) guidance on risk assessment for birds and mammals states that population models may be used as refinements, on a case-by-case basis (EFSA, 2009) Still, there is currently no guidance on requirements for effect model development or how to apply them to risk assessments.

The aim of this study was to explore the role and the potential applicability of mechanistic effect models in ERA of pesticides under the European regulation No 1107/2009, as perceived by potential model users, reviewers, creators, and evaluators. Partially employing the policy arrangements (PA) approach, we studied perspectives of different stakeholder groups (actors) on effect models and modeling. PA provide a framework to analyze shifts and changes in environmental policy. The framework, coined by Arts et al. (2006) has been used as a concept linking long-term policy changes (e.g., the shift toward more environmentally concerned and "green" policy and legislation in recent years), with particular decision-making processes (see e.g., Veenman et al., 2009). The concept allows focusing on both institutional (i.e., involved stakeholders and their interactions) and discursive (i.e., views and opinions of involved parties) characteristics of a policy change (Wiering and Arts, 2006). Because mechanistic effect models are both gaining popularity in ecotoxicology, and also slowly getting on the political agenda (EFSA Panel on Plant Protection Products and their Residues, 2010; Forbes et al., 2011), we employed the PA as a framework able to grasp long-term policy change. We decided to study both stakeholders' interactions and discourse in ERA toward the integration of effect models into the current ERA of pesticides scheme. The PA framework comprises four tightly interwoven dimensions:

- *Actors* (stakeholders) and their coalitions;
- The division of *power* between actors and influence over policy outcomes;
- *Rules of the game*, which are forms of interaction, both formal and informal in pursuit of decision-making;
- *Discourse*, which is the narrative and views of involved parties "i*n terms of norms and values, definitions of problems and approaches to solutions*"

We identified the key actors in the European ERA for registration of pesticides and interrelations among them. We also followed closely the current state of effect modeling in ERA of pesticides and the general organization of the decision-making processes behind pesticide authorization. Moreover, we sought answers for two main questions: (1) what criteria does an effect model need to fulfill to be used in ERA?, and (2) what prevents models from being used to predict effects of pesticides?

Although similar issues are emerging in other ERAs within the European legislation, such as under REACH and the Water Framework Directive, we focused on pesticide regulation as a case study, because exposure scenarios and focal species are better developed than in other regulatory frameworks for chemicals.

2.2 Methods

Our aim was to reach a wide variety of stakeholders directly or indirectly involved with ERA for registration of pesticides across Europe, and we found the key informants approach best suited for the purpose. The key informants technique originates in anthropology and ethnographic studies, where a key informant is a source of detailed information based on the expert knowledge of a particular subject (Marshall, 1996). Hence, our sampling methods were strictly purposive, rather than random, because we sought participants with predefined characteristics—in this case expertise in ERA of pesticides. First, we identified key actors and continuously sampled by means of snowball sampling (Goodman, 1961), where each stakeholder identified as important by others was interviewed and asked to suggest other key stakeholders to be interviewed.

We interviewed participants from the pesticide industry, academia, and national and Pan-European regulatory authorities involved in the registration of pesticides. In addition, we had invited a number of large, international nongovernmental organizations, actively involved in agrochemical campaigns; however, none of these accepted our invitation to participate. We recruited not only risk assessors and mechanistic effect modelers, but also risk managers, policy makers, fate modelers and some contract researchers from10 different European countries and the United States. In total, more than 60 prospective participants were invited, out of which 43 were interviewed: 15 participants from industry, 14 from regulatory bodies, and 14 from academic institutions.

To gather a variety of responses, we prepared an interview guide comprising 15 open topics. Only a part of the interview guide was directly related to models in ERA (see Appendix 1 for a complete interview guide). The focus was on the use of models and recent changes in guidance documents and pesticides legislation, however, participants were encouraged to share their own views and stories. We also defined "models" very broadly, asking our participants whether they used/came across models at all, and whether they used or developed models to assess any potential

effects of pesticides.

All interviews were confidential and conducted by the same person, as different interviewers could set a different tone and influence the answers. The interviewer was not professionally involved with either pesticide registration or the development of population models, but trained in gathering interviews and survey data. The interviews lasted 40 minutes on average and they were recorded with the participants' permission, after which the texts were transcribed verbatim and submitted to the participants for approval. We divided the interviewees into the three abovementioned groups and analyzed the transcripts accordingly, assuring the full anonymity of our participants. Two researchers worked on coding and organizing the anonymous transcripts, grouping and cross referencing similar pieces of text to identify main themes and key-words related to modeling. Working separately al-lowed controlling for potential biases in a single re-searcher, but the coders discussed and compared their separate results afterwards. Transcripts were first divided into the smallest logical units (couples of sentences forming single ideas), which were then grouped together. We cross-referenced them both within a single interview and against other interviews. The method we used here is commonly employed in qualitative data analysis in social, psychological, and ethnological studies, and it is known as the "grounded theory approach," because of the way text data are treated (Corbin and Strauss, 2008; Glaser and Strauss, 1967). The approach is only used to answer broad, open-ended research problems, instead of testing any predefined hypotheses. The authors, Glaser and Strauss (1967), proposed this method to uncover any common, repeating themes "grounded" in qualitative data. The technique is not meant to achieve the best possible statistical representativeness, but to obtain a wide range of responses and in-depth perspectives (Glaser and Strauss, 1967). We also based our methods on discourse analysis for the parts of interviews revolving around interactions among stakeholders. Discourse analysis is, to a great extent, similar to the grounded theory approach as a method (grouping raw text data into larger, meaningful units), but the stress is on social interactions (Gasper and Apthorpe, 1996). A researcher also looks for emerging themes (called discourses), but the main focus is on inconsistencies, repetitions, citing others to support one's own views, etc. The purpose is to study, for instance, power relations between different stakeholders (social groups; Hutchby and Wooffitt, 1998).

We continued to invite new participants up to the point of theoretical saturation in the data. Theoretical saturation is the point at which further interviewing does not add to the findings, or repeats already collected information. It is commonly used in the grounded theory approach (Corbin and Strauss, 2008) and helps researchers to decide when to stop recruiting new participants.

Our qualitative text analysis is accompanied by quantitative data, as we also calculated frequencies of keywords that we identified during the coding phase with TextSTAT 2.8 software (TextStat is open source software, created by Matthias Hüning, Freie Universität Berlin, Berlin, Germany).

We counted the keywords—in this case the key criteria for accepting models, first automatically, then manually. Keywords and their equivalents (e.g., validation, validated, validate) were counted together. As we primarily used the keywords count to get a better understanding of criteria a model needs to fulfill, according to our respondents, to be used/accepted we excluded some keywords from the count due to their context, for example the ones that occurred in phrases like "models cannot be validated", without contending whether such phrases were true/false.

2.3 Results

We first focus on general patterns, common for all participants, before moving to a detailed analysis of common themes and differences between the three groups (coded as academia, industry, and regulators). To protect anonymity of the participants, we present the results in a narrative form, without any direct quotations. However, all themes, trends, and patterns described in the next sections were directly derived from and supported by text (transcribed interviews) data.

We asked the respondents about modeling in risk assessment first, and then in very broad terms about "the use of models to predict effects." Our aim was to keep the interviews open and let the participants decide for themselves what they understood by an "effect model." Therefore, we obtained various reactions, although only four respondents from our sample did not associate "effect model" with some kind of mechanistic effect modeling approach. The best-known approach for the participants turned out to be individual-based population models (see Schmolke et al., 2010b). This type of ecological model was also the only one that respondents thought tended to have a history of use in pesticide ERA.

General patterns

In our sample, there were only eight active modelers, working with different kinds of effect models: individual- or agent-based, matrix, energy budget, or meta-population models. On the other hand, our interviewees were familiar with environmental fate modeling, and a common pattern during the course of an interview was to assume that by "models" the interviewer meant "fate models" only. Table 2.1 presents a summary of this pattern among our participants.

Effect models were not as familiar to our participants as were fate models. The regulatory authorities working with risk management were the least likely to work with or use models at all, followed by those academics exclusively devoted to experimental work and consultants from contract research companies. Skepticism about models among the participants did not follow exactly the same pattern. The most positive group turned out to be regulators—both risk assessors

and risk managers, while some skepticism about effect models was present among the academics involved in experimental research. In some cases modeling was mentioned as a threat to the experimental approach in terms of funding priorities. Still, models seemed to be rather warmly expected, however, as shown in Table 2.1 they are yet to be widely used. In the whole group, eight participants were skeptical about the usefulness of effect models in ERA: four academics, three industry representatives and one regulator.

The last result is contrasted with the popularity of fate models. Not only were such models familiar to the majority of our participants, but they were often set as an example for effect models, mainly of robustness, user-friendliness, and as having well-documented version control. Participants, particularly from industry and regulatory authorities, stressed that, provided population models were developed in a similar manner as fate models, they would quickly become more commonly used. At the same time, skepticism of models estimating pesticide effects was matched by skepticism of experimental results estimating pesticide fate. Many participants noticed that the outcome of environmental fate models to predict exposure to pesticides (e.g., FOCUS Groundwater models) was generally more trusted and preferred over results of field experiments.

*Table 2.1. Fate and effect modelling in ERA of pesticide registration. First columns show numbers of respondents expressing agreement (*true*) or disagreement (*false*) with the heading statements. Second section shows how the agreement (*true*) with each heading statement is distributed in each group.*

	I use fate models	**I use effect models**	**I hardly ever come across effect models**	**I am an active modeller in the effect area**
TRUE	29	15	9	8
FALSE	14	28	34	35
Breakdown of "TRUE" by each group				
Regulators	14	4	5	0
Industry	11	3	1	1
Academia	4	8	3	7

Discourse on Modeling: Common Themes

There were three main themes present in our data: expectations associated with models, obstacles in their implementation, and criteria that population models had to meet in order to be accepted in ERA of pesticides.

Expectations

Both industry and regulators put a lot of hope in population models as a tool to reduce the costs and increase cost-effectiveness of risk assessment and management measures. Industry representatives saw models as a way to reduce the amount of required testing, which would have

benefits for animal welfare and reduce costs, especially for expensive field experiments performed for higher tier assessments. Regulators, on the other hand, expected modeling to help them reduce the costs of expensive monitoring programs.

Yet, our analysis showed that the expectations that models should meet were contradictory. First, participants in every group expected models to be simple—easy to understand, create, re create, and control. They should be transparent in terms of allowing much control over the input parameters and representing processes that should be easy to follow. At the same time, every group put much hope in modeling as a tool to tackle problems that were complex and/or not yet addressed in ERA of pesticides. Therefore models were expected to be complex enough to address several problems mentioned by the respondents: the issue of mixture toxicity (regulators), allowing extrapolations to different conditions, time spans and species (all three groups), aiding understanding of real populations (academia), and accommodating landscape changes (all three groups). Second, ecological models were expected to be specific, in terms of addressing precisely some explicit questions of higher tier assessments (regulators,

industry), but also generic enough to be applicable internationally, represent average situations, landscape and climatic conditions, even generic species (all three groups). Third, models were supposed to solve the current problems of risk management by providing different scenarios and a whole range of probability estimates (all three groups). At the same time, some stakeholders also expected the models to produce a binary output, in terms of allowing the decision maker to answer yes/no or below/above safety threshold questions (regulators).

Obstacles

The three groups were surprisingly unanimous on obstacles to population models becoming accepted for regulatory use. The main problems mentioned by respondents were: lack of trust in modeling accompanied by lack of models suited for decision-making purposes, and the problem of uncertainty present in modeling output.

1. Vicious circle. The main obstacle that every group mentioned preventing the use of ecological models in ERA was a lack of trust in them. Models were rarely submitted as part of the risk assessment, as participants from industry stressed, because they were not trusted and tended to be rejected by the regulatory authorities. As familiarity breeds trust, and working with models breeds familiarity, at the moment ecological modeling is in a Catch-22 – not used because of the lack of trust, and not trusted because of the lack of use.

2. Regulatory question. Another obstacle, particularly apparent to our respondents from regulatory authorities was that models very often failed to sufficiently address the issues regulators expected them to address. Participants mentioned several instances of such a situation. First, an ecological model could use an inappropriate species to show potential effects, for example, a

species that did not represent a worst-case scenario. Second, a model could fail to provide a clear answer about meeting the cut-off criteria, for example, by providing a probability, instead of a yes/no result. Finally, many participants from all three groups mentioned that models would not be able to answer regulatory questions due to procedural factors. Regulatory respondents, for instance, said that the protection goals were not clear, as the current pesticide legislation states these goals in very general terms (e.g.,...shall have no unacceptable effects on the environment; Regulation (EC) 1107/2009). Broad legal boundaries made it challenging to adapt modeling outputs to regulatory problems.

3. Black box. All three groups stressed that understanding what happened inside a model was key, and that the lack of transparency in modeling was a serious obstacle. Two main reasons for such a situation were mentioned: the lack of necessary documentation, which was stressed by modelers themselves, and the lack of control over the input parameters, which was especially important for regulators. Interestingly, an understandable model did not necessarily have to be simple. Regulators actually preferred transparency in terms of clear documentation and control of modeling input over simplicity.

4. Uncertainty. Uncertainty of a future outcome is built into the definition of the word "risk." Still, it turned out that uncertainty that is built into a model's outcome could be a serious obstacle in accepting effect models. Uncertainty and probability inherent in models were identified as an issue by all three groups. Regulators would ideally like to see modeling that reduces uncertainty as much as possible, or otherwise, they preferred the most conservative model. Both industry representatives and academics stressed that probability distributions produced by models needed to be communicated properly, but a model's output could not replace a final decision on management and protection goals, which had to be made by risk managers themselves. Several participants from regulatory authorities stressed that the emphasis on reducing uncertainty is strongly interwoven with social and psychological factors accompanying management decisions. Respondents mentioned that registration of pesticides was "about life", so the final decision-making process could not be based on scientific criteria alone – for instance, they stressed that decisions, risk managers had to make were strongly connected with valuing the environment (from an anthropocentric or ecocentric perspective), so the ERA scheme should accommodate that fact.

5. ERA is flawed. Many participants mentioned that mechanistic effect modeling was the future of ERA of pesticides. Still, the problems of uncertainty and acceptance of models were reinforced by the current risk assessment scheme, namely by the lack of clear protection and management goals. Both modelers and model reviewers found it problematic to translate some exceptionally vague legal texts into modeling input and output. Illustrating the problem, participants gave two particular examples of "protecting ongoing behaviour of (…) species" and "no unacceptable effects on the environment" (Regulation (EC)1107/2009). Participants stressed that the future acceptance

of models had to be preceded by having clear and specific protection goals.

Criteria to meet

We asked the participants what formal criteria a model had to fulfill to be accepted. All three groups mentioned validation among the main criteria. A realistic output turned out to be important as well, and was mentioned more frequently than, for instance, transparency. Models also needed to be calibrated and accurate, in terms of being able to repeatedly return similar output results from the same input parameters. Stakeholders from industry also stressed that a model needed to be scientifically robust.

Model validation was one of the most frequent issues addressed by our interviewees. All three groups expected models to be validated somehow, but the detailed discourse on validation varied greatly. First, modelers themselves presented a rigid definition of validation, and at the same time, they were the most skeptical about it. Some modelers mentioned that it might not be possible at all to thoroughly validate a model against experimental data. If a model was expected to be realistic, extrapolate to different conditions, and project results over long time spans (e.g., 100 years), such extensive experimental designs were either not available at the moment or virtually impossible to conduct.

Modelers also stressed that a model needed to be verified and tested in the first place, and parts of it should be validated wherever possible, but the end users had to be aware that there were limitations to validation possibilities. In contrast, the majority of the non modelers expected that effect models would be thoroughly validated against experimental data. Validation was the most important criterion for our regulatory respondents, followed closely by academics and industry. Yet, "validation" had different meanings for different participants—some of them defined it in very general terms, as "testing the model." In Table 2.2 we compared the most important criteria mentioned by our respondents across the three groups.

Not taking into account the obvious between-group differences in the absolute number of keywords, it was apparent that key criteria were different in each group. Academics put understanding of models in first place, followed by realism, validation, and simplicity. For industry, the need to have their models understood was the most important factor just before validation. Models also needed to be simple and scientifically robust. For regulators, the most important criteria were validation, understandable interface, and realistic output.

"Rules of the game" – who can change the status quo?

According to our participants, communication of models and modeling was key. There were, however, two things that needed to be accounted for beforehand. First, although informal op-

portunities for communication were, according to our interviewees, sufficient, communication flow between the three groups was perceived as flawed. Respondents from industry pointed out that they did not get enough opportunities to discuss and exchange feedback on their submissions. Regulatory representatives mentioned that the risk assessment scheme was getting more and more complicated and overly scientific, while the reality of management decisions was not taken into account. Respondents from academia told us that even though their models were used in regulatory submissions, they hardly ever received any feedback on them. Second, although the discourse on ecological models was in principle similar in all three groups, there were some important differences, which might hinder communication.

Table 2.2. "What criteria does a model need to fulfill to be used?" Numbers are frequencies of keywords present in raw text data, taking into account the context the keywords appear in. Interviews were divided into three groups (regulators, academia, industry) before the keywords were counted for each group separately. We also counted the number of interviews where keywords were present, separately for each group. Keywords are sorted alphabetically.

Keywords	Academia/number of respondents	Industry/number of respondents	Regulators/number of respondents
Calibrated	3/3	2/1	1/1
Realistic	18/13	3/2	6/5
Robust	2/2	4/3	0/0
Simple	14/12	10/10	4/3
Tested	10/10	2/2	0/0
Transparent	12/10	5/5	4/3
Understandable	42/13	42/15	19/14
Validated	15/9	15/14	22/14
Verified	9/7	2/2	0/0

Shared views and different priorities

After a closer look at Table 2.2 it is apparent that, although in general our participants talked about similar things and shared the same views, they prioritized different issues. For example, modelers—both from academia and industry—put much effort on making models user-friendly, that is, simple, understandable, and transparent. But for the users from the regulatory community, simple models were not a top priority, as long as they were reliable to run—thoroughly validated, understandable, and allowing flexibility of input parameters.

The call for realistic output of ecological models was another shared view. Again, every group wanted an increased realism in ERA, but it turned out that "realistic" meant different things to different stakeholders. Academics wanted realistic models that would be ecologically relevant, that is to say, showed what happened in real ecosystems as accurately, as possible. Regulators, in

their need for realism stressed that a realistic model was as conservative as possible, and, especially in case of many uncertainties allowed to run a worst-case scenario. For industry, "realistic" was something that was not overly simplistic, scientifically robust, and most importantly aiding the decision-making process in the best possible way.

Yet, another issue important for every participant was the need for better communication of models. The majority of stakeholders believed that improved communication is key to widespread acceptance of population models. However, each group had their own take on communication, and perceived the role of the two other groups according to their own communication preferences. Academics presented a slightly top-down approach—they wanted to educate others about modeling and to "make them" understand the principles of effect models. Participants from industry expected communication to take a form of training in models. At the same time regulators looked forward to having an open dialogue on models and modeling—they not only wanted to understand models but also to take a more active part as a communication partner.

What/who prevents the use of effect models in ERA?

Who has the power to change the current situation? Is mechanistic effect modeling the future of ERA? The majority of our participants agreed that modeling was the way forward. Regulators were especially optimistic and believed that sooner or later ecological models would be used on the effect side of ERA, the same way as fate models are used on the exposure side. Our participants mentioned several ways to change the *status quo*.

Academics perceived themselves as a group that slowed down models' acceptance. They stressed that first, there was no agreement on modeling approaches. In addition, the way research was funded and organized naturally encouraged diversity—it was easier to get funding for innovations than for applying established methods. Some participants from academia pointed out that they perceived their role as constantly coming up with new modeling methods for ERA. On the other hand, respondents said that if some models were a part of widely accepted mainstream science, they would be easily accepted by other stakeholders that were generally expected to keep up with scientific developments. On the other hand, industry participants pointed out that effect models suffered from lack of trust—they believed that models could be perceived as a way of "massaging" data in higher tier risk assessment, and that modeling approaches were not accepted because they were always submitted by industrial applicants.

All three groups agreed that the best way to introduce ecological models into ERA of pesticides for good would be to work on guidance documents for them. Although models could be used in higher tier assessment, their legal status was not clear to many regulators we interviewed. Ideally, our participants would like to see all three-stakeholder groups working together on guidance for ecological models similar to the process under which the FOCUS models were developed.

2.4 Discussion

Perhaps the most important take-home message for modelers in our study is to be aware of expectations end-users and evaluators have about mechanistic effect models. Our results show that attitudes toward models among the majority of risk assessors and managers are generally positive, but whether a model is going to be successfully used in pesticide ERA, depends on whether the model meets the specific wants and needs of the regulatory community.

Modelers have to be aware of contradicting expectations about their models as well. The need for complex scenarios and simplicity at the same time could be, for instance solved by restricting the simplicity to a transparent and easily accessible documentation, and user-friendly interface, while the model itself could operate on a complex scenario and many input parameters. The need for realistic and generic situations at the same time is more difficult to address. Our participants themselves proposed a way to solve this problem by suggesting that effect model development should follow FOCUS group footsteps, where generic landscape and climate scenarios were developed for different parts of Europe. Combining the probabilistic output with the requirement of answering binary questions seems to be impossible to address at this moment, although there have been some attempts to provide a framework for addressing uncertainty in ERA (Hart et al., 2006). Whereas the FOCUS group was suggested as a good example to follow in model development, we believe that the familiarity of FOCUS models can be partially blamed for the expectations of simplified output from effect models. Probably an increased overall confidence and general acceptance of effect models would partially solve the problem, but the desire for an output similar in certainty to the FOCUS-type fate models is one of the more serious obstacles in effect models' use. However, we need to keep in mind that effect models are just a tool, and they can only aid, but never replace the decision-making process in risk management.

The discourse on effect modeling has a sound basis in a growing number of models available and current modeling developments (Grimm et al., 2009), yet our study shows that the three stakeholder groups involved in modeling speak different languages: it is important to mention that some of the obstacles we found may be triggered mostly by different understanding of certain concepts, such as "validation." Thus, despite the declared enthusiasm, we found some serious obstacles that need to be addressed before mechanistic effect modeling can be implemented in ERA. Interestingly, as the majority of our respondents were positive about effect modeling, the obstacles we identified were, in most cases, external to models and modeling itself. Models were generally perceived as something useful and benign, whereas what hinder their acceptance were human and procedural factors.

Most importantly, the lack of guidance on effect models and modeling, which is additionally reinforced by the flaws in the ERA procedures, was identified as one of the main issues by our

regulatory respondents. Whereas we found a number of significant attempts to provide documentation protocols on effect models for ERA (Grimm et al., 2009; Schmolke et al., 2010b; Topping et al., 2010), our respondents from regulatory authorities sometimes shunned effect models only because there was no guidance available on how to use and evaluate them. On the other hand, the wish for clear guidance can easily lead to very prescribed solutions, which leave no place for development of new models. Moreover, stakeholders agreed that they did not want more complicated procedures to follow and paperwork to handle, yet they wanted more guidance documents on modeling. It is then a challenge for modelers to provide documentation which would fulfill both criteria—setting out an understandable framework, without being overly complex and bureaucratic, whereas the actual development of regulatory guidelines for using effect models in ERA obviously has to be carried out in a forum with the relevant stakeholders (i.e., including modelers, regulatory authorities and other end users).

The second problem that is vital for effect models' popularity is the issue of protection goals. Risk managers expect modelers to provide them with models returning applicable, clear-cut answers, whereas modelers expect risk managers to provide them with clear management goals that can be modeled. Neither can be achieved as long as protection goals for pesticide ERA are not clearly set. The EFSA Panel on Pesticides provided some solutions with the ecosystem services concept (EFSA Panel on Plant Protection Products and their Residues, 2010). Hopefully the guidance on protection goals can help to solve the problem for risk managers and modelers alike. The issue of protection goals is strongly linked with general attitudes toward the environment and its use (Butler and Acott, 2007). As some risk managers mentioned, decisions they were making were, first "*about life.*"Effect models, especially population or individual-based models in many cases provide an output of recovery probability after a pesticide application, so the implicit assumption is indeed about survival ("*life*") of the modeled species. Growing environmental concern (Stern and Dietz, 1994), if combined with uncertainty in the model outcome, is an additional obstacle that can prevent model acceptance. Empirical information on pesticide effects clearly seemed to carry more weight for our regulatory respondents than the information provided by outputs of effect models.

Finally, we found that although model makers and model users are concerned about similar problems, their values and priorities differ. This was especially visible in the validation discourse. Although all respondents stressed that validation was at the top of the modeling agenda, the frequency of keywords proved otherwise. It seems that the problem lies in different "validation discourses" each group is using. A validated model for a modeler does not have to be the same as for a stakeholder with a regulatory perspective. The problem clearly has to be addressed, but looking at our results it seems that, for instance, regulators expect effect models to be well tried and tested, reliable to run, based on sound scientific principles, and widely accepted by the

scientific community.

Perhaps one of the most practical and applicable findings of our study is the call for the next FOCUS-like group working on population models, worded by many of our interviewees. The FOCUS group worked from1992 (Boesten et al., 1995) until 2009, developing fate models and scenarios, which are presently used in the majority (but not all) of the EU member states. The FOCUS group was a community of academics, industry representatives, risk assessors, and managers working together on addressing the problems of pesticide fate in different environmental compartments. Our respondents wanted effect models to be developed in a similar manner. One has to be cautious, however, of the different communication requirements. In our study, regulators are the group expecting something closest to a round-table dialogue, whereas academics would rather take a role of teachers, and industry provides training. Finding a common platform for communication is key, and it seems that the FOCUS group indeed set an example for effect models in ERA.

Overall, the stakeholder processes in ERA of pesticides are no different from other decision-making areas where all interested parties are somehow involved in reaching a final consensus (Beierle and Cayford, 2002). The main issue present in all stakeholder-based decisions is the variety of factors influencing the final outcome (Beierle, 2002). Our respondents wish for the ecotoxicological decisions to be based on purely scientific results, but there are many other, socioeconomic, psychological, and political factors that are accounted for when the final consensus is reached. There are, however, no systematic studies analyzing the exact share of all components in the decision-making process. In the area of technological hazards, one of the more important issues is social amplification of risk (Kasperson et al., 1988). Pesticides risk can be amplified, for instance, by mass-media focusing on catastrophic events. In turn, perceived risks influence the stakeholders' take on the ERA of pesticides (Kraus et al., 1992). Therefore, a participatory approach in environmental matters is sometimes questioned for its quality and efficiency. For instance, in a study of regulatory decisions concerning hazardous waste, Viscusi and Hamilton (1999) found that the efficiency of decisions decreased with the increasing emphasis on political power. However, Beierle (2002) conducted a large, comparative case study into the quality of stakeholder-based decisions. He found that stakeholders' involvement in fact results in overall improved decisions (compared to *status quo*). Moreover, he also found that different stakeholders have access and use technical and scientific resources in various environmental decisions.

In our study, we tried to reach as many stakeholders as possible and collect a wide variety of responses. However, there are some limitations to every qualitative research design. We have to take into account, for instance, that our respondents were volunteers and they had not received any remuneration for their time. It may have biased the overall positive attitude toward effect modeling in pesticide ERA, because our interviewees took part in the study due to their own

interests in mechanistic effect models.

We tried to balance that effect by continuous recruitment during the data collection process, where we started working with text data and kept interviewing new participants at the same time (Corbin and Strauss, 2008). Moreover, we tried to reach a wide variety of prospective respondents during ecotoxicology-related conferences, workshops, and project meetings, which allowed us to recruit not only modelers, but also respondents, who have never come across effect models in ERA.

There are many questions with regard to the use of effect models that seem worth answering. One of the possible research directions would be to study the connection between the development of protection goals and guidance on effect modeling. The majority of our regulatory respondents pointed out that both the guidance on modeling and opinions on protection goals (EFSA Panel on Plant Protection Products and their Residues, 2010) are much awaited and it would be interesting to see how the protection goals (EFSA Panel on Plant Protection Products and their Residues, 2010) can be accommodated into population modeling.

The need for further guidance and more dialogue among stakeholders, which is apparent from our analysis, is being more and more recognized, and several initiatives to solve these issues are taking place, for instance within the Society of Environmental Toxicology and Chemistry (SETAC). SETAC workshops have already been the arena for discussions among stakeholders within the fields of ecotoxicology and ecological risk assessment of chemicals, with outcomes that directly led to guidance documents currently being used in ERA of pesticides (see for instance Barrett et al., 1994). The SETAC Europe advisory group MEMORISK (Preuss et al., 2009) is now active as a forum for communication through the organization of group meetings and workshops as well as a real advisory group for regulators willing to use models in risk assessment. Their latest effort is the organization of a workshop to provide a transparent overview of the current state of science related to ecological modeling and developing experimental/regulatory guidance for when and how to apply ecological models to regulatory risk assessment of pesticides.

In summary, effect modeling can play an important role in ERA of pesticides. However, it may take years before the models are widely used and accepted. The process can be accelerated by a closer cooperation between the most important stakeholder groups. Our analysis suggests that the needs of different stakeholders often overlap and thus that there is a good chance that consensus on the role and requirements of ecological modeling for risk assessment can be reached, but at the same time modelers have to revise their own priorities to meet the expectations of model users. Although we have used ERA for pesticide registration as a case study, most, if not all, of the issues raised by stakeholders will apply to effect models used in ERA for other legislative purposes (e.g., REACH, Water Framework Directive, etc.), and we believe that our findings have broad relevance.

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Chapter 3

Population-level consequences of spatially heterogeneous exposure to heavy metals in soil: An individual-based model of springtails

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Abstract

Contamination of soil with toxic heavy metals poses a major threat to the environment and human health. Anthropogenic sources include smelting of ores, municipal wastes, fertilizers, and pesticides. In assessing soil quality and the environmental and ecological risk of contamination with heavy metals, often homogeneous contamination of the soil is assumed. However, soils are very heterogeneous environments. Consequently, both contamination and the response of soil organisms can be assumed to be heterogeneous. This might have consequences for the exposure of soil organisms and for the extrapolation of risk from the individual to the population level. Therefore, to explore how soil contamination of different spatial heterogeneity affects population dynamics of soil invertebrates, we developed a spatially explicit individual-based model of the springtail, *Folsomia candida*, a standard test species for ecotoxicological risk assessment. In the model, individuals were assumed to sense and avoid contaminated habitat with a certain probability that depends on contamination level. Avoidance of contaminated areas thus influenced the individuals' movement and feeding, their exposure, and in turn all other biological processes underlying population dynamics. Model rules and parameters were based on data from the literature, or were determined via pattern-oriented modelling. The model correctly predicted several patterns that were not used for model design and calibration. Simulation results showed that the ability of the individuals to detect and avoid the toxicant, combined with the presence of clean habitat patches which act as "refuges", made equilibrium population size due to toxic effects less sensitive to increases in toxicant concentration. Additionally, the level of heterogeneity among patches of soil (i.e. the difference in concentration) was important: at the same average concentration, a homogeneously contaminated scenario was the least favourable habitat, while higher levels of heterogeneity corresponded to higher population growth rate and equilibrium size. Our model can thus be used as a tool for extrapolating from short-term effects at the individual level to long-term effects at the population level under more realistic conditions. It can thus be used to develop and extrapolate from standard ecotoxicological tests in the laboratory to ecological risk assessments.

3.1 Introduction

Heavy metals are common soil contaminants resulting from anthropogenic activities such as smelting of ores, municipal wastes, use of fertilizers and pesticides (Leyval et al., 1997; Nursita et al., 2005). They pose a major threat to the continued capacity of soil to sustain its biological productivity, maintain the quality of the surrounding air and water environments, and promote plant, animal, and human health (Doran et al., 1996). To accurately evaluate this threat, just determining the total metal concentrations in soils is not sufficient since some of the metal may be in a form that is not available for uptake by organisms (Loureiro and Nogueira, 2005). Bioassays are therefore widely used for accurately assessing soil quality and potential toxicity of contaminants in that they measure the bio-available metal fraction (Boiteau et al., 2011). Standardized soil ecotoxicology tests have been developed using soil-dwelling invertebrates such as earthworms and collembolans (springtails) (Løkke et al., 1998). Collembolans are one of the most abundant groups of arthropods on Earth (Fountain and Hopkin, 2005). They play an important role in ecosystems functioning (Hopkin, 1997) and are vulnerable to soil contamination (Fountain and Hopkin, 2005; Crouau et al., 1999). The abundance and diversity of collembolans have been widely used to assess the environmental impacts of a range of pollutants on soils (Fountain and Hopkin, 2005; Crouau et al., 1999).

In particular, interest in the collembolan *Folsomia candida* Willem 1902 has been increasing in recent years. It is a very common species and has been found in a variety of habitats including soil, caves and glasshouses. *F. candida* has been used extensively as a model arthropod in many ecological and evolutionary studies (see Hopkin, 1997 and references therein). Moreover, it is used as a standard test organism for toxicity tests: a 28-day reproduction test (ISO, 1999; OECD, 2009) is included in the refinement options for ecological risk assessment of plant protection products to soil organisms (EC, 2009). However, one of the limitations of virtually all standard toxicity tests with soil organisms is that soil contamination is assumed to be homogeneous, whereas the heterogeneous nature of soil is well known. Spatial heterogeneity in soils occurs at widely different scales, from continental and regional to micro aggregates within specific soil horizons. Common soil properties such as clay and organic matter concentrations often show clearly defined spatial patterns that vary depending on the scales at which they are considered (Lavelle and Spain, 2001). Moreover, contamination of soils is heterogeneous as well because the distribution of chemicals in soil depends on the source of contamination (i.e., point vs. non-point source) and on specific soil properties that result in different interactions between chemicals and soil particles.

Studies exploring the ecological relevance of local variability in soil conditions are rare. Palmqvist and Forbes (2008) examined the influence of contaminant spatial heterogeneity in sediment systems and found that contaminant hotspots led to lower equilibrium population sizes

and longer recovery times compared to homogeneously contaminated sediment, despite that the total amount of contamination in the former scenario was less than the latter. Understanding such effects and predicting the consequences of spatially heterogeneous contamination are not straightforward. On the one hand, unpolluted patches in a matrix of polluted soil can be beneficial in order to sustain metapopulations. However, if soil organisms actively aggregate in unpolluted patches, and if such patches are small, density can become too high to sustain local populations.

Several studies have been conducted to investigate avoidance behaviour of *F. candida* in the presence of heterogeneously contaminated soil, and have had mixed results. For instance, *F. candida* does not avoid naphthalene (Boitaud et al., 2006), while (Aldaya et al., 2006) observed a good correlation between avoidance and toxicity for substrates with a high content of polycyclic aromatic hydrocarbon (PAH) compounds. Greenslade and Vaughan (2003) compared avoidance and reproduction for soils with heavy metal contamination and found that some substances, such as cadmium salts, were not perceived as repellent, and therefore were not avoided, whereas other metals, such as inorganic copper, were avoided by *F. candida* at concentrations below those having effects on reproduction. Similarly, Filser and Holscher (1997) observed that *F. candida* is capable of discriminating between Cu-contaminated and uncontaminated areas. A 2-day avoidance test with collembolans has also been proposed as an early screening tool to assess toxic effects of chemicals and soil contamination (da Luz et al., 2004).

To obtain a more comprehensive understanding of how behavioural responses such as avoidance affect population dynamics, population structure, and distribution of individuals in soils with heterogeneous contamination, population models can help to overcome the logistical constraints of short-term laboratory experiments. We therefore developed an individual-based model of laboratory populations of *F. candida* in heterogeneously contaminated soils. We used copper sulphate (CuSO4) as a model contaminant. It is proven to have toxic effects on *F. candida* survival and reproduction, and to elicit behavioural responses like avoidance (Boiteau et al., 2011). Moreover, it is the main ingredient of Bordeaux mixture, a commonly used fungicide (Barker and Gimingham, 1911).

Our model, incorporating information on behaviour and life history, is designed to represent *F. candida* realistically enough to be used for evaluating and improving standard ecotoxicological tests based on this species. Parameter values were taken directly from the literature or determined inversely by making the model reproduce several patterns observed in laboratory populations atdifferent scales and levels of biological organization ("pattern-oriented modelling"; Grimm et al., 2005; Grimm and Railsback, 2012; Railsback and Grimm, 2012). The structural realism of the model, i.e. its ability to make valid independent predictions, was tested. In this paper we focus on the design, parameterization, and understanding of the model, and present first results regarding the population-level effects of different levels of heterogeneity in soil contamination.

More specific analyses related to ecotoxicological tests will be published elsewhere.

3.2 Methods

Biological background

The genus *Folsomia* includes species in the family *Isotomidae* that have a well-developed furca (springing organ), no anal spines, and an abdomen with the posterior three segments fused (Fountain and Hopkin, 2005). Like all other collembolans, *F. candida* has a pair of thin-walled, closely apposed, eversible vesicles on the ventral side of the first abdominal segment. This structure is commonly known as the ventral tube, or collophore, and is involved in fluid exchange with the external environment (Hopkin, 1997). The ventral tube is an important exposure route for chemicals dissolved in soil pore water (Lock and Janssen, 2003). Mature individuals of *F. candida* are 1.5-3.0 mm long; the species feeds preferably on fungal hyphae, and populations exclusively consist of parthenogenetic females. The species can inhabit caves and mines, agricultural systems, soils with a high level of organic matter, forests, and the edges of streams. *F. candida* is occasionally the dominant collembolan, and population densities commonly reach 105 m-2 in soil and leaf litter layers in many ecosystems. The average lifespan of a female at 15°C under laboratory conditions is 240 days, but decreases when temperature increases (e.g. lifespan is 111 days at 24°C: Marshall and Kevan, 1962). At 20°C females reach sexual maturity around 15-20 days after hatching (Fountain and Hopkin, 2005; Krogh, 2008).

F. candida can be exposed to contaminants via the soil and/or food in a battery of tests that examine life-history parameters, bioaccumulation, and/or effects on behaviour. Such tests are used to assess the toxicity of a wide range of organic and inorganic pollutants and have been used as bioassays to monitor the success of remediation of contaminated soils (Fountain and Hopkin, 2005).

The model

The model description follows the ODD (Overview, Design concepts, Details) protocol fordescribing individual-based models (Grimm et al., 2006; Grimm et al., 2010). The model was implemented in NetLogo 5.0 (Wilensky, 1999), a free software platform for implementing individual-based models.

Purpose

The purpose of the model is to simulate Folsomia candida population dynamics and to inve-

stigate how they are affected by spatial distribution of toxic contamination in soil, with a special focus on interactions with food availability and local population density.

Entities, state variables and scales

The model includes three kinds of entities: eggs, female springtails (juveniles and adults), and grid cells they live on. Eggs are immobile and are characterized by age (in days) and position (continuous coordinates). Springtails are represented as mobile individuals with state variables for their age (in days), position (continuous coordinates), direction for movement, energetic status (days-to-death), cumulative distance (in cm) walked in each hourly time-step (which affects the energy used for movement), and time (h) spent on contaminated grid cells. Grid cells are characterized by their food level and concentration of toxicant (mg kg^{-1} soil). The model world is two-dimensional. Each cell of a 100x100 cells square grid represents a square patch of soil of 1 cm^2.

The global environment is characterized by six "seasons" (spring and fall are divided into "early" and "late"), which determine the temperature-dependent life-cycle parameters of the springtails: data from literature allowed the implementation of four different parameter sets, reflecting the temperature ranges 0-5°C (winter), 12-15°C (early spring and late fall), 19-21°C (late spring and early fall) and 24-26°C (summer).

Process overview and scheduling

Each of the following processes are run, in the given order and by the category of entities given in parentheses, once per day, except for the foraging procedure, which is executed at hourly time-steps (Figure 3.1). If no executing category of entities is given in the list of processes, the process is run by the program, or "observer" (Wilensky, 1999). The order in which the model entities are processed is randomized at each time step, and state variables are updated immediately. The submodels representing the processes are described in detail in Section "Submodels".

Seasons: At the beginning of a new season, individuals get a new set of life-cycle parameters, whose values reflect the change in the temperature range.

Foraging (springtails): Individuals move to look for food, but also to avoid contaminated patches of soil.

Re-growth of food (grid cells): When the amount of resource on a food cell is depleted, it isrestored at the beginning of the next day.

Ageing/growth (springtails): Age is increased by one day. Based on the age, the hatching time and the maturation time, springtails are divided into three stages: eggs, juveniles and adults. When an egg hatches, its age is set to 0.

Reproduction (springtails): Springtails may reproduce when they reach maturity, and afterwards reproduce according to the values of the parameters "time between broods" and "number of broods".

Hatching (eggs): Eggs hatch according to their viability when they reach an age equal to the hatching time. Hatching success depends also on the concentration of toxicant of the grid cell on which the eggs are laid.

Density-dependence and starvation effects (springtails): Fecundity of springtails is reduced when they experience high population density on their grid cell, due to jostling effects. If they do not feed, their energetic status decreases, with consequences for fecundity and survival. Because reproduction requires energy, and *F. candida* do not lay eggs while they are feeding, this procedure is scheduled so that they first look for food and afterwards check for local population density.

Mortality (springtails): Two different rules, based on survival parameters, are implemented for juveniles and adults. In addition to a background rate of mortality, survival depends on the concentration of toxicant and the amount of time the organism spends on contaminated patches.

Update output: The last action executed at daily intervals is an update of model outputs, i.e. plots are updated as well as summary statistics.

Design concepts

Emergence: Population dynamics and the spatial arrangement of individuals emerge from the behaviour of single organisms, their interactions with each other and their habitat: population dynamics are regulated by the number of reproducing individuals, which themselves depend on population density and the amount of food resources. Life cycle, reproduction, and survival rates are partly imposed via empirical rules and parameters; partly emerge from the movement path taken by an individual, which will differ among individuals and in terms of contamination, density and resource availability experienced.

Stochasticity: Values of almost all parameters are drawn from uniform or normal probability distributions, in order to reflect heterogeneity among individuals (Table 3.1). Stochasticity is also used for initializing springtails' starting positions, as well as causing individual behaviours (movement, reproduction, hatching, mortality) to occur with specified frequencies, which depend on the values of said parameters.

Sensing: Individuals sense the amount of food and the presence of other individuals within a defined distance. They also sense whether or not the grid cell they are currently on, and the grid cell which is ahead in their direction of movement, is contaminated.

Adaptation: Individuals implicitly try to optimize their fitness by preferentially selecting cells with high food resources and by avoiding both cells occupied by too many other individuals and too high contamination levels.

Interaction: Individuals compete for food and space; competition is assumed to be of the scramble type.

Observation: Size and structure of the population as well as spatial distribution of the individuals

for different concentrations of toxicant, food resource amounts and distributions are compared.

Initialization

A simulation starts the first day of the year, and therefore in the winter season. Usually, 5% of the grid cells, which are randomly chosen, are made to be "food cells", with maximal food levels as determined in Section "Pattern-oriented parameterization". Simulations start with 10 randomly distributed juvenile springtails; values for their state variables are drawn from the distributions reported in Table 3.1. Four different scenarios for the extent and spatial distribution of contaminated areas are used (see Section "Simulation experiments").

Input data

This model has no time-series inputs or external environmental drivers.

Submodels

All parameters, their meaning, range of possible values, and source for parameterization are listed in Table 3.1.

Seasons: Individual variability is represented by independently drawing, for each individual, at the beginning of a new season, parameter values from a certain interval corresponding to a different temperature range (Table 3.1). When the temperature is too low, springtails are inactive. Joosse and Testerink (1977) observed that below 10°C the percentage of *Orchesella cincta* individuals in a fed state decreases dramatically, while Takeda (1984) reported that in a population of *Folsomia octoculata* overwintering adults were in an immature state, and they became mature with the stimulation of increasing temperature. Verhoef (1996) noted that during the winter period nearly all the adults of the collembolan *Anurida maritima* died, and it appeared that this was due to starvation caused by low locomotor activity in situations of low temperature. Therefore during the time interval corresponding to winter, individuals in the model do not execute any actions except for ageing and mortality: all adults die during winter, while 50 % of the eggs survive, as it is typical of many insects that embryos tolerate cold better than the other life stages.

Foraging: This submodel is comprised of two parts: first, organisms check whether they are on a contaminated grid cell. If one of the neighbouring cells has a lower concentration, the springtail moves onto it with a chance equal to its avoidance probability, which is proportional to the toxicant's concentration (Table 3.2). The second part of the submodel contains rules for feeding. Movement is triggered by the reduction of the collembolan's energy level. This process is executed with a frequency determined by a probability of movement, which includes two components: a baseline probability and a multiplier (up to two) proportional to the olfactory stimulus representing the amount of food present within the range of perception. This multiplier

Table 3.1. Parameters and values used in the Folsomia candida *model.*

Parameter	Units	Temperature (°C)	Distribution	Value	References
Maturation time: time to reach adulthood (matur_ time)	Days	12-15	Uniform	30-40	Milne, 1960
		19-21		13-29	Snider, 1973
		24-26		11-30	Marshall and Kevan, 1962
Hatching time: time needed for the eggs to develop and hatch to juveniles (hatch_time)	Days	5	Uniform	90	Milne, 1960
		12-15		13-19	Milne, 1960; Fountain and Hopkin, 2005
		19-21		7-15	Marshall and Kevan, 1962
		24-26		7-9	Milne, 1960
Number of eggs per brood, general value for the season (nr_eggs_season)	Number	12-15	Uniform	19-98	Milne, 1960;Snider, 1973; Grimnes and Snider, 1981
		19-21		30-50	Fountain and Hopkin, 2005
		24-26		26-68	Snider, 1973; Green, 1964b
Nr of broods per female: max number of reproductive events (max_num_repr)	Number	12-15	Uniform	9-16	Milne, 1960;Snider, 1973; Grimnes and Snider,1981
		19-21		3-20	Snider, 1973
		24-26		4-6	Snider, 1973; Green, 1964b
Time between broods (repr_interv)	Days	12-15	Uniform	13-15	Snider and Butcher, 1973
		19-21		6-16	Marshall and Kevan, 1962
		24-26		11-13	Marshall and Kevan, 1962
Egg viability: percentage of eggs that successfully hatch (egg_viab)	Number	12-15	Normal	Mean 94.50% S.D 5%	Snider and Butcher, 1973
		19-21		Mean 92% SD 5%	Snider and Butcher, 1973
		24-26		Mean 81% SD 9%	Snider and Butcher, 1973
Juvenile survival., expressed as probability to survive until age at maturity (j_surv)	Number	12-15	Normal	Mean 98% SD 2%	No reference for this temperature; value has been derived from other temperatures
		19-21		Mean 95% SD 2%	Marshall and Kevan, 1962
		24-26		Mean 83.30% SD 2%	Snider, 1973
Adult survival., expressed as the age of death of the individual (a_surv)	Days	12-15	Normal	Mean 241 SD 50	Snider and Butcher, 1973
		19-21		Mean 140 SD 25	Snider and Butcher, 1973
		24-26		Mean 73 SD 26	Snider and Butcher, 1973
Probability to reproduce at every reproductive instar (repr_probab)	Number	12-15	Uniform	96 - 100%	Milne, 1960; Snider, 1973; Grimnes and Snider, 1981
		19-21		95 - 99%	Milne, 1960; Snider, 1973; Grimnes and Snider, 1981
		24-26		94 - 98 %	No reference for this temperature; value has been derived from other temperatures
Distance within which food and conspecifics are sensed	Cm	Independent from temperature	Constant	2.5	Auclerc et al., 2010

Parameter	Units	Temperature (°C)	Distribution	Value	References
Energy level (energy)	Days-to-death	Independent from temperature	Constant	Initial values Max: 30 Min: 0	Final values determined by calibration (see Results section)
Energy reduction per time-step (en_reduce_hour)	Days-to-death	Independent from temperature	Constant	Initial value 0.042	Final value determined by calibration (see Results section)
Energy gained by food intake (food)	Days-to-death	Independent from temperature	Constant	Initial value 0.5	See Results section
Energy reduction per step moved (en_reduce_mov)	Days-to-death	Independent from temperature	Constant	Initial value 0.01	See Results section
Probability to move at each time-step (probab_mov)	Number	Independent from temperature	Constant	Initial value 0.1	Final value determined by calibration (see Results section)
Maximum energy spent for foraging at each time-step (tradeoff_mov)	Days-to-death	Independent from temperature	Constant	Initial value 0.2	See Results section
Tradeoff between energy and reproduction (tradeoff_repr)	Days-to-death	Independent from temperature	Constant	Initial value 20	See Results section
Maximum energy spent for avoiding high density at each time-step (tradeoff_dens)	Days-to-death	Independent from temperature	Constant	Initial value 0.1	See Results section

Table 3.2. Equations for the linear regressions used in the model.

Independent variable	Dependent variable	Regression	R^2	References
ln concentration	Reduction of survival	$y = 0.0824x - 0.1366$	0.847	Sandifer and Hopkin, 1996
ln concentration	Reduction of fecundity	$y = 0.2189x - 0.8743$	0.919	Sandifer and Hopkin, 1996
ln concentration	Nr of hatched eggs (Normalized to the control)	$y = -0.2243x + 1.8893$	0.932	Xu et al., 2009
ln concentration	Percentage of avoidance	$y = 5.7475x - 1.4235$	0.926	Boiteau et al., 2011
Local density	Normalized nr of eggs	$y = 1.0637*\exp(-0.305x)$	0.942	Green, 1964a
Energy	Normalized nr of eggs	$y = 0.01*\exp(4.6052x)$	1	Assumed

has been introduced to represent the characteristic periods of activity/inactivity shown by several collembolan species (de With and Joosse, 1971). From experimental observations reported in the literature, it is known that collembolans go through periods of inactivity (i.e. they do not move and do not feed), for instance during the moulting process (Joosse and Testerink, 1977; Marshall and Kevan, 1962). Therefore, in order to account for these periods of inactivity, individuals in the model do not moveat each time-step, but according to a given probability (*probab_mov*), which is proportional to the amount of food sensed by the individual (i.e. to the strength of the attractive olfactory stimulus). The minimum value for *probab_mov* occurs when the organism does not sense any food; the maximum value for *probab_mov* is twice the minimum. The value for minimum *probab_mov* was determined via sensitivity analysis and pattern-oriented parameterization (details in Sections " Energy-related parameters and sensitivity analysis" and "Pattern-oriented

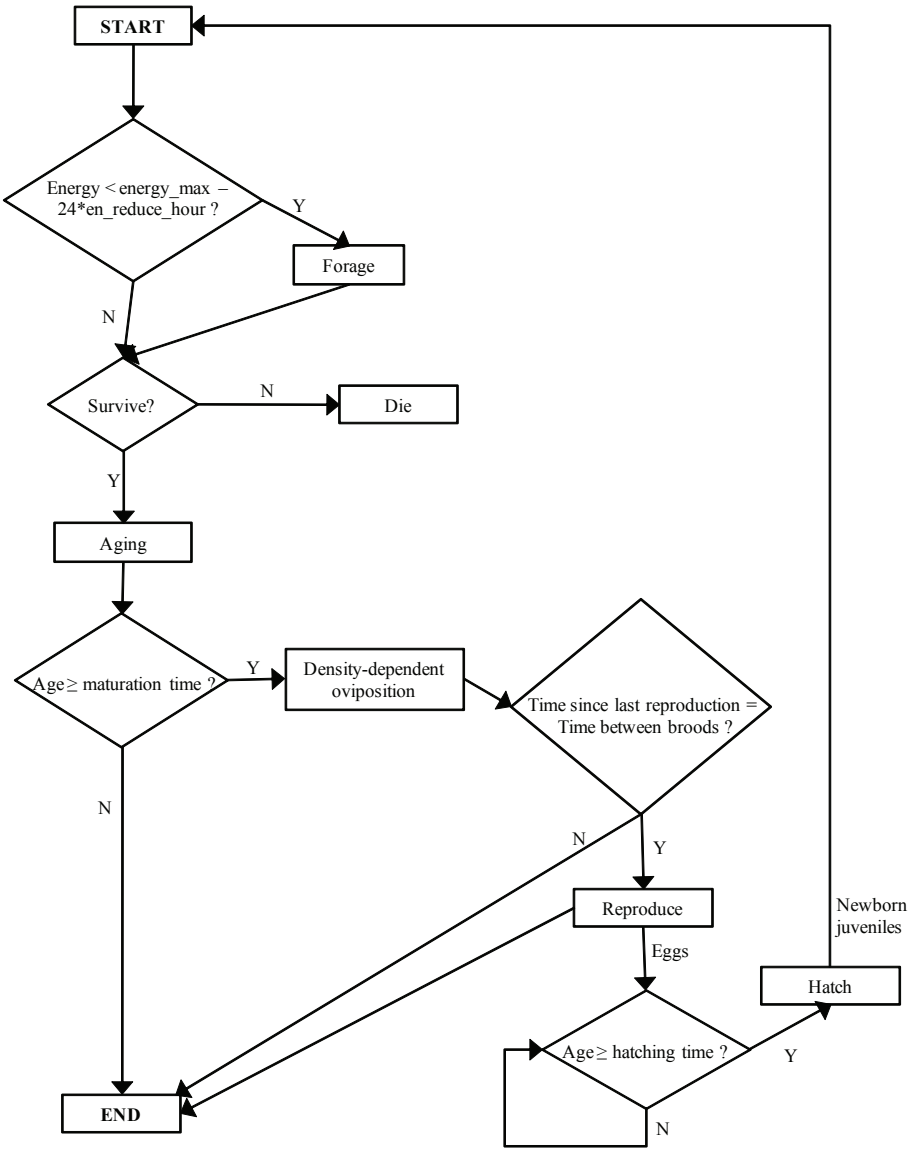

Fig. 3.1. Flow chart representing the processes executed by individuals in the model.

parameterization"), but has initially been set to 0.1. While springtails forage, they decrease the stock on the food cell on which they are feeding by one food item per time step. The probability of movement is calculated as:

$$probab\_mov = 0.1 * \left(1 + \frac{\text{mean } \textit{amount of food on food cells within sensing range}}{\text{maximum } \textit{amount of food initialized on a food cell}} \right)$$

The foraging submodel is described below using pseudo-code. The rationale for each part of the code and the values of parameters involved and the equations used are explained in more detail in Appendix 2. A visualization of the resulting movement patterns is shown in Fig. 3.2.

Pseudo-code:

```
for all springtails
   if current cell is contaminated and concentration  on one of the neighbouring
   cells is lower

      move towards it according to p_avoid
   if current energy reserve is below energy_max – 24*en_reduce_hour
      if any food patches in a 2.5 cm radius and if total food in a 2.5 cm
      radius is at least 1 food item

         Set movement probability dependent on average food in 2.5 cm radius
      else
         Set movement probability to minimum movement probability
      While no food found and energy spent for foraging (nr steps moved *
      en_reduce_step) is below threshold (tradeoff_mov)

         if food on current patch is at least 1
            Eat
         if no food on current patch and food on one of the grid cells in the
         semicircle of radius 2.5 cm the individual is facing to contains more
         food than 1
            Turn towards one of these grid-cells
         else
            Turn randomly by 0-359°
         if cell ahead 1 cm is contaminated
            Move towards it according to p_avoid
            Update exposure counter
         else
            Move towards one of the uncontaminated neighbour cells
         Calculate energy loss due to movement
   Update energy reserves: old value plus food intake minus energy loss
Update grid cell variable "local_density" for all grid cells.
```

Reproduction, density dependence and starvation effects: Individuals, after they reach maturity, have a certain probability to reproduce at every reproductive instar (Table 3.1), which is drawn from a specified distribution for every season. They lay a predetermined number of eggs, which depends not only on the season but also on the local density (i.e., number of organisms on the

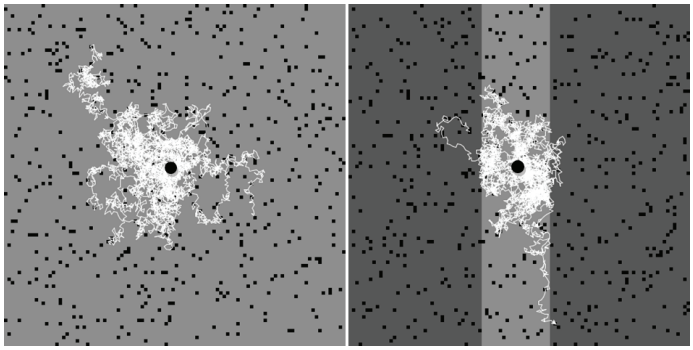

Fig. 3.2. Movement paths of 10 springtails after 20 days of simulation in absence (left panel) and presence (right panel) of toxicant on 80% of the area (dark grey), with a concentration of 3200 mg kg^{-1}. Five percent of the grid cells of 1 cm^2 are food cells (in black). Initially, the springtails are released in the uncontaminated region (light grey), in the centre of the simulation grid (black dot).

same patch) and the energy level of the organism. As shown by Green (1964a), fecundity of springtails is reduced when they experience high population density, due to jostling effects: the effect of crowding upon fecundity has been calculated as an exponential function (Table 3.2) that interpolates data from Green (1964a). The same type of mathematical relationship has been assumed to exist between energetic status of the organism and number of eggs laid (Table 3.2). In addition to the effect on fecundity, the energetic status affects the survival of an organism: if energy level is below the minimum (*energy_min*), the individual dies.

The number of eggs laid also depends on the contamination experienced by the organism, in terms of concentration and time spent on a contaminated patch. From literature data (Sandifer and Hopkin, 1996), a log-linear regression (Table 3.2) between concentration and reduction of fecundity has been calculated. Dose-response curves for reproduction or survival are usually modelled as logistic functions, but have been implemented as log-linear regression because published data did not allow further analysis and, in order to keep the model as simple and easy to re-implement as possible, only data already available have been used. The performance of the model with these data has been tested and will be discussed later.

To account for the fact that the toxicity data used for this regression are the result of 28 days of exposure to homogeneous contamination, it has been corrected by the ratio of the toxicity counter (number of hours spent on contaminated patches) and the number of hours in 28 days, i.e. 672 hours. When the toxicity counter of an individual is greater than 672, this coefficient is set to one.

F. candida can sense the presence of conspecifics (Leonard and Bradbury, 1984) and therefore they move to look for a less crowded area if on the current cell other individuals are present. In the model it is assumed that the range within which the olfactory stimulus of other individuals is perceived is the same as for food. This process is described using pseudo-code below; the underlying

assumptions are explained in more detail in Appendix 2.

Pseudo-code:

```
for springtails with energy above tradeoff_repr and age above matur_time
   if local_density on any cell in a radius of 2.5 cm is lower than local_density
   of current cell

      while local_density on any cell in the semicircle of radius 2.5 cm the
      individual is facing is lower than local_density of current cell, and
      energy spent for moving (nr steps moved * en_reduce_step) is below
      tradeoff_dens

            Turn towards one of these grid-cells
            if cell ahead 1 cm is contaminated
               Move towards it according to p_avoid
               Update exposure counter
            else
               Move towards one of the uncontaminated neighbour cells
            Calculate energy loss due to movement
   Update energy reserves: old value minus energy loss
Update grid cell variable "local_density" for all grid cells.
```

Hatching (eggs): Hatching success of eggs, besides the natural viability, depends on the concentration of toxicant of the grid cell on which the eggs are laid. From the data reported in Xu et al. (2009), the concentration-effect relationship for the reduction of egg viability caused by copper has been derived (Table 3.2). When an egg hatches, it changes its status to "springtail"; age is set to 0, and energy level is set to maximum.

Mortality: Juvenile survival is implemented as the probability to survive each day until maturation:

$$probability\ to\ survive = \left(juvenile\ survival \right)^{1/maturation\ time}$$

Adult survival is implemented via the age of death: every organism, when it hatches and again when the season changes, draws a value for this parameter from a normal distribution, which is different for every season of the year, and every day it checks if its own age is still below this value, otherwise it dies.

Survival is reduced by exposure to the toxicant. From the literature data (Sandifer and Hopkin, 1996), a linear regression between the logarithm of the concentration and reduction in survival (where 0 equals no reduction, 1 equals no surviving organisms) has been calculated (Table 3.2) and applied to both juveniles and adults. The same coefficient used for the regression between concentration of toxicant and reduction of fecundity, which takes into account the amount of

time spent on a contaminated patch, was applied.

Energy-related parameters and sensitivity analysis

As shown in Table 3.1, for some parameters it was not possible to find values in the literature. These are all related to the energy level of individuals and their movement. Initial values have been indirectly estimated from observations reported in the literature. A sensitivity analysis was used to identify those parameters having the strongest effect on model output, and these were selected for inclusion in the pattern-oriented parameterization described below.

The initial values assigned to these parameters were used as a pivot point, and for the sensitivity analysis the parameters were adjusted independently to ±10, ±20, ±30, ±40, ±50 % of their pivot point values. Linear and second order polynomial regressions were calculated between the relative changes in each parameter value and two model outputs, final population size and average weekly population growth rate. For this analysis, 40 replicate simulations of 120 days were run for each parameter value, and, in order to simplify interpretation of the results, all simulations were run for season parameters corresponding to a constant temperature interval of 19-21°C, i.e. late spring/early fall.

Pattern-oriented parameterization

Following Wiegand et al. (2003), we use different patterns to determine unknown parameters using an inverse modelling approach. The central idea of pattern-oriented parameterization is to make the model produce multiple patterns simultaneously, so that the structural realism of the model is increased, i.e., the internal organization of the modelled system is more likely to be captured sufficiently for the intended purpose of the model. As an indicator of structural realism, model output is checked for secondary, independent predictions, i.e., system-level patterns observed in reality, which were not used for model design or parameterization.

After a thorough literature search, we found five suitable patterns. We chose two patterns for parameterization and the remaining three for testing secondary predictions. The patterns we identified and their intended use were determined before the model was formulated and analyzed in detail, to avoid ad hoc parameterization issues.

Pattern 1: Food-dependence (Usher et al., 1971). Three different observations describe this pattern: population growth with excess food, with marginally limiting food and with limiting food supply. Usher et al. (1971) observe that when food is not a limiting factor or is only marginally limiting, and when food is being supplied in proportion to population density, the establishment of an equilibrium population size is achieved, but the speed of establishment is proportional to

the rate at which food is supplied, and population densities approach those reached with excess food. When the food supply is independent of density and limiting, equilibrium population size is reduced.

Pattern 2: Population growth rate and density dependence (Seifert et al., 1979). Microcosm experiments on *F. candida* by Seifert et al. (1979) show that population growth rates have decreased in all cultures before the termination of the experiments after 43 days, which indicates density-dependent effects. Estimates of exponential rates of increase are based on population increases from the 7th through the 31st day from the beginning of experiment.

The three observations that comprise the first pattern were used as filters to progressively exclude combinations of parameter values: 10 replicate simulations with every combination of the relevant parameters within a range of ±20% around the initial value were run and then compared to the first observation (population growth with excess food) using Chi-square statistics. The 40 best combinations were chosen, and the same procedure repeated for the other two observations (population growth with limiting and slightly limiting food). Sets of values that met all of the three observations were then used to simulate Pattern 2. Simulated ranges of final population size and exponential growth rate were compared to the observation from Seifert et al. (1979), and the parameter set which gave the best fit, in terms of overlapping ranges, was chosen (Further details in Appendix 2). The resulting final parameter set was used in all subsequent simulations.

Pattern 3: Number of generations per year (Marshall and Kevan, 1962). The authors observe that in a greenhouse (constant temperature 22° C) *F. candida* can have as many as 12 generations per year.

Pattern 4: Seasonal variation in population size in the soil of a temperate forest (Klironomos and Kendrick, 1995). In this study, a 100 m^2 plot was set up in a sugar maple forest in Canada. The soil profile was divided into layers (i.e. litter, 0-10 cm, 10-20 cm and 20-30 cm) and sampling of microarthropods was carried out four times throughout the year (May 1991, July 1991, October 1991 and February 1992) to account for seasonal variation. For comparison with the model, data for the litter layer were considered. Results of this survey show that the highest population density is reached in October, with a relatively high peak also in May, while in July and February population abundance is very low.

Pattern 5: Instantaneous rate of population increase, ri, under homogeneous copper contamination (Herbert et al., 2004). Soil concentrations of copper up to 12,800 μg g^{-1} were tested. Calculated ri values ranged from -0.086 (extinction) to 0.077 (in one replicate at 200 μg g^{-1}). The mean control ri was calculated as 0.041, although the authors note that adult survival and juvenile production in the controls were lower than specified in the ISO guidelines. Copper significantly affects ri with significant differences found between the control and treatment at concentrations of 3200 μg g^{-1} and higher. For comparison with this pattern, the model was initialized with the

same conditions as the empirical study in terms of temperature, size of model arena and initial population, and predicted values of r_i were calculated using the equation provided in Herbert et al. (2004):

$$r_i = \frac{\left(\ln\left(n_f + 1\right) / \left(n_0 + 1\right)\right)}{\Delta T}$$

with n_f and n_0 being final and initial number of animals, respectively, and ΔT the difference in time (number of day the experiment was run).

Simulation experiments

Simulations with homogeneous contamination and with two different heterogeneous scenarios were conducted (Figs. 3.2 and 3.3), using the parameter set chosen using via pattern-oriented parameterization. In these scenarios, the spatial arrangement and connectivity among contaminated cells is different, but the percentage of contaminated area is the same, 80%, while the remaining 20% is either uncontaminated or has a lower Cu concentration (Fig. 3.3). Two different combinations of concentrations, referred to as combination A and B, were tested (Table 3.3). The total amount of toxicant was equal in both combinations, and the average contamination was the same as in the homogeneous scenario. The level of heterogeneity (i.e. the difference among patches with high and low concentration) was lower in combination B. The total amount of food available was kept constant among simulations, but the distribution of food resources on the grid cells (Fig. 3.2) was randomized at the beginning of every model run.

Two sets of experiments were performed. In the first set, the temperature, or season, was kept constant, in order to compare growth rates and carrying capacity in the different scenarios and with increasing concentrations of toxicant. The length of these simulations was 200 days. In the second set of experiments, temperature was changed with season as described in Section "Submodels". The length of these simulations was 365 days, starting the 1st of January. In all simulation experiments, five replicate runs were performed both for the control (no toxicant) and for all treatments, and the initial position of the organisms was randomized at the beginning of each model run.

Table 3.3. Concentrations used in the homogeneous and heterogeneous scenarios; the total amount of toxicant present in the system is the same.

Homogeneous scenario concentrations (mg kg^{-1})	Heterogeneous scenarios combination a (mg kg^{-1})	Heterogeneous scenarios combination b (mg kg^{-1})
0	0 – 0	0 – 0
125	0 -160	12.5 – 153.1
500	0 - 625	50 – 612.5
2500	0 - 3125	250 – 3062.5

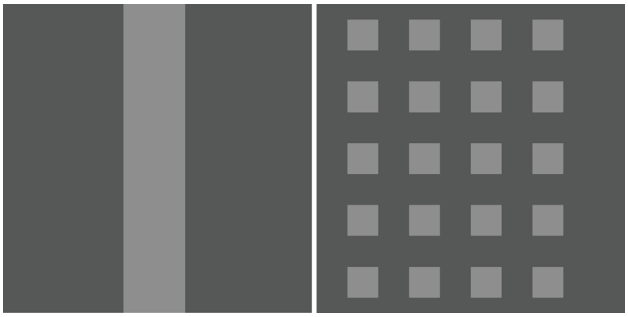

Fig. 3.3. Spatial arrangement of contaminated patches (dark grey) in Scenario 1 (left) and 2 (right).

3.3 Results

Sensitivity analysis

Among the parameters included in the sensitivity analysis, only those for which the regressions were statistically significant ($p < 0.01$) for both dependent variables were selected for calibration, i.e., energy maximum and minimum, metabolic rate, maximum energy spent to forage at each time-step and probability to move at each time-step.

Pattern-oriented parameterization

The final parameter set, after using Patterns 1 and 2 as filters, was: *energy_max* = 30, *energy_min* = 4, *en_reduce_hour* = 0.0462, *tradeoff_mov* = 0.18 and *probab_mov* = 0.12. This parameter set was used to test the performance of the IBM against the other patterns described in the "methods" Section.

For Pattern 3, the mean number of generations produced during model simulations lasting one year at constant temperature range (19-21°C) was compared to the number of generations obtained in a greenhouse (Marshall and Kevan, 1962), also at constant temperature (22°C). The model output ranged from 11 to 13 generations per year, with an average of 11.6 compared to the 12 generations found by Marshall and Kevan (1962).

A comparison of the population abundance (individuals m^{-2}) predicted by the model with the data reported by Klironomos and Kendrick (1995) (Pattern 4, Fig. 3.4) shows a good fit for the data for spring, summer and winter, whereas the fall peak predicted by the model was lower. The highest peak in the simulated population abundance occurred in June, but since there were no data points for this month in Klironomos and Kendrick (1995), it is not possible to compare

this model prediction with a field observation.

Finally, we tested the performance of the IBM in predicting population-level effects of copper on *F. candida* (Pattern 5). Toxic effects were implemented using only individual-level data (Table 3.2), with endpoints on fecundity, survival, hatching success and avoidance; therefore we compared model output to the data presented in Herbert et al. (2004), where the authors measured the instantaneous rate of population increase, r_i, after exposure to different copper concentrations. There was a higher simulated growth rate for the control and the two lowest concentrations, however for higher toxicant levels the model output and data matched well (Fig. 3.5).

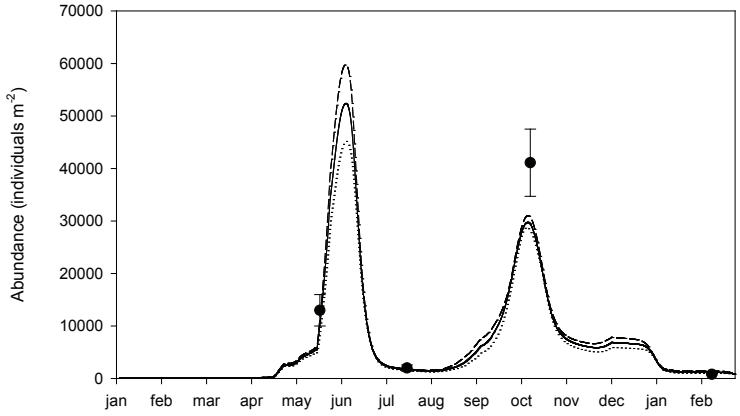

Fig. 3.4. Pattern 4: population abundance of F. candida in different seasons. Solid and dashed lines represent respectively mean and range of model simulations; dots represent Klironomos and Kendrick (1995) data.

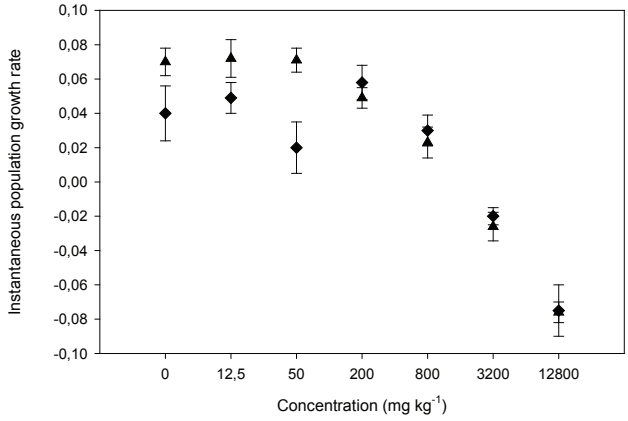

Fig. 3.5. Pattern 5: mean (±SEM, four replicates) instantaneous rate of population increase of F. candida exposed to different copper concentrations. Simulation results represented with (▲), Herbert et al. (2004) data with (♦).

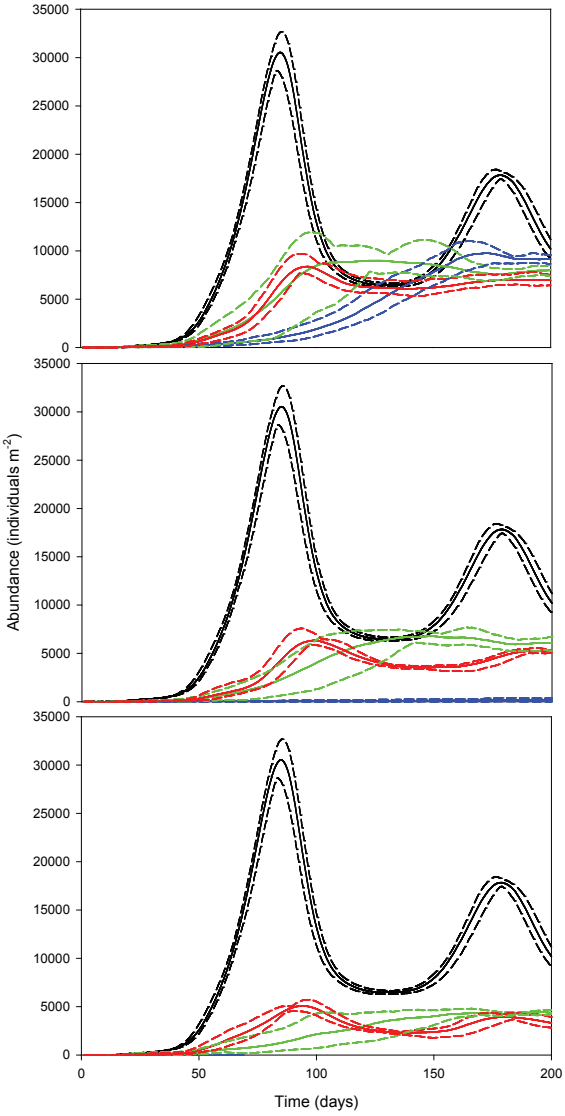

Fig. 3.6. (a–c) Population abundance in the control (black line), and under homogeneous (blue line) and heterogeneous contamination, combination A (green line Scenario 1, red line Scenario 2) at constant temperature range. Average concentrations are 125 mg Cu kg^{-1} (a), 500 mg kg^{-1} (b) and 2500 mg kg^{-1} (c). Solid lines represent averages, dashed lines minimum and maximum simulated values.

Simulation experiments

Results of the first set of simulation experiments are shown in Fig. 3.6a-c. Fluctuations in population abundance were more marked in the control, because of the explosive growth that

leads to food limitation. Longer simulations with constant temperature (not shown) indicated that abundance tended to stabilize after a few, dampened oscillations. At the lowest simulated contaminant concentration (125 mg kg^{-1}; Fig. 3.6a), the final population size reached in the three different scenarios was in the same range. Time to reach equilibrium population size, which is defined as the size reached when population growth rate (*r*) is equal to zero, i.e. the population does not grow or decline, was longer under homogeneous contamination. In all scenarios, initial growth rates were smaller than in the control, as were the final population sizes.

At the average concentration of 500 mg kg^{-1} (Fig. 3.6b), the population exposed to homogeneous contamination survived until the end of the simulation, but was barely growing (mean final size 160 individuals), whereas in the heterogeneous scenarios abundance was around 5000 individuals m^{-2}. At the highest concentration (2500 mg kg^{-1}, Fig. 3.6c) the population exposed to homogeneous contamination went extinct, while in both heterogeneous scenarios a population of almost 3000 individuals m^{-2} was sustained.

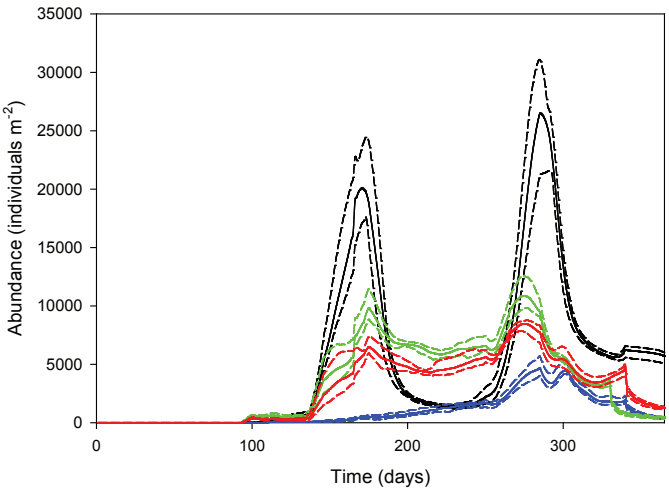

Fig. 3.7. Population abundance in the control (black line), and under homogeneous (blue line) and heterogeneous contamination, combination A (green line Scenario 1, red line Scenario 2) at varying temperature ranges. The average concentration is 125 mg Cu kg^{-1}. Solid lines represent averages, dashed lines minimum and maximum simulated values.

In the second set of simulation experiments, for the lowest concentration used seasonal fluctuations were less marked in the treatments than in the control, and the spring peak was completely missing under homogeneous contamination, due to the slow initial population growth rate (Fig. 3.7). Finally, Fig. 3.8 compares for Scenario 2 population growth with average concentration of 500 mg kg^{-1} at constant temperature, exposed to two different combinations of concentrations: combination A where 20% of the grid cells are uncontaminated, and combination B, where they

have a lower concentration of toxicant but are nonetheless contaminated. Population growth strongly is reduced for combination B compared to combination A.

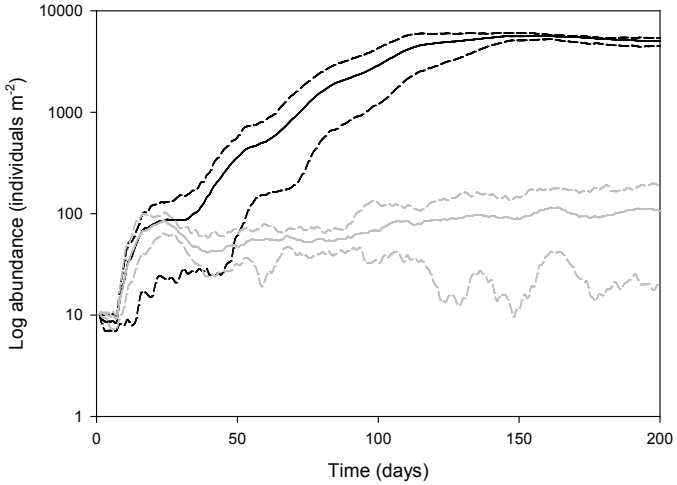

Fig. 3.8. Logarithm of population abundance under heterogeneous contamination with average concentration of 500 mg Cu kg^{-1} in Scenario 2, at constant temperature range and different combinations of concentrations, i.e. combination A (black line) and B (grey line, see text). Solid lines represent averages, dashed lines minimum and maximum simulated values.

3.4 Discussion

Our model describes a method for using data from standard laboratory tests to investigate effects of heavy metal contamination on long-term population dynamics of springtails under spatially heterogeneous contamination scenarios. The model is spatially explicit and represents single individuals. In particular, processes which contribute to simulate the *F. candida* avoidance behaviour and understand the consequences of a heterogeneous soil contamination at a population level are represented. Since springtail behaviour is hard to observe directly, quite a few model assumptions had to be based on general principles of energy budgets, foraging, sensing, and adaptive movement in a heterogeneous arena. In particular, foraging activity is regulated by a probability of movement, which is proportional to the amount of food sensed by the individual. This is an unusual assumption, as typically movement depends on an individual's energy level, i.e. organisms forage more when hungry. While in our model foraging is also initiated and terminated by energy levels, we additionally introduced the effect of the amount of food sensed to represent the periods of activity/inactivity shown by several collembolan species. In particular, it has been shown by empirical studies (Westerberg et al., 2008; Auclerc et al.,. 2010) that activity is reduced in presence of food, increased in absence of it, and in general movement patterns (i.e. turning

angles, step length and direction of movement) change when organisms can sense the presence of food. In the model, movement activity is also reduced in presence of food (i.e. when individuals are located on a food cell), directionality of movement is increased when food is sensed, and to reflect the fact that food acts as an olfactory stimulus for *F. candida* (i.e. organisms are attracted to it), probability to move in presence of this stimulus is higher than in its absence.

Furthermore, energy budgets were represented very coarsely in terms of days that an individual could survive under starving conditions (see Appendix 2). All the parameters involved in energy-related processes had to be determined indirectly via sensitivity analysis and pattern-oriented parameterization.

Nevertheless, testing of the model showed that it reproduces patterns observed both in laboratory cultures and natural populations of *F. candida*. Comparison with data from Herbert et al. 2004) demonstrates that the chosen parameter set for the individual processes related to energy expenditure results in a population growth very close to the observed values, especially with exposure to increasing copper concentrations (Fig. 3.5). Making a model simultaneously reproduce several patterns, observed at different levels of organization, scales, and under different environmental conditions, is harder than fine-tuning a model towards one single pattern (Grimm et al., 2005; Grimm and Railsback, 2012). In POM, when testing secondary predictions, no calibration is involved, and inferences about the realism of a model are not based on single, but multiple patterns. Thus, even though, for example, Pattern 4 (Fig. 3.4) is not reproduced by the model in detail, it does still provide additional evidence of structural realism. We therefore conclude that our model is realistic and flexible enough for its purpose of estimating the effects of toxicants at the population level under different patterns of spatial distribution, and representing *F. candida* realistically enough to be used for evaluating and improving standard ecotoxicological tests based on this species.

Empirical evidence regarding the drivers of springtail population dynamics is unequivocal. Some laboratory observations (Green, 1964a) show that behavioural effects such as avoidance of crowding influences oviposition, whereas other studies (Ferguson and Joly, 2002) suggest that changes in springtail numbers may be explained primarily on the basis of temperature and competition for food. Therefore, our model includes both competition for food and behavioural responses to crowding, as well as temperature-dependent life cycle parameters.

The restocking of food resources on grid cells after they have been consumed, which happens instantaneously within one 24-hour time-step after food has been depleted, certainly is an over-simplified representation of food dynamics. However, data for more realistic assumptions seem not to exist and we found that for the model purpose our approach was satisfactory. Nevertheless, since food is an important driver in determining the spatial distribution of collembolans (Usher and Hider, 1975), further systematic empirical studies on foraging and toxicant avoidance would

be worthwhile.

Simulation results showed that for the two heterogeneous scenarios used (Fig. 3.3), the spatial arrangement of contaminated patches of soil had only moderate effects on growth and maintenance of the population *F. candida*, given the same percentage of contaminated area. Looking only at the averages of model output, it would appear that the heterogeneous Scenario 2, where the uncontaminated cells are connected in one big patch of suitable habitat, is, especially with increasing copper concentrations, more favourable during the population growth phase (Fig. 3.6). Nevertheless, the ranges between minimum and maximum population abundance for the two heterogeneous scenarios are to a large extent overlapping.

For a more systematic and comprehensive analysis of the effects of heterogeneous contamination, algorithms used in landscape ecology to generate heterogeneous landscapes with given properties should be used (With, 1997). In particular, the midpoint displacement algorithm (Saupe, 1988; Hargrove et al., 2002; Koerner and Jeltsch, 2008; dos Santos et al., 2011) would be suitable, which allows to control the spatial autocorrelation of contaminated areas by one parameter.

In both heterogeneous scenarios, as well as for homogeneous contamination, equilibrium population sizes decreased with increasing copper concentration under the constant temperature condition (Fig. 3.6a-c). However, whereas in the homogeneous scenario the population goes extinct already at a concentration of 500 mg kg^{-1}, the equilibrium population size is around 5000 individuals m^{-2} at 500 mg kg^{-1} of copper, and just below 3000 individuals m^{-2} at 2500 mg kg^{-1} in both heterogeneous scenarios. Avoidance is not very well studied for many invertebrate species, but can have important consequences for population-level toxic effects. As shown in Fig. 3.6b and c, a five-fold increase in concentration only corresponds to a two-fold decrease in equilibrium population size. This is due to the fact that, even at 2500 mg kg^{-1} of copper, avoidance probability is still less than 50% and organisms entering contaminated areas are exposed to a lethal concentration of the toxicant. Nevertheless, being able to detect toxicants at all and having clean habitat patches which act as "refuges" allows the reduction in population size to be much less than proportional to the increase in concentration.

It is essential to bear in mind, though, that these considerations apply only if the species in question is able to sense and avoid the toxicant. In their approach to modelling the effects of heterogeneous distribution of a contaminant in sediments on populations of aquatic invertebrates, Palmqvist and Forbes (2008) assumed that the modelled species did not avoid the toxicant, and they found that under this assumption the population was more affected in a heterogeneous scenario, where the contaminated patches acted as population sinks.

Additionally, also the heterogeneity in contamination levels seems to be very important. At the same average concentration of 500 mg Cu kg^{-1} soil, which is close to the EC50 for reproduction (i.e. concentration that causes 50% effect, in this case reduction of oviposition), in a homogeneously

contaminated scenario the population goes extinct, whereas in both heterogeneous scenarios and both combinations of concentrations, viable metapopulations are formed in the more suitable soil patches. From Fig. 3.8, it is obvious that if no clean habitat is offered to the individuals (combination B), population growth is substantially more reduced than if clean habitat is offered, but still the population persisted whereas it went extinct in the homogeneous scenario within a few weeks.

Our model allows for the first time to extrapolate effect of avoidance behaviour, which has been observed and quantified only recently and which is highly variable (Boiteau et al., 2011; Boitaud et al., 2006; Greenslade and Vaughan, 2003; Filser and Holscher, 1997) to effects at the population level. Currently, in ERA both the distribution of chemicals in soil and the possible avoidance behaviour are disregarded, while our results showed that they can have an important influence on the actual risk to the populations. Therefore, based on the results of our model, we suggest that an avoidance test, as it is already standardized (ISO, 2011), should be performed when lower tier risk assessment is not passed. Our model can be used to study the relevance of avoidance behaviour for population dynamics and ecological risk assessment in more detail (Meli et al., unpublished manuscript).

Furthermore, we showed that particular attention should be paid to the spatial distribution of chemicals when assessing risk, as the species represented in our model is used in standard ecotoxicological tests where homogeneous contamination is assumed. It is thus important to know whether the compound under investigation can be sensed by the organisms, and whether the concentrations in the test soils are really homogeneous, otherwise toxicity might be overestimated. Overestimation of environmental risk may have relevant consequences from a societal and economical point of view, as the use of chemicals in agriculture is beneficial to food production.

Our model is designed to interact with laboratory tests and experiments, and ultimately help to improve standard tests and increase the ecological relevance of such tests. We deliberately implemented our model so that it can easily and directly be used by others, by using the software platform NetLogo (Wilensky, 1999), and by providing the NetLogo program implementing our model in Appendix 3. Future studies based on our model will explore effects of heterogeneity more systematically and relate the model more directly to risk assessment of chemicals and to ecologically relevant endpoints.

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Chapter 4

Comparing microscale patterns of habitat fragmentation and disturbance events: A modelling study of the effects on **Folsomia candida** *(Collembola) populations*

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Abstract

In the present study, we implemented a fractal algorithm in a spatially explicit individual-based model, in order to generate landscapes with different microscale patterns of habitat fragmentation and disturbance events, and focused on their effects on population dynamics of the collembolan *Folsomia candida*. Among the different human activities that may cause habitat destruction, we focused on agricultural practices, as it is especially relevant for the selected species. Soil organisms that live in a cultivated field are subjected to different patterns of habitat loss and fragmentation as well as disturbance events generated by the application of agrochemicals and related activities (e.g. tillage). In addition, they are exposed to natural stressors, which might also influence the effects of chemicals on populations. We designed simulation experiments that incorporate these three different factors, and investigated their effects on populations of *F. candida*. Furthermore, we ran simulations with and without behavioural avoidance of contaminated habitat. Simulation results show that spatial autocorrelation of contamination has different effects on population growth and equilibrium size according to the percentage of clean habitat. This pattern changes when avoidance behaviour is excluded from the model, as does population recovery after a series of disturbance events. The model suggests that a combination of heterogeneous contamination and multiple stressors can lead to unexpected effects of toxicants at the population level. Individual-based models can help to understand these effects and therefore can add ecological realism to environmental risk assessment of chemicals, as well as explore the effects of different risk management options.

4.1 Introduction

Several studies have highlighted that landscape structure, such as habitat amount and fragmentation, has important effects both on diversity (Fahrig, 2003; Chisholm and Gonzalez, 2011) and on persistence of populations and communities (Davies and Margules, 1998). However, several aspects of the relationship between landscape complexity and population dynamics are still not well understood (Nabe-Nielsen et al., 2010).

Species-specific characteristics such as body size or dispersal ability are likely to be crucial in determining which aspects of habitat heterogeneity (e.g. availability, patchiness, structural diversity) are relevant and at which spatial scales individuals perceive landscape structure, or are affected by habitat fragmentation(Roland and Taylor, 1997; Steffan-Dewenter et al., 2002; Chust et al., 2003; Dauber et al., 2005; Wiens and Milne, 1989). Furthermore, responses to habitat heterogeneity may also be scale-specific (Vanbergen et al., 2007). For example, carabid beetle assemblages have been shown to be positively influenced by habitat heterogeneity at micro-scales (0.25 m^2) and mesoscales (500-1000 m^2) but not at macroscales (10 km^2) (Brose, 2003; Tews et al., 2004).

The influence of habitat fragmentation on population dynamics has often been confounded with that of habitat composition (i.e. habitat amount and quality; Ewers and Didham, 2006) and habitat loss (Fahrig 2003). Empirical studies have shown that fragmentation may have positive or negative effects on populations, but in order to determine the factors that lead to such consequences, more studies that separate effects of habitat loss from fragmentation are needed (Fahrig, 2003). Spatially-explicit simulation models, in which these two factors can be manipulated independently (e.g., Nabe-Nielsen et al., 2010) can be the key to produce the required evidence.

Neutral landscape models (NLMs), in particular, provide a good modelling framework to study impacts of habitat fragmentation on population dynamics. NLMs are grid-based maps in which complex habitat distributions are generated by random, hierarchical, or fractal algorithms. Because the artificial landscapes are generated with analytical algorithms, they are thus "neutral" to the biological and physical processes that shape real landscape patterns. In an NLM cells are identified by habitat type or some other landscape feature, and using fractal algorithms to generate NLMs allows simple control over spatial autocorrelation. This way complex landscapes can be generated systematically just by varying the degree of spatial autocorrelation of habitat patches (With, 1997). Neutral landscape models were introduced to generate spatial patterns in the absence of any structuring process (Hargrove et al., 2002) and provide null models for predicting when habitat fragmentation occurs and is likely to affect population dynamics (With, 1997).

Microarthropod communities have often been used as model systems to study effects of habitat fragmentation, and results of several studies indicate their usefulness for this purpose. For instance, microarthropods have been used to investigate how autocorrelation of disturbance events affects

time to extinction of populations (Pike et al., 2004), or how habitat loss and fragmentation affects population viability and ecosystem functioning (Astrom and Bengtsson, 2011).

Habitat fragmentation may be the result of natural phenomena such as fire (Wright, 1974) and windfall (Foster, 1980). However, the most important and large-scale cause of habitat fragmentation is the expansion and intensification of human land use (Burgess and Sharpe, 1981).

Among the different human activities that may cause habitat destruction, we focused on agricultural practice, as it is especially relevant for soil organisms. Robertson et al. (1993) showed that cultivation changes the spatial structure of important soil properties, and several field studies have investigated the relationship between soil metal pollution and microarthropod distribution (see e.g. Bengtsson and Rundgren, 1988; Hagvar and Abrahamsen, 1990; Salminen and Haimi, 1999; Gongalsky et al., 2010).

In most studies, heterogeneity in the distribution of toxic metals in soil has been found to be significantly related to spatial changes in the community structure of springtails and mites (Caruso et al., 2009).

In most agricultural practices, both inorganic and organic compounds are applied to protect crops from pest species. Among inorganic chemicals, copper is widely used as a fungicide on a number of crops. Due to its long degradation time, sites where copper has been applied for several years can reach very high concentrations in soil, and this causes a permanent loss of habitat quality for soil organisms. In contrast, organic agrochemicals are often easily degradable, but are generally more toxic to microarthropods than metals. Their application thus acts as a disturbance event for these communities, killing a smaller or larger fraction of exposed populations, and quickly degrading afterwards.

Different crops and modes of application of agrochemicals can cause different patterns of distribution of the compounds in soil. For instance, a pesticide which is sprayed on homogeneously distributed crops is more likely to have a random distribution in soil, whereas a treatment in bands (e.g. potato furrows) will have a spatially correlated distribution. Furthermore, another source of disturbance that comes with agricultural land use is the mechanical stress caused by tillage and other uses of machinery. Maraun et al. (2003) suggested that Collembola are sensitive to mechanical disturbances, and according to several studies reviewed by (Petersen, 2002) collembolan density reported for some cultivated fields was generally low compared with data from natural or semi-natural sites.

Beside chemical and mechanical stressors, populations can be exposed to physical ones, such as periods of drought, especially during summer, to which collembolans have little resistance (Holmstrup, 1997).

In the present study, we implemented a fractal algorithm in a spatially explicit individual-based model, and focused on the effects of different patterns of microscale habitat fragmentation and

disturbance events on the population dynamics of the collembolan *Folsomia candida* (Willem). For this purpose we designed simulation experiments in which three different factors interact with individuals in the model: 1) different spatial patterns of copper contamination represent permanent loss of habitat, 2) a realistic implementation of a summer drought period is used to investigate the influence of natural stress on population-level effects of copper, and 3) different levels and numbers of disturbance events represent the other stressors (i.e., pesticide applications, tillage, etc.) to which springtail populations are exposed in the field.

Furthermore, we ran simulations with and without avoidance behaviour, as it has been shown that *F. candida* avoid copper but do not detect all toxicants (Greenslade and Vaughan, 2003). This allowed us to investigate which effects habitat loss and fragmentation would hypothetically have on populations in cases in which contaminated habitat is not avoided, and allowed us to test whether knowing if a compound is avoided or not would change the estimation of risk posed by the toxicant.

An important aim of our study was to explore hypotheses and scenarios to show how different management strategies could reduce long-term risks of agricultural practices for soil invertebrates.

More specifically, we tested the following hypotheses: (i) reduction in population abundance caused by a progressive habitat reduction is more than proportional to the habitat loss; (ii) collembolan populations are more affected by habitat loss when the degree of fragmentation of remaining habitat is higher (i.e. patches are spatially uncorrelated or poorly correlated), especially if the percentage of available habitat is low; (iii) if individuals cannot avoid contaminated patches of soil, population-level effects of habitat loss and fragmentation are worse, especially if the percentage of available habitat is low; (iv) intensive agricultural practices, exemplified by a generally reduced habitat quality and repeated disturbance events, will in combination with natural physical stress (i.e., exemplified by drought) adversely affect population recovery and potentially lead to population extinction.

4.2 Methods

The species used in the simulations is *Folsomia candida* Willem 1902, which belongs to the order Collembola, suborder Entomobryomorpha, family Isotomidae. This species is used as a standard test organism for toxicity tests: a 28-day reproduction test (ISO Guideline 11267: International Organization for Standardization, 1999; OECD Guideline 232: Organisation for Economic Co-operation and Development, 2009) and is included in the refinement options for ecological risk assessment of plant protection products to soil organisms in the EU (Santé des Consommateurs, 2002; European Commission, 2009).

Copper sulphate ($CuSO_4$) was used as a model contaminant to simulate permanent loss of habitat quality, as it is proven to cause toxic effects to *F. candida* survival and reproduction, and to elicit behavioural responses like avoidance (Boiteau et al., 2011). Moreover it is used as fungicide on a variety of crops, is one of the most widely distributed pollutant among metals, and therefore relevant for ecological risk assessment.

Full descriptions of both the biology of *Folsomia candida* and the individual-based model are found in Meli et al. (2013). Therefore, in the following section we give only a brief overview of the model itself, while we focus on the submodels that have been added to the original model in order to test the hypotheses tested here.

Individual-based model overview

The purpose of the model is to investigate how populations of *F. candida* are affected by spatial distribution of toxic contamination in soil, with a special focus on interactions with food availability and local population density (Meli et al., 2013). The model comprises the entities eggs, juvenile and adult female springtails, and grid cells. Springtails are mobile and are characterized by the state variables age (days), position (continuous coordinates), direction of movement, energetic status (days-to-death), cumulative distance (in cm) walked in each hourly time-step, and time (h) spent on contaminated grid cells. Grid cells are characterized by their food level and concentration of toxicant (mg kg^{-1} soil). The model world is a two-dimensional grid of 100x100 square grid cells representing 1 cm^2 of soil. The global environment is characterized by six "seasons", that determine the temperature-dependent life-cycle parameters of the springtails. The model proceeds in daily time steps comprising the following processes: updating the season, foraging including avoidance of contaminated and densely populated grid cells (hourly time steps), re-growth of food, ageing and growth, reproduction, hatching, density dependence on fecundity and survival, and mortality.

Values of almost all parameters are drawn from uniform or normal probability distributions, in order to reflect heterogeneity among individuals. Stochasticity is also used for initializing springtails' starting positions, as well as causing individual behaviours (movement, reproduction, hatching, mortality) to occur with specified frequencies depending on the values of the parameters.

At the beginning of a model run, food resources are also randomly assigned to grid cells that are initialised to be food sources, with different maximal food levels.

A full description of the model following the ODD format (Grimm et al., 2006; 2010) is provided in the Supplementary Material.

Submodel generation of fractal patterns of habitat destruction

This submodel is based on a NetLogo implementation of the midpoint displacement algorithm (Saupe, 1988) included in the individual-based model "TraitScape" developed by Jackson and Fahrig (2012), which has been modified to fit our purpose.

Midpoint displacement is a well-known algorithm that produces random, realistic-looking, fractal landscapes (Saupe, 1988). The amount of spatial autocorrelation of the fractals generated by this algorithm can be adjusted by varying the value of a parameter (*H*). The fractal dimension (*D*) of the landscape is a property of *H* such that $D = 3 - H$ (Jackson and Fahrig, 2012). Another characteristic that makes this algorithm especially suitable to study natural phenomena is that it allows independent control of habitat amount and configuration. The main differences among landscapes in our runs are the amount and the configuration of habitat, which are driven by the following parameters:

Habitat cover. Proportion of landscape cells that are clean habitat. The user can control habitat cover by adjusting a parameter (user-cover) to the desired amount. For instance, a user-cover value of 0.3 means that 30% of the habitat is without contamination.

Spatial autocorrelation of habitat (H). Degree of aggregation of habitat cells, i.e. the opposite of habitat fragmentation. *H* can assume values between 0 and 1; given the same habitat cover, low *H* will result in many small patches and low inter-patch distances, whereas high *H* will result in a few large patches with high average inter-patch distances (Fig. 4.1a-c).

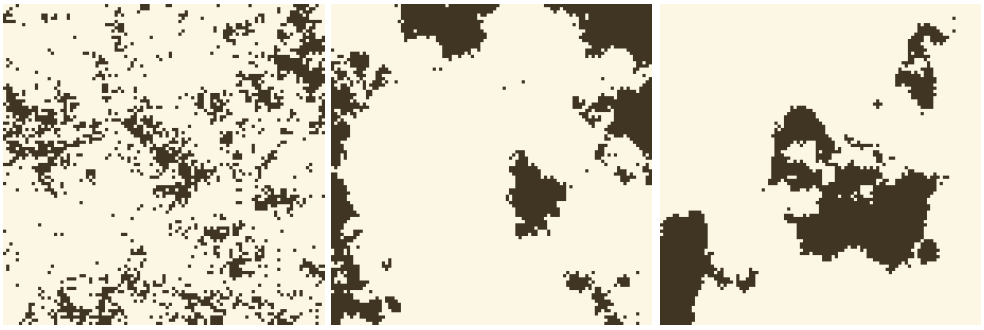

Fig. 4.1. Examples of fractal landscapes with 20% habitat cover (dark grey), and different degrees of spatial autocorrelation of the clean habitat: uncorrelated (H = 0: a), moderately correlated (H = 0.72: b) and completely correlated (H = 1: c).

Submodel disturbances

Disturbance events are characterized by disturbance level, number of events in a year, days of occurrence of disturbance events, and spatial autocorrelation of disturbed area. The disturbance

level is defined as the proportion of area disturbed per single disturbance event. The shape and placement of disturbed areas are randomized, in the case of uncorrelated disturbances. In the case of spatially autocorrelated disturbances the disturbed area is distributed in a circle proportional to the disturbance level and located around the central grid cell. Disturbances occur with a frequency defined by the specified number of events, removing all individuals (eggs, juveniles and adults) on the disturbed grid cells, and can hit both habitat cells and unsuitable (i.e. contaminated by copper) cells.

Submodel summer drought

Implementation of the summer drought submodel is based on data from Waagner et al. (2011). In this study, the authors performed a long-term experiment tin which the water potential of soil was slowly decreased, to reproduce the natural condition that occurs when soil dries out. Relative humidity (RH) was progressively decreased during 12 days, and the target level was maintained for 20 days. Exposure to RH > 98.2% had no significant effect on survival, whereas below 99.4% RH oviposition stopped. Among the different target RHs tested in this study, 97% RH has been chosen for implementation, as the range 99.8 to 97% RH represents a realistic RH regime in soil during periods of natural drought (Holmstrup, 1997; Hojer et al., 2001). Furthermore, data from Holmstrup (1997)showed a reduction in drought tolerance caused by copper when the desiccation stress was higher than 97.8% RH.

Therefore, based on these observations, the implemented drought effects reflect the decline in relative humidity shown in Fig. 4.2, and the following assumptions have been made:

Four days after the beginning of the drought period, corresponding to a decline of RH below 99.4%, all eggs die and both juvenile and adult survival begin to be affected by drought.

As reported by Waagner et al. (2011), mean survival at the end of the exposure period to 97% RH is 32%. Survival is therefore implemented as the probability to survive each day until the end of the drought period (i.e. 25 days, from day 5 to day 29):

$$probability\ to\ survive = \left(drought\ survival\right)^{1/length\ of\ drought\ period}$$

To account for the variability in drought tolerance, individual survival follows a normal distribution with the same mean and standard deviation as recorded in Waagner et al. (2011).

Starting from the seventh day of drought, equivalent to a RH value of 97%, drought tolerance is reduced by 30% if the individual has cumulatively been exposed to copper for at least a week, according to Holmstrup (1997). This rule does not apply to the first six days of drought, as no reduction of drought tolerance was observed for RH values above 97.8% (Holmstrup, 1997).

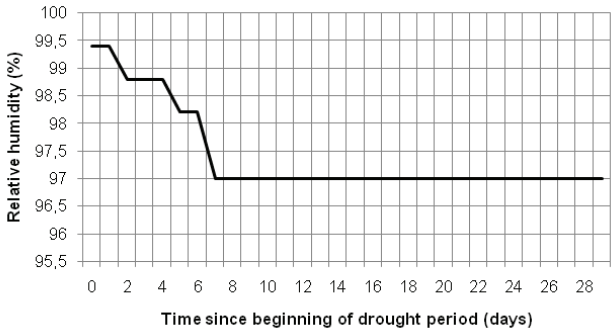

Fig. 4.2. Profile of relative humidity decrease over time during the simulated period of drought.

Simulation experiments

We designed four sets of simulation experiments to separately test the hypotheses defined above (see Introduction). The first three sets of simulations were conducted at a constant temperature range of 19-21ºC, with a duration of 300 days, whereas the fourth used variable temperature ranges, according to the implemented seasons submodel (see Meli et al., 2013), and a duration of 730 days (two years).

Hypothesis i: simulations with a percentage of contaminated habitat ranging from 70 to 100%, at a chemical concentration that causes an avoidance response of 50%, were run. It is assumed that there is no spatial autocorrelation among contaminated patches of soil ($H = 0$). In the following, these simulations will be referred to as simulation set A.

Hypothesis ii: while in the first set of simulation experiments no spatial autocorrelation ($H = 0$) is assumed, in this second group we have tested the effects of moderately ($H = 0.72$) and completely autocorrelated ($H = 1$) contamination. All three degrees of autocorrelation were tested for the same rangeof habitat availability as in the previous set of simulations (contaminated habitat ranging from 70 to 100%). In the following, these simulations will be referred to as simulation set B.

Hypothesis iii: in the previous sets of simulations, it was assumed that individuals can sense and avoid contaminated patches of soil. Here we ran simulations with the same setup as in i and ii, but excluding avoidance behaviour from the model. In the following, these simulations will be referred to as simulation set C.

Hypothesis iv: the purpose of the last set of simulation experiments was to test the effects of disturbance events on a population already subjected to habitat fragmentation, and under more realistic field conditions. Two separate subsets of simulations were performed to test this hypothesis: in the first subset temperature was maintained constant and the only disturbance added, besides copper contamination, was a period of drought. In the following, these simulations will

be referred to as simulation set D. For the second subset seasonal temperature variation and disturbance events were added. These simulations were run with either two or 14 disturbance events per year, to compare two rather extreme scenarios. We chose to set the number of disturbances to 14 as this is the average number of pesticide applications on vineyards during a growing season (Cerruto et al., 2010). Disturbances were either spatially autocorrelated or uncorrelated, and with disturbance levels ranging from 30 to 90%. All disturbance events happened between spring and summer. In the following, these simulations will be referred to as simulation set E. In both subsets, uncontaminated habitat was set to 20% of the total simulated area, with a coefficient of spatial autocorrelation of either 0 or 1.

In all simulations, 10% of the grid cells were initialized as food sources, with a stock of 20 food items each. The initial population comprised 100 individuals of random age (juveniles and adults), which occupied randomized locations on the model grid. Ten replicate model runs were executed for each set of simulation experiments.

Due to the fact that the percentage of available habitat in the fractal landscapes generated at the beginning of each model run may differ slightly from the specified value, actual values were recorded for each model run, and results were analyzed by clustering the simulations in groups within 2% habitat ranges, as shown in Table 4.1.

Table 4.1. Groups of percentages of available habitat in which simulations have been clustered.

Group nr	lower limit (% habitat)	upper limit (% habitat)
1	0	2
2	2	4
3	4	6
4	6	8
5	8	10
6	10	12
7	12	14
8	14	16
9	16	18
10	18	20
11	20	22
12	22	24
13	24	26
14	26	28
15	28	30

4.3 Results

Results of simulation set A, plotted as population abundance over time, are presented in Fig. 4.3. Results of model runs were grouped as shown in Table 4.1; therefore each line in the graph represents the average over 10 runs with habitat availability varying within a 2% range. These simulations were conducted with different percentages of habitat availability, spatial correlation coefficient equal to 0 (i.e. spatially uncorrelated clean habitat) and including avoidance behaviour.

From the graph it is apparent that the effect on population size of a progressive reduction in the availability of clean habitat becomes stronger as the percentage of clean habitat reduces. For instance, a reduction of habitat availability from 18-20% (Group 10) to 14-16% (Group 8) corresponds to a decrease in equilibrium population size of about 25%, whereas a reduction of habitat availability from 6-8% (Group 4) to 2-4% (Group 2) corresponds to a decrease in equilibrium population size of about 80%.

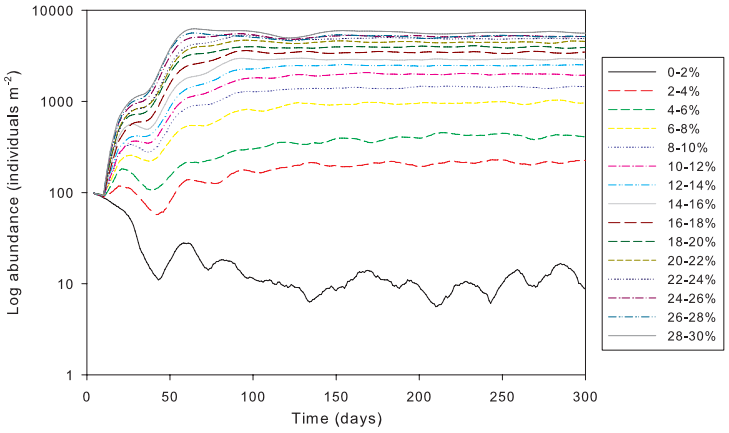

Fig. 4.3. Results of simulation set A: mean population abundance over time for different percentages of clean habitat, grouped within 2% ranges.

In Fig. 4.4a final population abundances averaged over the last 10 days of simulation set B are shown. Bars represent mean population abundance of each group (Table 4.1) and error bars represent standard errors of means. Simulations were conducted with different percentages of clean habitat and different spatial arrangements of the contaminated areas, ranging from completely scattered (H = 0) to completely aggregated (H = 1).

The results show that fragmentation has opposite effects at low and high percentages of clean habitat. In fact, at the lowest simulated levels of habitat availability, when clean habitat is between 0 and 10% of the total area, population growth is enhanced when clean habitat is arranged in a spatially autocorrelated way. At the highest simulated levels of habitat availability, when clean

habitat is above 20% of the total area, the population reaches a higher equilibrium size when the clean habitat is uncorrelated.

The same simulations were then run excluding avoidance behaviour from the model, and results of simulation set C are presented in Fig. 4.4b. In comparison with Fig. 4.4a, these simulations show generally higher variability among replicate runs, as illustrated by the error bars which represent standard errors of the mean for 10 replicates. Furthermore, the relationship between degree of fragmentation and population abundance described above is no longer visible when avoidance behaviour is switched off, as abundance in the spatially uncorrelated scenarios is always lower than in the correlated ones, given the same habitat availability.

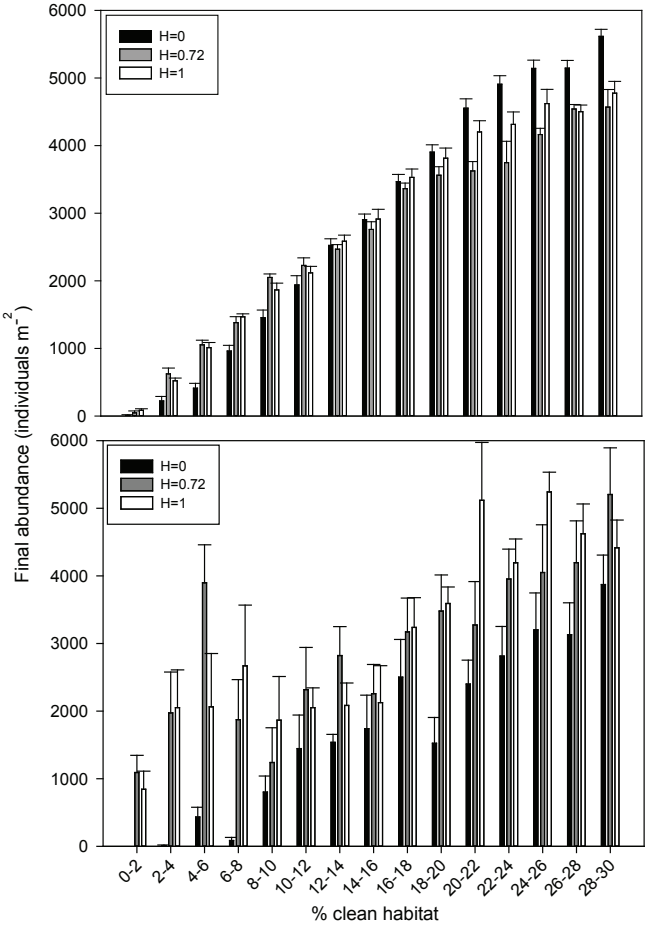

Fig. 4.4. Results of simulation sets B and C: mean final population abundance for different percentages and levels of spatial autocorrelation of clean habitat, in the presence (a) and absence (b) of avoidance behaviour. Error bars represent standard error of mean for 10 replicates.

Fig. 4.5 presents results of simulation set D. From the graph it is apparent that time to recovery from a severe drought period depends on the level of fragmentation of the available habitat. In fact, given the same amount of clean habitat, recovery is slower when the level of fragmentation is higher (H = 0).

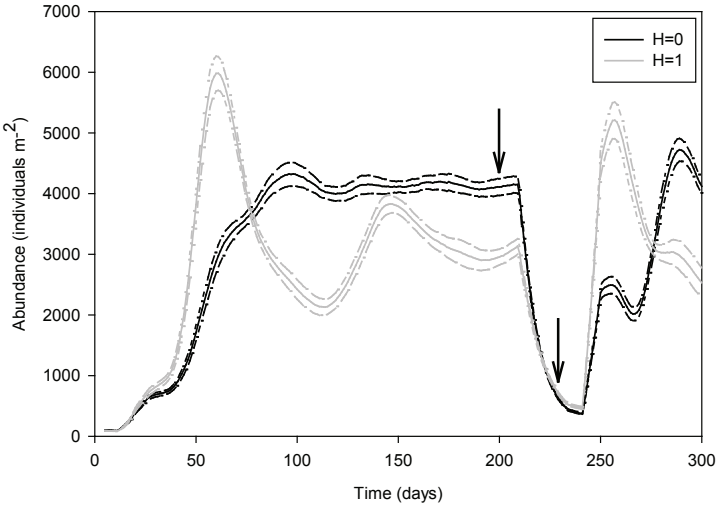

Fig. 4.5. Results of simulation set D: population recovery after a period of drought stress under two different spatial configurations of clean habitat. Arrows indicate beginning and end of the drought period.

Results of simulation set E, where different patterns of disturbance events were tested, are shown in Fig. 4.6. In these simulations, copper contamination is fixed in terms of contaminated area (20% clean habitat), but two spatial distributions were tested ("uncorrelated" (H = 0) and "correlated" (H = 1) on the x-axis of Fig. 4.6). In both spatial distributions of copper contamination, three patterns of disturbance events were tested: two spatially uncorrelated events (Fig. 4.6a), 14 spatially uncorrelated events (Fig. 4.6b), and 14 spatially correlated events (Fig. 4.6c). The amount of area affected by these disturbances ranged from 30 to 90%. In the graphs, the total abundance over one year (measured as the sum of juveniles and adults from day 1 to day 365) has been calculated for two consecutive simulation years.

In all cases population abundance decreases with an increase in the percentage of disturbed area. A similar trend in the effects of disturbance events and contaminated habitat is noticeable: for all three patterns of disturbance events, population abundance is higher in the case of correlated copper contamination, given the same amount of disturbed area. Similarly, given the same amount of disturbed area and distribution of copper, population abundance is higher if disturbance events are spatially correlated (Fig. 4.6b vs. c).

Furthermore, it is important to note that in the worst-case scenario (14 spatially uncorrelated

disturbance events applied on at least 70% of the modelled area)populations decline dramatically over consecutive simulation years, and even go extinct. In all other cases the total abundance reached in the second year of simulation is equal to the first year.

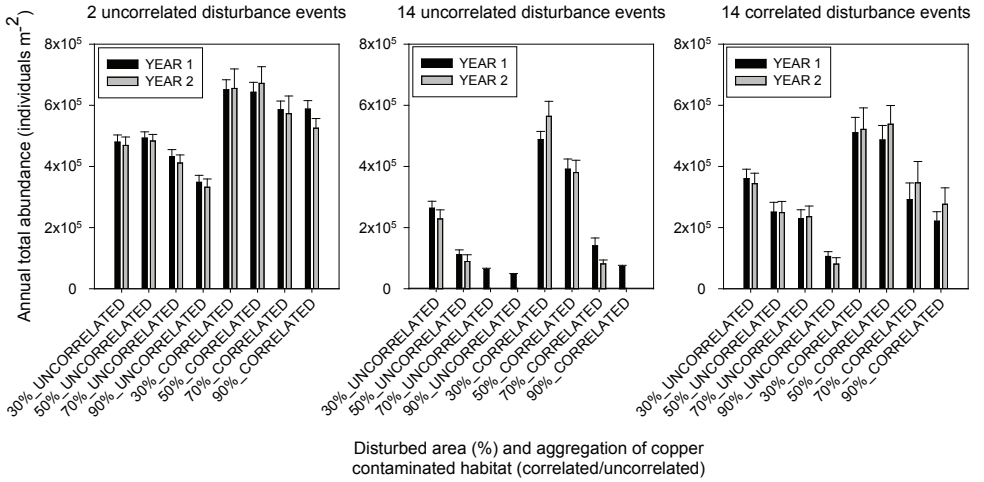

Fig. 4.6. Results of simulation set E: mean annual abundance of populations exposed to different patterns of disturbance events and spatial configurations of clean habitat.

4.4 Discussion

In this study we used a spatially-explicit individual-based model, fully described in Meli et al. (2013), to investigate the effects of different microscale patterns of habitat fragmentation and disturbance events on populations of the collembolan, *Folsomia candida*. The model has been developed, parameterized and tested following the Pattern-Oriented Modelling (POM) framework (Grimm et al., 2005). Model rules and parameters are based on data and empirical observations reported in the literature, and the model includes behavioural responses such as avoidance (of both the model contaminant and high population densities) and foraging behaviour. These responses have been shown to significantly influence model outcomes when predicting population-level effects of copper sulphate in simple heterogeneous exposure scenarios (Meli et al., submitted).Therefore for this study we implemented a midpoint displacement algorithm to generate fractal landscapes with different degrees of spatial autocorrelation and percentages of contaminated habitat, in order to further investigate the effects of heterogeneous exposure combined with avoidance.

We hypothesized that reduction in population abundance caused by a progressive habitat reduction is more than proportional to the habitat loss. Previously published results of model simulations with simpler toxicant distributions and more coarse variations in clean habitat avai-

lability suggested that the percentage decline in population abundance is more than proportional to the decline in percentage of available habitat (Meli et al., submitted). Furthermore, in another model study, (Fahrig, 1997) found that habitat loss has a large effect on population persistence.

Outcomes of simulation set A (Fig. 4.3) show that only in landscapes with low percentages of clean habitat is a further reduction in habitat reflected in a more than proportional effect on the population. In such landscapes reduction in available habitat results in an exponential increase in distances between clean patches: in fact, in this situation available habitat is already scarce and therefore highly fragmented, with small patches of clean soil scattered in a matrix of unsuitable habitat. Further removal of clean patches thus strongly increases isolation of remaining patches. As available habitat becomes more abundant, the same reduction results in a progressively lower impact on population abundance, suggesting a threshold effect of habitat loss.

In our second hypothesis we assumed that collembolan populations are more affected by habitat loss when the degree of fragmentation of remaining habitat is higher (i.e. patches of clean habitat are spatially uncorrelated or poorly correlated), especially for lower percentages of available habitat.

In fact, findings of some theoretical studies suggest that the effects of fragmentation per se should become apparent only at low levels of available habitat, below approximately 20-30% habitat on the landscape (Fahrig, 1998; Flather and Bevers, 2002), whereas above this threshold population responses should depend only on pure habitat availability effects.

Whereas in simulation set A no spatial autocorrelation ($H = 0$) was assumed, in simulation set B we tested the effects of moderately ($H = 0.72$) and completely autocorrelated ($H = 1$) contamination. All three degrees of autocorrelations were tested at different percentages of habitat availability, to understand whether the above-mentioned threshold effect of fragmentation would emerge from the simulations.

Our results (Fig. 4.4a) are in accordance with the findings reported in the studies listed above and in the review by Andrén (1994), despite the species and modelling approach being different. The difference between spatially autocorrelated and uncorrelated contamination is apparent for habitat availability lower than 10-12% (groups 1-6). In these simulations population growth is higher when clean habitat is spatially correlated, i.e. when size and connectivity of patches is greater. Nevertheless, when habitat availability is above 20% we see the opposite tendency (i.e., higher population growth at lower connectivity/autocorrelation). Therefore, it seems that including behavioural responses, such as avoidance and foraging, in the simulations makes interactions with distribution of toxicants more complicated than what has been suggested in theoretical studies. When individuals avoid contaminated areas, they tend to spend more time on clean patches of soil, which in turn can get overcrowded, and high population density affects the number of eggs laid by individuals in the model, leading to reduced reproductive output. It appears from Fig. 4a that this happens more evidently in simulations with a high percentage of spatially correlated

available habitat, where clean patches are larger than in simulations with uncorrelated contamination. On these large patches of soil populations can reach locally high densities, resulting in a negative relationship between population density and patch size.

We also hypothesized that if individuals cannot avoid contaminated patches of soil, population-level effects of habitat loss and fragmentation are worse, especially for lower percentages of available habitat. In the previous sets of simulations, it was assumed that individuals can sense and avoid contaminated patches of soil. However, it has been shown that *F. candida* do not detect all toxicants (Greenslade and Vaughan, 2003), and thus we ran simulations without avoidance, in order to understand the effects of habitat loss and fragmentation on populations in both cases.

If we assume that individuals cannot sense the contaminant, the variation among replicates is much higher than in analogous simulations with avoidance (simulation set C; Fig. 4.4b). This implies that survival of individuals and population growth are subjected to higher stochasticity, related to the fact that the choice of clean vs. contaminated habitat is purely accidental. In this situation it appears that the aforementioned threshold effect (Andren, 1994; Fahrig, 1997; With, 1997) does not apply, as the population is affected more at higher fragmentation ($H = 0$) compared to spatially autocorrelated habitat ($H = 0.72$ and $H = 1$), also when available habitat is more than 20% of the total area.

In general, in uncorrelated simulations ($H = 0$) all abundances are lower without avoidance than with avoidance, given the same percentage of clean habitat: not surprisingly, in situations where the risk of moving out of the clean habitat and into contaminated habitat is larger due to the higher degree of fragmentation, populations perform better when they can sense and avoid the contaminant.

Contrasting simulations with and without avoidance behaviour also clearly shows that the flexibility given by the possibility of switching certain submodels on and off, and by the adaptability of the model to new scenarios and conditions, is one of the strengths of IBMs as a tool for hypothesis testing.

In simulation set D, all simulations were run at constant temperature (19-21°C) and with only one persistent contaminant, simulating the effects of a permanent habitat loss. However, vital rates change with temperature, and populations in agricultural landscapes may often be exposed to multiple chemicals at the same time. Furthermore, natural stressors may interact with chemicals in a variety of ways. For instance, *F. candida* is very sensitive to soil moisture, and summer drought periods can cause declines of natural populations. Therefore to study the effects of different chemical application regimes in combination with a natural stressor, we combined contamination by copper with the effects of drought. In addition, we added various disturbance events that hit defined percentages of the modelled area, killing all the organisms (eggs, juveniles and adults) within this area. These simulations were performed with a percentage of clean habitat (20%) that

in previous simulations ensured a stable population abundance of around 4000 individuals m^{-2}.

One of the endpoints currently considered by risk assessors to determine whether the risk to soil invertebrates is acceptable is population recovery within a growing season. From simulation set D, where natural stress is added to the effect caused by the model contaminant, it can be implied that in a scenario more realistic than what is currently considered in terrestrial risk assessment of plant protection products, this recovery might not be reached. In fact, even if temperature is held constant at the optimal range for this species, it takes around 70 days to fully recover from the drought stress under spatially uncorrelated copper contamination, whereas under spatially correlated contamination this time decreases to 20 days. However, introducing seasonal variations of temperature and disturbances (simulation set E), it appears that only when 14 spatially uncorrelated disturbance events are applied on at least 70% of the modelled area populations decline dramatically over consecutive simulation years, while in all other tested cases the total abundance reached in the second year of simulation is equal to the first year.

From this results it appears that, while *F. candida* is widely used in risk assessment because it is sensitive to the effects of a wide range of chemicals and is easy to rear in the laboratory (Fountain and Hopkin, 2005), populations of this species are not particularly vulnerable even when they are reduced to very low numbers, as they have rapid population growth (Gregoire-Wibo and Snider, 1977) and reproduce parthenogenetically. Therefore, as has also been recommended by Krogh (2008) especially for chemicals that are suspected to interfere with the reproductive biology of sexually reproducing species, another species such as *Folsomia fimetaria* should be used in combination with, or instead of, *F. candida*, to ensure that risk assessment covers more vulnerable soil invertebrate species.

Another point that emerges from the graphs in Fig. 4.6 is the difference between the effect of disturbance events under correlated and uncorrelated habitat destruction (Cu contamination). Given the same pattern of disturbances, these events have a much higher effect in simulations with spatially uncorrelated copper contamination. The same trend is visible when disturbance events are correlated, i.e., given the same distribution of contaminated habitat, spatially uncorrelated disturbances have a higher effect on population size than correlated ones.

This example illustrates the possibility offered by the model to explore the effects of different management options. For example, one could use the model to explore if and how modifying the application of the chemical of concern, as well as its spatial distribution could influence risks to soil invertebrate populations. Such information could be helpful in identifying suitable mitigation options. Therefore mechanistic effect models could represent not only a good tool for the refinement of risk assessments, but also for risk management.

4.5 Acknowledgment

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Chapter 5

Two pairs of eyes are better than one: Combining individual-based and matrix models for ecological risk assessment of chemicals

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Abstract

Current chemical risk assessment procedures may result in imprecise estimates of risk due to sometimes arbitrary simplifying assumptions. As a way to incorporate ecological complexity and improve risk estimates, mechanistic effect models have been recommended. However, effect modeling has not yet been extensively used for regulatory purposes, one of the main reasons being uncertainty about which model type to use to answer specific regulatory questions. We took an individual-based model (IBM), which was developed for risk assessment of soil invertebrates and includes avoidance of highly contaminated areas, and contrasted it with a simpler, more standardized model, based on the generic metapopulation matrix model RAMAS. We then explored consequences of model aggregation in terms of assessing population-level effects for different spatial distributions of a toxic chemical. For homogeneous contamination of the soil, we found good agreement between the two models, whereas model output differed for heterogeneous contamination. In particular, as RAMAS did not allow to represent avoidance behavior, for high concentrations and percentages of contaminated areas, RAMAS output was similar to the IBM only if the latter did not include avoidance. Overall, RAMAS was less sensitive than the IBM in detecting population-level effects of different spatial patterns of exposure. We conclude that choosing the right model type for risk assessment of chemicals depends on whether or not population-level effects of small-scale heterogeneity in exposure need to be detected. We recommend that if in doubt, both model types should be used and compared. Describing both models following the same standard format, the ODD protocol, makes them equally transparent and understandable. The simpler model helps to build up trust for the more complex model and can be used for more homogeneous exposure patterns. The more complex model helps detecting and understanding the limitations of the simpler model and is needed to ensure ecological realism for more complex exposure scenarios.

5.1 Introduction

What is the risk that chemicals released into the environment have unacceptable effects on populations and ecosystems? In current regulatory environmental risk assessment (ERA) of chemicals, ecological effects are determined indirectly. Threshold exposure concentrations for detectable effects on individuals measured in the laboratory are extrapolated to populations in real landscapes by dividing them by so-called assessment, or safety, factors, which are supposed to take into account ecological characteristics of the species, landscape, and ecosystem under consideration. However, whether or not these factors are over- or under-protective remains an open question (Forbes and Calow, 2002).

As a way to incorporate ecological complexity and bridge the gap between laboratory tests and effects on the ecological entities that current risk assessment schemes aim to protect, ecological mechanistic effect models (MEMs) have been recommended as they provide a tool for expressing ecological risks in a way that informs the environmental management process (Forbes et al., 2010) and increases the ecological relevance of risk assessments (Forbes et al., 2008; Thorbek et al., 2009; European Food Safety Authority, 2009; EFSA Panel on Plant Protection Products and their Residues, 2010).

Nevertheless, in contrast to exposure modeling (Boesten et al., 1995), effect modeling has not yet been extensively used for regulatory purposes (Schmolke et al., 2010a; Schmolke et al., 2010b). A main reason for this was identified in a survey among stakeholders from academia, industry, and regulatory authorities involved in ERA (Hunka et al., 2013): the lack of official guidance for developing and using mechanistic effect models. This includes choosing the model types to be used, which is influenced by contradicting expectations (Hunka et al., 2013): models are supposed to be simple and user-friendly enough to be easily understood, parameterized, and used in a standardized way, but at the same time complex enough to be realistic and capable of capturing a wide range of ecological scenarios.

Thus, in addition to developing ecological models for chemical risk assessment, which just have a certain level of complexity, the costs and benefits of this particular level of complexity for ERA procedures need to be demonstrated more often, by contrasting more simple and more complex models. Fully independent comparisons, though, would require that the models were developed by different modelers with no direct or indirect interactions whatsoever, which would be difficult and so far has never been tried. An alternative is starting with a more complex model and then aggregating it into a simplified one. For mechanistic effect models, this was done by Topping et al. (2005), who compared a very complex spatially explicit IBM to a very simple non-spatial matrix model.

Here we take a recent spatially explicit individual-based population model, which was de-

veloped for risk assessment of soil invertebrates (Meli et al., 2013), and contrast it with a simpler, more standardized model, which is based on the generic metapopulation matrix model RAMAS Metapop 5.0 (Akçakaya and Root, 2005). RAMAS falls into the family of "canned" programs (Reed et al., 2002), which corresponds to the widely held believe among the stakeholders involved in ERA of chemicals that using standardized software is the best way to establish MEMs for regulatory risk assessment.

In our example models, we focus on soil invertebrates, which are key drivers of important ecosystem services such as nutrient cycling and soil formation (Lavelle et al., 2006). For these species, an important ecological factor that is largely ignored in current regulatory risk assessments is spatial heterogeneity in exposure. It is well known that in soils both natural properties, such as moisture and organic matter concentrations, and chemical contamination are heterogeneously distributed (Lavelle and Spain, 2001; Becker et al., 2006), which has important consequences for the distribution and functioning of populations of soil organisms (Hoy and Hall, 1998). Thus, the real risk posed by the use of chemicals in agricultural practices or industrial activities is likely not to be adequately captured by current risk assessment procedures.

The two models we are contrasting are mostly based on the same input data and, similarly to Topping et al. (2005), the IBM is used to determine some of the parameters of the metapopulation model, as it was not possible to find appropriate values in the scientific literature. Therefore in this study we are not trying to compare independent predictions of two models, but to explore the consequences of model aggregation. Aggregating a complex individual-based model into a metapopulation matrix model, where all the individuals within a grid cell are not treated as separate entities anymore and the spatial resolution is lower, will allow us to understand whether it really is necessary to look at single individuals for a species with a relatively simple life-cycle in order to assess toxic effects at the population level. Furthermore, we will explore which benefits contrasting more simple and more complex models can have within a regulatory perspective, for instance in terms of model acceptance.

5.2 Methods

The species used in the simulations is *Folsomia candida* Willem 1902, which belongs to the order Collembola, suborder Entomobryomorpha, family Isotomidae. This species is used as a standard test organism for toxicity tests: a 28-day reproduction test (International Organization for Standardization, 1999; Organisation for Economic Co-operation and Development, 2009) is included in the refinement options for ecological risk assessment of plant protection products to soil organisms. A more detailed description of *Folsomia candida* is given in Meli et al. (2013).

Copper sulfate ($CuSO_4$) was used as a model contaminant: it is proven to cause toxic effects to *F. candida* survival and reproduction, and to elicit behavioral responses like avoidance (Boiteau et al., 2011). Moreover it is the most widely distributed pollutant among all metals, and therefore it is relevant from the practical point of view of ecological risk assessment.

Individual-based model

The purpose of the model is to investigate how populations of *F. candida* are affected by spatial distribution of toxic contamination in soil, with a special focus on interactions with food availability and local population density (Meli et al., 2013).The model comprises the entities eggs, juvenile and adult female springtails, and grid cells. Springtails are mobile and are characterized by the state variables age (days), position (continuous coordinates), direction for movement, energetic status (days-to-death), cumulative distance (in cm) walked in each hourly time-step, and time (h) spent on contaminated grid cells. Grid cells are characterized by their food level and concentration of toxicant (mg kg^{-1} soil). The model world is a two-dimensional grid of 100x100 square grid cells, whereas each grid cell represents 1 cm^2 of soil. The model proceeds on two time scales: hourly time steps are used for the foraging procedure, while the following processes are repeated at daily time steps: updating the season re-growth of food, ageing and growth, reproduction, hatching, density dependence on fecundity and survival, and mortality.

Values of almost all parameters are drawn from uniform or normal probability distributions, in order to reflect heterogeneity among individuals. Stochasticity is also used for initializing springtails' starting positions, as well as causing individual behaviors (movement, reproduction, hatching, mortality) to occur with specified frequencies. Simulations start with 1,000 individuals located on the upper left corner of the model arena, in order to simulate a recolonization scenario. The initial population is divided in a stage distribution that randomly varies around the mean values of all the stable stage distributions used for the metapopulation model. Food resources are also randomly assigned at the beginning of a model run to grid cells which are initialized to be food sources, with different maximal food levels. Four different scenarios for the extent and spatial distribution of contaminated areas are used (see Section "Simulation experiments", below). A key feature of the model is that it represents avoidance behavior: individuals can, depending on the toxicant's concentration, sense and avoid contaminated areas. The stage distributions used to initialize the model and the TRACE documentation of the model (Schmolke et al., 2010a), which includes a full description following the ODD protocol, are provided in Appendix 2.

Metapopulation model

The model is based on RAMAS Metapop 5.0, a software platform designed to build age- or stage-structured, spatially explicit metapopulation models, to run simulations, and to predict the risk of extinction, time to extinction, expected metapopulation abundance, its variation and spatial distribution (Akçakaya and Root, 2005). In the following we describe the model according to the ODD protocol (Grimm et al., 2006; 2010) to facilitate direct comparison to the IBM.

Purpose

The purpose of the model is to simulate *Folsomia candida* population dynamics and to investigate how they are affected by the spatial distribution of toxic contamination in soil.

Entities, state variables and scales

The basic model for the dynamics of local populations is based on a stage-based Lefkovitch matrix, where the matrix elements incorporate fecundity, mortality, and growth rates of the three stages eggs, juveniles and adults. *Folsomia candida* is parthenogenetic, therefore only females are included in the model. The life-cycle parameters used to calculate the matrix elements are based on data collected from the literature and refer to a temperature range of 19-21°C. The model world is two-dimensional and consists of 5×5 square grid cells, each of which is inhabited by one sub-population and represents an area of 20 cm^2 of soil, so that the total modeled area is 1m^2. One model run lasts for 300 days; one time-step corresponds to one day.

Process overview and scheduling

Changes in population size and structure are determined by multiplying, each time step, the vector characterizing the stage structure of the sub-populations by the Lefkovitch matrix (see Section "Submodels", below). The matrix elements are not constant but can depend on local population size and structure and include random variation. At each time-step, the following processes are executed; the submodels representing the processes are described in detail in Section "Submodels".

Population dynamics: At each time-step and for each sub-population, the population vector is multiplied by the corresponding stage matrix. Different subpopulations are characterized by different stage matrices, according to the level of contamination they are initialized with.

Contamination effects: To simulate toxic effects of copper sulfate on vital rates, different stage and standard deviation matrices have been implemented for different copper concentrations. Therefore, for sub-populations on contaminated patches, these matrices are used.

Density-dependence: To model density dependence, each time step certain elements of the stage matrix are multiplied by a variable representing sub-population density abundance at that time

step. The stages affected by population density are juveniles and adults.

Standard deviation matrix and stochasticity: Using the option for demographic stochasticity in RAMAS, each time-step the number of survivors and dispersers (emigrants) is sampled from binomial distributions, and the number of young is sampled from a Poisson distribution. Furthermore, to represent environmental stochasticity, at each time step the program draws the vital rates from a normal or lognormal distribution; the mean of this distribution is taken from the stage matrix, and its standard deviation is taken from the standard deviation matrix.

Dispersal: Dispersing individuals have a higher chance of ending up in a closer patch than a distant one; dispersal is implemented in RAMAS as a negative exponential function of distance.

Design concepts

To represent environmental noise, i.e. stochastic variation in the population's growth rate, stochasticity has been incorporated by using the standard deviation matrix option that in RAMAS is meant for purely environmental sources of variation. Interaction among individuals is indirectly included in the model as density-dependence of juvenile survival and adult fecundity and survival, and density-dependent dispersal. To observe model output, size and structure of the population, elasticities of individual life-history parameters, as well as spatial distribution of the individuals for different concentrations of toxicant are compared.

Initialization

The model is initialized with 1000 organisms, divided into the three stages according to the stable stage distribution defined by the corresponding stage matrix (see Appendix 3). All individuals are placed in sub-population number one, i.e. in the top left corner, therefore simulating a recolonization scenario.

Input data

This model has no time-series inputs or external environmental drivers.

Table 5.1. Parameters used in the individual-based and in the RAMAS model.

		Temperature = 19-21°C			
Parameter	**Units**	**Empirically recorded range**	**Empirically recorded mean value**	**Use of the parameter in IBM**	**Use of the parameter in RAMAS**
Maturation time: time to reach adulthood	Days	13-29		Empirically observed range of values directly implemented	Used to calculate, through resampling, stage and standard deviation matrices elements S_2 and F_2
Hatching time: time needed for the eggs to develop and hatch to juveniles	Days	7-15		Empirically observed range of values directly implemented	Used to calculate, through resampling, stage and standard deviation matrices elements S_1 and F_1

Parameter	Units	Temperature = 19-21°C: Empirically recorded range	Empirically recorded mean value	Use of the parameter in IBM	Use of the parameter in RAMAS
Number of eggs per brood, general value for the season	Number	30-50		Empirically observed range of values directly implemented	Used to calculate, through resampling, stage and standard deviation matrices element F_3
Nr of broods per female: max number of reproductive events	Number	3-20		Empirically observed range of values directly implemented	Not used
Time between broods	Days	6-16		Empirically observed range of values directly implemented	Used to calculate, through resampling, stage and standard deviation matrices element F_3
Egg viability: percentage of eggs that successfully hatch	Number	0.75- 0.97		Empirically observed range of values directly implemented	Used to calculate, through resampling, stage and standard deviation matrices elements S_1 and F_1
Juvenile survival, expressed as probability to survive until age at maturity	Number		0.95	Empirically observed range of values directly implemented	Used to calculate, through resampling, stage and standard deviation matrices elements S_2 and F_2
Adult survival, expressed as the age of death of the individual	Days	6-198	140	Empirically observed range of values directly implemented	Used to calculate, through resampling, stage and standard deviation matrices element S_3
Probability to reproduce at every reproductive instar	Number		0.98	Empirically observed range of values directly implemented	Used to calculate, through resampling, stage and standard deviation matrices element F_3
Distance within which food and conspecifics are sensed	Cm	2.5		Empirically observed range of values directly implemented	Food is not included in the RAMAS model
Energy level	Days-to-death	Max: 30 Min: 0		Parameter value directly used in the IBM	No energy budget implemented; energy level is assumed to be optimal and constant
Energy reduction per time-step	Days-to-death	0.042[1]		Parameter value directly used in the IBM	No energy budget implemented; energy level is assumed to be optimal and constant
Energy gained by food intake	Days-to-death	0.5[1]		Parameter value directly used in the IBM	No energy budget or foraging behavior implemented
Energy reduction per step moved	Days-to-death	0.01[1]		Parameter value directly used in the IBM	No energy budget implemented; energetic cost of movement implicit in the dispersal function
Probability to move at each time-step	Number	0.1[1]		Parameter value directly used in the IBM	Implicit in the dispersal function
Maximum energy spent for foraging at each time-step	Days-to-death	0.2[1]		Parameter value directly used in the IBM	No energy budget implemented; energy level is assumed to be optimal and constant

Parameter	Units	Temperature = 19-21°C: Empirically recorded range	Empirically recorded mean value	Use of the parameter in IBM	Use of the parameter in RAMAS
Tradeoff between energy and reproduction	Days-to-death	20[1]		Parameter value directly used in the IBM	No energy budget implemented
Maximum energy spent for avoiding high density at each time-step	Days-to-death	0.1[1]		Parameter value directly used in the IBM	No energy budget implemented; energetic cost of movement implicit in the dispersal function

[1] Values determined via sensitivity analysis and parameterization, see Meli et al. (2013)

Submodels

Population dynamics. The stage matrix is:

$$\begin{pmatrix} S_1 & F_2 & F_3 \\ P_1 & S_2 & 0 \\ 0 & P_2 & S_3 \end{pmatrix} \quad [1]$$

where S is the probability of remaining in the corresponding stage, F fertility, and P the probability of moving from one state to the next state.

Following Crouse et al. (1987), in RAMAS the probabilities of remaining in the same stage through the next time step and of moving from one stage to the next for eggs and juveniles, have been calculated as:

$$S_i = \frac{1 - p_i^{d_i - 1}}{1 - p_i^{d_i}} * p_i \quad [2]$$

$$P_i = \frac{p_i^{d_i} * (1 - p_i)}{1 - p_i^{d_i}} \quad [3]$$

Where p_i is the probability of surviving to the next time-step and d_i the stage duration in days.

We have only direct information on the egg viability, i.e. the percentage of eggs that have been vital during the hatching period d_1. Therefore, we can calculate daily survival p_1 as

$$p_1 = egg\ viability^{(hatching\ time)^{-1}} \quad [4]$$

Similarly, for juveniles survival until maturation is p_2^{d2}, d_2 maturation time, and

$$p_2 = juvenile\ survival^{(maturation\ time)^{-1}} \quad [5]$$

Juveniles are not fertile, thus $F_2 = 0$.

For adults, fecundity F is expressed as the number of eggs produced per time step, which can

be approximated by dividing the number of eggs per brood by the time between broods.

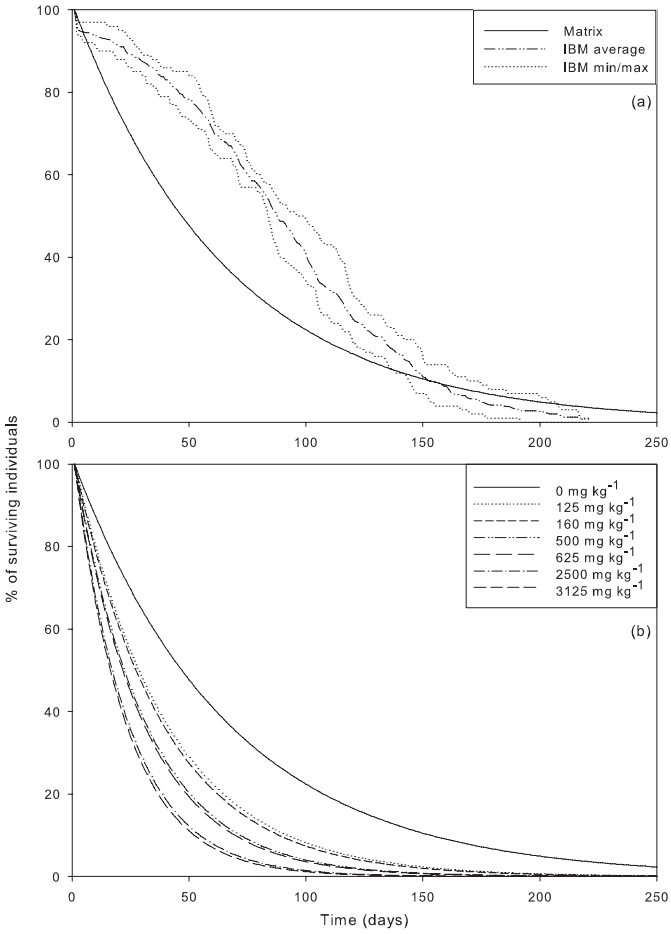

Fig. 5.1. (a) Comparison of survival curves for individuals in the adult stage implemented in RAMAS (solid line) and in the IBM (dashed line (mean), dotted lines (minimum and maximum)). (b) Survival curves for individuals in the adult stage implemented in RAMAS for different copper concentrations.

Concerning adult survival, the only information available in the literature is for typical lifespans (in days). Therefore we inversely determined S_3 by making the corresponding survival curve as similar as possible to the corresponding curves of the IBM, which were based on empirically observed distributions of lifespans (Fig. 5.1a). The reason for the difference between the survival curves of the IBM and RAMAS is that the only option to model survival in RAMAS is an exponential function, whereas in the IBM survival emerges from the implemented lifespan distribution.

Since egg viability and the other measures show large variation, we used the following strategy to take this variation into account. We drew the parameters in equations [2]-[5] from a uniform

distribution limited by the minimum and maximum reported value (Table 5.1) 1000 times and calculated the corresponding S_i values. We derived from this sample the mean S_i, which was then used in the stage matrix, and the standard deviation, which was used in the standard deviation matrix.

Contamination effects. To simulate toxic effects of copper sulfate on vital rates, different stage and standard deviation matrices have been implemented for different copper concentrations. The elements of these matrices have been calculated following the same rationale as described above for the control matrices, using the same dose-response relationships for each affected life-cycle trait as in the IBM. The life-cycle traits affected by copper are:

- Egg viability. For each copper concentration used in the simulations, egg viability values derived from the concentration-response curve (Table 5.2) have been used to calculate new values of the matrix elements S_1 and P_1 through equations [2] and [3].
- Fecundity. The number of eggs per brood is reduced by copper contamination, therefore the matrix element F_3 is also reduced accordingly.
- Juvenile survival. In the stage matrix, the sum of the S_2 and P_2 elements gives the total survival for the juvenile stage of the population. Because no other information is available, we assumed that the proportion of individuals remaining in the same stage and the proportion of individuals moving to the next stage at each time-step remains the same under copper contamination, i.e. that copper does not affect maturation time, as no evidence for this was found in the literature. Therefore, for each copper concentration used in the simulations, the new total survival (S_2 + P_2) has been determined from the dose-response regression for survival (Table 5.2), and S_2 and P_2 have then been calculated using the same proportion as in the control: S_2 is 95.1% of total survival and P_2 4.9% of total survival.
- Adult survival. Values for the S_3 matrix element for the different concentrations were selected on the basis of the observation that the survival rate implemented for the control (Fig. 5.1a) resulted in a residual survival of about 10% after a time equal to the average of the empirically observed lifespan range (Table 5.1). To represent effects of copper sulfate, we used the same approach, using lifespans deduced from the dose-response regression (Table 5.2). For instance, for individuals exposed to a copper concentration of 500 mg kg^{-1}, the average lifespan is 62 days. Therefore, after trying different values for the survival rate, we chose the one that resulted in a residual survival after 62 days of 10%, as in the control. The survival curves for control and the simulated copper concentrations are reported in the graph in Fig. 5.1b.

Density dependence. To model density dependence in RAMAS, each time step the elements of the stage matrix are modified according to the density of the sub-population at that time step.

We assumed that density dependence is based on the abundance of juveniles and adults, and that density dependence influences fecundity and survival. We assumed scramble competition for most simulations with the exception of the *F. candida* 2500 mg Cu kg^{-1} matrix, since the corresponding λ value (i.e. population growth rate expressed as population multiplication rate) was less than one, and that is not compatible with the scramble model. Thus, for this high contamination level, we assumed ceiling-type density dependence.

Scramble-type density dependence is defined by maximum population growth rate (R_{max}) and carrying capacity (K). It was not possible to find a precise value for carrying capacity in the literature, but some observations (Hopkin, 1997; Fountain and Hopkin, 2005) show that the highest recorded *F. candida* population densities are in the range of 10^5 individuals m^{-2}. Therefore this value, equally divided among sub-populations, was used in the model as K. R_{max} was set to the same value as the eigenvalue of the corresponding stage matrix. For the ceiling-type density dependence, the same carrying capacity used as in the scramble model was used as the ceiling density.

Table 5.2. Dose-response regressions used in the metapopulation model.

Independent variable	Dependent variable	Regression	R^2	Reference
ln concentration	Reduction of survival	$y = 0.0824x - 0.1366$	0.847	Sandifer and Hopkin, 1996
ln concentration	Reduction of fecundity	$y = 0.2189x - 0.8743$	0.919	Sandifer and Hopkin, 1996
ln concentration	Nr of hatched eggs (Normalized to the control)	$y = -0.2243x + 1.8893$	0.932	Xu et al., 2009

Standard deviation matrix and stochasticity. Stochasticity can be incorporated in RAMAS as demographic or environmental stochasticity. For the former, the program does not have any customization options, but only allows the number of survivors and dispersers (emigrants) to be sampled from binomial distributions, and the number of young to be sampled from a Poisson distribution.

To account for environmental stochasticity, RAMAS has the option to fill in a standard deviation matrix, which has the same structure as the stage matrix and should contain standard deviations of the vital rates. Then, each time step the program draws the corresponding vital rates from a normal or lognormal distribution; the mean of this distribution is taken from the stage matrix, and its standard deviation is taken from the standard deviation matrix. To avoid bias in the estimation of vital rates due to truncation above 1.0, a lognormal distribution has been specified, rather than a normal one. This means that survival rates are sampled from a lognormal distribution if the mean is less than 0.5, and from a "mirrored" lognormal if the mean is above 0.5. Fecundities are always sampled from a regular lognormal distribution, since they are not truncated above 1.0.

Dispersal. Dispersal is distance-dependent, i.e. dispersing individuals have a higher chance of ending up in a closer patch than a distant one. The generic dispersal-distance function imple-

mented in RAMAS has the form:

$$dispersal\ rate = a * \exp\left(-\frac{distance}{b}\right), \text{if distance} \leq D_{max}$$

$$dispersal\ rate = 0, \text{if distance} > D_{max}$$

The dispersal-distance function assumes the negative-exponential form, which has been shown to be a generally appropriate model of dispersion (Wolfenbarger, 1946; Kitching, 1971).

D_{max} is assumed to be equal to 40 cm: from 20 IBM simulations it has been measured that the maximum net distance moved by an individual during a simulation time equal to one day (measured as the number of grid cells from the original position; each grid cell is 1 cm wide) is 22 cm. Therefore, since the spatial scale introduced by dividing the whole population into discrete sub-populations was 20 cm, the dispersal function implemented in RAMAS should allow dispersal from a population to the eight neighboring patches, but not further (Fig. 5.2a).

The value for the parameter *b*, set to 17.24, has been chosen so that the corresponding negative exponential function would give a dispersal rate of about 0.2 to the population situated on the diagonal in respect to the original population, as in the IBM only a small fraction of the observed individuals covered an equivalent linear distance.

The parameter *a* is a coefficient that multiplies the exponential function. When there are several populations within dispersal distance of each other, dispersal rates to single populations are summed up, and the total rate of dispersal must be less than or equal to one, otherwise the number of dispersers would be higher than the actual number of individuals. Therefore, the value for *a* has been set to 0.2 to meet this condition.

A model summary generated by RAMAS for the control, which includes all our parameter settings, is included in Appendix 4.

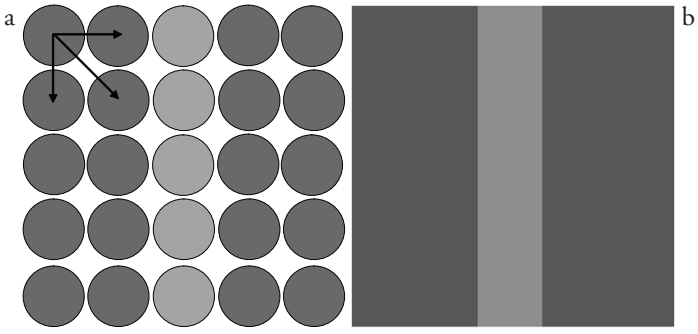

Fig. 5.2. Heterogeneous contamination scenario in the metatpopulation (a) and individual-based (b) model. Contaminated areas (dark grey) are equal to 80% of the total modeled area. Arrows in (a) indicate sub-populations within dispersal distance from the upper-left corner sub-population during one time-step.

Simulation experiments

For the simulations with homogeneous contamination, the concentrations tested were 0, 125, 500 and 2500 mg Cu kg^{-1}. In the first set of experiments with heterogeneous contamination, the contaminated area in the scenario was 80%, corresponding to 20 sub-populations in the metapopulation model (Fig. 5.2); the concentrations used were 160, 625 and 3125 mg Cu kg^{-1}, so that the average contamination was the same as in the homogeneous scenario.

In the second set of experiments with heterogeneous contamination, the effects of progressively increasing contaminated habitat and the smallest area needed to sustain a viable population were investigated, starting with 20 sub-populations with toxicant (equal to 80% of the total metapopulation), and progressively increasing this number to 21 (84%), 22 (88%), 23 (92%) and 24 (96%). The concentrations tested in this set of experiments were the same as in the first one, i.e. 160, 625 and 3125 mg Cu kg^{-1}. To better understand how avoidance, which is implemented in the IBM but not in the metapopulation model, influences the comparison between results of the two models, in a third experiment simulations of the IBM were run both with and without avoidance.

Furthermore, for all three experiments, different levels of food abundance were tested in the IBM; results were compared to the metapopulation model, where food resources are not explicitly modeled, and are assumed to be optimal and homogeneously distributed. For each level of food abundance simulated, the same percentage of grid cells was initialized to be food sources, so that the total amount of food was kept constant among simulations, but the distribution of food resources on the grid cells was randomized at the beginning of every model run.

5.3 Results

Homogeneous contamination

Fig. 5.3 shows the results of the first simulation experiments, conducted with homogeneous concentrations of 0, 125, 500 and 2500 mg Cu kg^{-1}. Three different food levels were simulated with the IBM by increasing the percentage of grid cells initialized as food sources from 10 to 25 and 50%, and in the following will be referred to as low, medium and high. With the metapopulation model it was not possible to explicitly represent foraging behavior, therefore optimal and homogeneously distributed food resources were assumed.

Fluctuations in population abundance were more marked in the control (0 mg Cu kg^{-1}) IBM simulations, due to the fast initial growth of the population that leads to food and space limitation, but abundance tended to stabilize after a few, dampened oscillations, especially for the high

food level. Population abundance in the RAMAS simulations with 0 and 125 mg Cu kg^{-1} reached the imposed carrying capacity (105 individuals m^{-2}) and therefore was stable for the length of the simulations. With 500 mg $kg^{-1}$of toxicant, population growth was much slower than in the control, both in RAMAS and IBM simulations, although in the latter the growth rate was lower, even at high food levels. At the highest simulated concentration, in both models the population goes extinct after about 100 days.

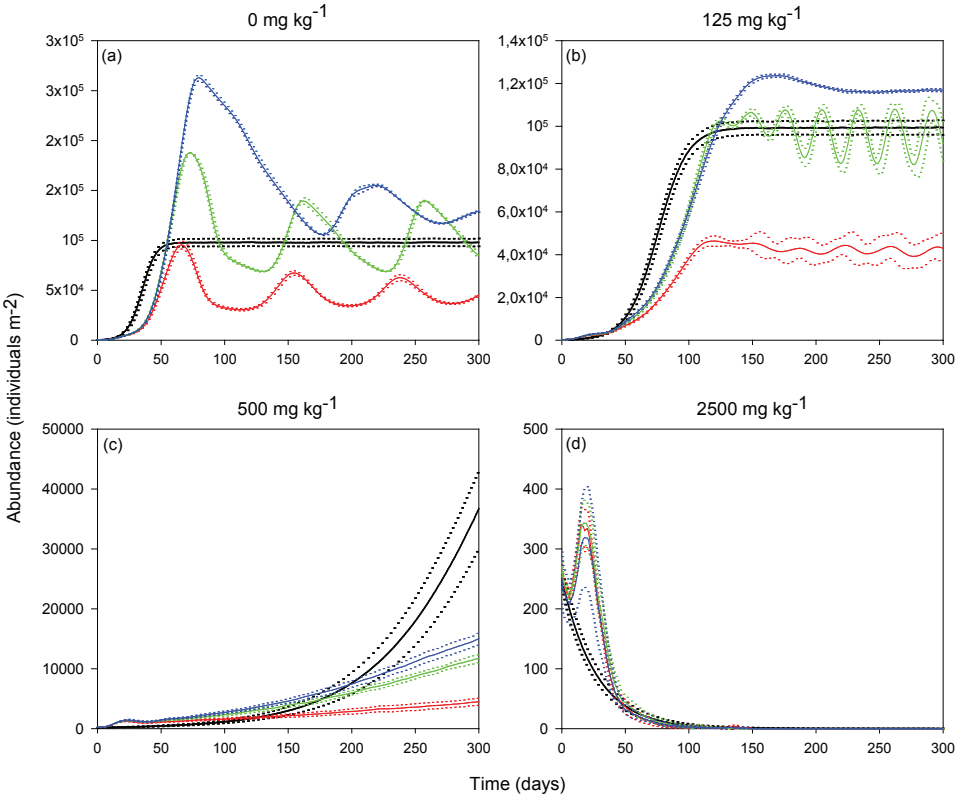

Fig. 5.3. Results of simulations with homogeneous contamination. Black lines represent RAMAS outputs, red lines outputs of IBM simulation with 10%, green lines with 25% and blue lines with 50% food cells. Dotted lines indicate one standard deviation around the mean (100 replicates with RAMAS, 10 replicates with the IBM).

Heterogeneous contamination

Fig. 5.4 shows the results of the second simulation experiment, conducted with heterogeneous local concentrations of 160, 625 and 3125 mg Cu kg^{-1}. The contaminated area was equal to 80 % of the simulation arena, therefore the average amount of toxicant the organisms were exposed to was the same as in the homogeneous scenario; despite this, both in the metapopulation and in

the individual-based model, the population abundance is generally higher for medium and high local contamination (Fig. 5.4c and d). Comparing the decrease in population abundance with increasing concentrations, it is apparent that this decline is more pronounced in RAMAS than in the IBM (Fig. 5.4).

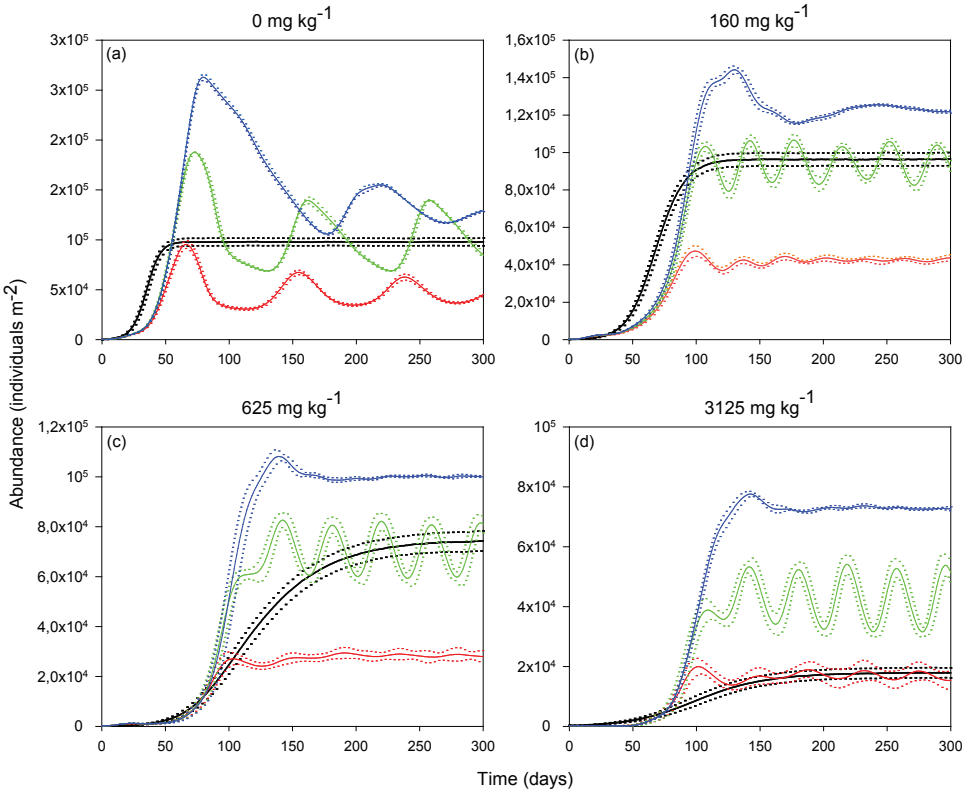

Fig. 5.4. Results of simulations with 80% of modelled area contaminated. Black lines represent RAMAS outputs, red lines outputs of IBM simulation with 10%, green lines with 25% and blue lines with 50% food. Dashed lines indicate one standard deviation around the mean(100 replicates with RAMAS, 10 replicates with the IBM).

In Fig. 5.5, histograms show the final population abundances for the third simulation experiment, where the contaminated area was 84, 88, 92 and 96%, while maintaining the same concentrations as in the previous set of simulations. Simulations with the IBM were run both with and without avoidance. In order to account for fluctuations in population size, the average abundance over the last 50 days of simulation has been considered as the final value. Fig. 5.5 shows that population-level effects of reducing the proportion of uncontaminated habitat increase with the concentration of toxicant present in the system: in both models, whilst at a concentration of 160 mg Cu kg^{-1} the same final population abundance is reached in all the different percentages of

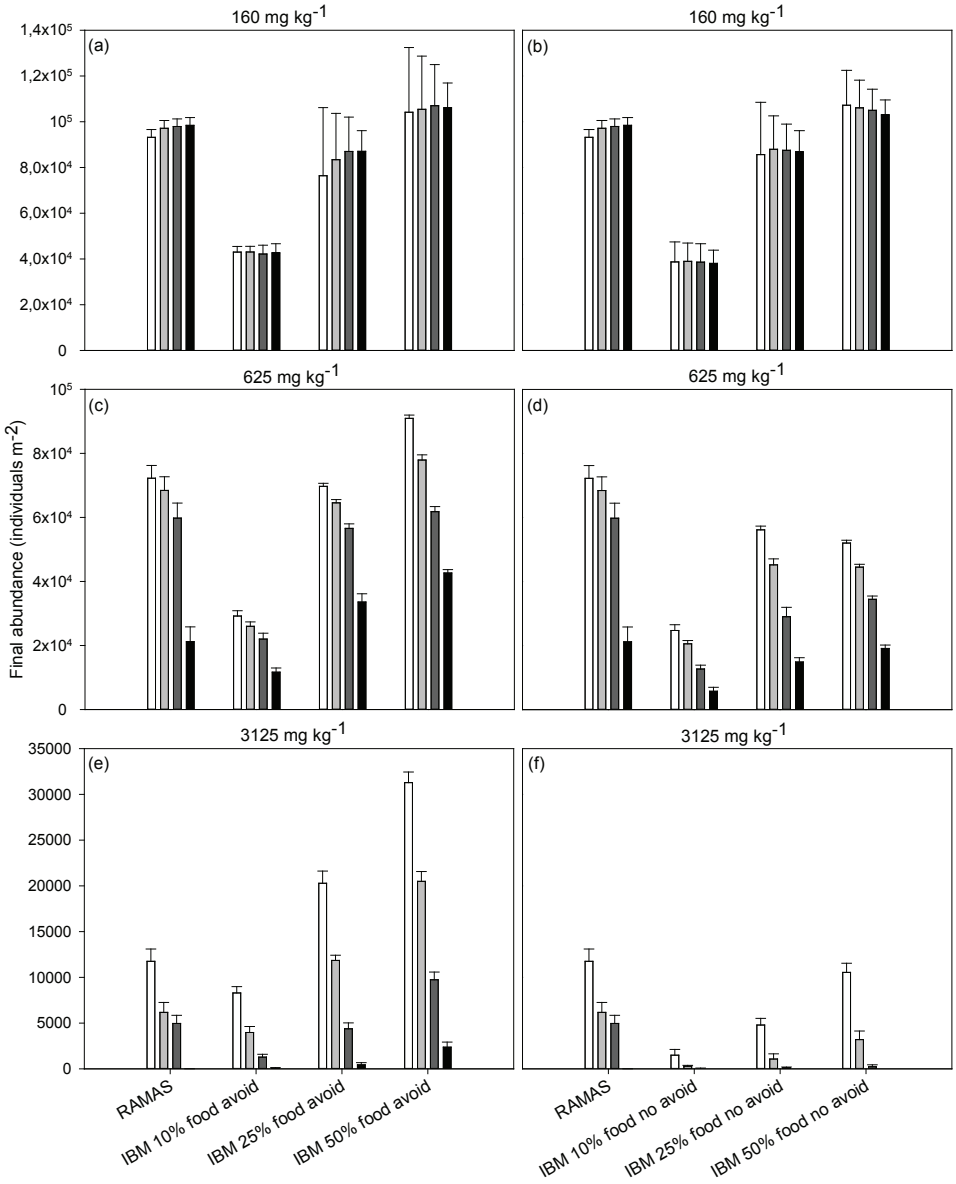

Fig. 5.5. Final population size (averages over the last 50 simulation time-steps), for different percentages of contaminated areas: 84% (white), 88% (light grey), 92% (dark grey) and 96% (black). Error bars represent standard deviations (100 replicates with RAMAS, 10 replicates with the IBM).

contaminated area, at 3125 mg Cu kg^{-1} the population declines with the increase of contaminated area. In the IBM the same trend is visible for the effects of including avoidance behavior: at 160 mg Cu kg^{-1}, simulations with and without avoidance reach the same final population abundance,

while at 3125 mg Cu kg^{-1} the population goes extinct in several food level/percent of contaminated area combinations if avoidance is not implemented.

Sensitivity of model outputs to different exposure patterns

Combining results from all three simulation experiments, we here compare scenarios without toxicant, with homogeneous (2500 mg Cu kg^{-1}) and with heterogeneous contamination (80, 84, 88, 92 and 96% of contaminated area; 3125 mg Cu kg^{-1}; Fig. 5.6). IBM results illustrated in this comparison refer to simulations with avoidance behavior and medium food level. The overall ranges of population abundance, between the most favorable set-up (no contamination) and the worst case scenario (homogeneous contamination), predicted by the two models, are for the most part overlapping. However, the sensitivity of the two models towards changes in spatial distribution of the toxicant is different: comparing simulations where the toxicant is distributed on 80% of the grid cells with simulations where the toxicant is present on 92% of the grid cells, the reduction in population density predicted by RAMAS is around 10,000 individuals m^{-2}, while the reduction predicted by the IBM is of 40,000 individuals m^{-2}. A further increase in the percentage of contaminated area, from 92 to 96%, led in RAMAS to a sudden decline of population abundance, to the point of extinction, whereas the reduction of population size predicted by the IBM is less dramatic.

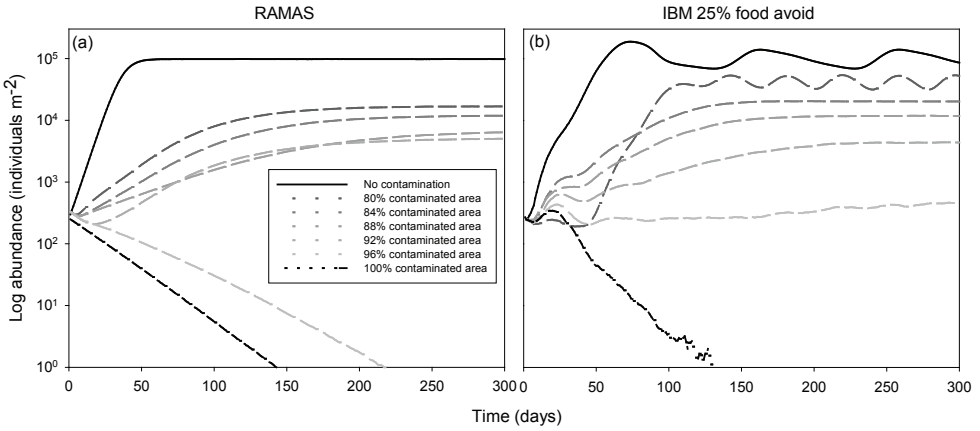

Fig. 5.6. Mean population size predicted by RAMAS (a) and the IBM with 25% food and avoidance behavior (b) for different percentages of contaminated areas (3125 mg Cu kg^{-1}).

5.4 Discussion

In the present study we aggregated a spatially-explicit individual-based population model into a stage-structured demographic metapopulation model, and compared population-level effects of different spatial distribution of a model contaminant (copper sulfate) predicted by the two models.

Individual-based population models are often seen as too complex and somewhat obscure, and questions are often raised about how much and which complexities need to be incorporated to get a robust estimate of population risk (Forbes et al., 2008). In contrast, matrix population models may offer a simpler approach, have a long history of use in applied ecology, and are easier to analyze (Pagel et al., 2008).

Results of the first set of simulations (Fig. 5.3) show that the two models' predictions are in good agreement for the control, the lowest, and the highest concentrations, at least when comparing IBM simulations with medium (25%) and high food level (50%), while the abundances reached with low food were much lower than the ones predicted by RAMAS. This was to be expected, as the carrying capacity implemented in the metapopulation model reflects an amount of food resources which is not limiting for the growth of the population. Overall, it should be kept in mind that the design of the two models was not independent: the RAMAS model was in fact designed, or calibrated, to mimic the behavior of the IBM for homogeneous scenarios. Fig. 5.3 shows that the aggregation towards the simpler model, which required quite a few assumptions, was appropriate, so that differences between the two models for other scenarios can be ascribed to factors which cannot be represented in the simpler model.

At the highest toxicant concentration simulated in both models, the populations go extinct after nearly the same interval of time; the peak at the beginning of IBM simulations can be explained by the fact that individuals are not instantaneously affected by the contaminant (i.e. toxic effect builds up over time), therefore at the beginning of a simulation their survival and fecundity allow for a positive growth rate, which is then disrupted by constant exposure to the toxicant.

The biggest difference between predictions produced by the two models appears to be at 500 mg Cu kg^{-1}, where RAMAS predicted a much faster population growth than the IBM. In all the graphs shown in Fig.3 the same pattern is repeated: at the beginning of the simulations the IBM curves are above the RAMAS one, then they switch, and in RAMAS simulations stable size is always reached before the IBM. For the control and low concentration this happens rather quickly, while in the 500 mg kg^{-1} simulations it takes longer because of the toxicant. Despite this difference in growth rate, longer simulations (not showed) demonstrated that eventually a similar stable population size is reached in the two models.

As all toxic effects implemented in the two models are based on the same empirical data, and population abundances reached in the homogeneous scenario with 500 mg Cu kg^{-1} are too low

for density-dependence to have a significant role that can justify the difference, we hypothesize that the disparity in population growth rate is due to the different level at which toxic effects act in the two models. In the metapopulation model toxicity is implemented as a reduction of vital rates, i.e. constant values that multiply the number of individuals present in the model at each time-step, whereas in the IBM, the toxicant influences single individuals, reducing their hatching success, fecundity and lifespan. As individuals are different from each other in terms of life-cycle parameters, at the beginning of simulations, when population density is low, application of toxic effects to single individuals enhances demographic stochasticity, causing the population to grow more slowly.

The main point that emerges from the results of simulations with heterogeneous contamination (Figs. 5.4 and 5.5) is that RAMAS and IBM predictions are not always consistent. This is apparent, for instance, from the results of the third set of simulations, shown in Fig. 5.5, where at different concentrations and percentages of contaminated area, RAMAS results are alternatively similar to IBM results with and without avoidance, and different food levels.

The RAMAS model does not include avoidance behavior, because only one dispersal function can be defined for the entire metapopulation. Therefore, the difference between RAMAS simulations and IBM simulations without avoidance can be explained mainly on the basis of how foraging behavior and density-dependence are implemented: dispersal in RAMAS has been modeled as an average of the movement procedure implemented in the IBM, but the finer scale of the latter, which varies from individual to individual and has a step-length of 1 cm, leads to high variability in the individuals' exposure. Furthermore, density-dependence is implemented in the metapopulation model via a ceiling carrying capacity for each subpopulation: until this is not reached there are no density-dependent effects. In the IBM, instead, density-dependence acts independently on each grid cell: this means that even though the global population abundance is low and would not give rise to density-dependent effects, locally situations of high density may occur (for instance, around food sources) that reduce the growth of the population.

In summary, the flexibility of the individual-based model makes it more suitable to investigate the effects of multiple stressors, for instance chemical contamination and food scarcity, on populations. In fact, to implement different food levels in a matrix model, it would be necessary to recalculate all the mean values and probability distribution of the matrix elements for each food level and concentration of toxicant. On the contrary, both these factors are implemented in the IBM as functions that dynamically modify the life-cycle parameters of the organisms; therefore it is not necessary to modify the model to test other combinations of the two stressors.

The importance of taking into account food availability on the growth of collembolan populations is apparent from several published studies. Usher et al. (1971), for instance, investigated the effects of food availability on the growth and production of *F. candida*, and found that food

appeared to play a major role in regulating the rate of population growth and in determining the maximum population density. Van der Kraan and Vreugdenhil (1973) demonstrated under field conditions that populations of Hypogastrura viatica are sometimes limited by food supply. Joosse and Testerink (1977) showed that natural factors, such as food and soil moisture, have a great influence on field populations of Orchesella cincta. Furthermore, results published by Bengtsson et al. (1985) indicate that survival and growth of the collembolan Onychiurus armatus in a metal polluted soil are dependent on the availability of food.

Another important take-home message from the comparison of model outcomes is that RAMAS is less sensitive than the IBM in detecting population-level effects of different spatial patterns of exposure (Fig. 5.6). The overall ranges of population abundance, between the most favorable set-up (no contamination) and the worst case scenario (homogeneous contamination), predicted by the two models, are for the most part overlapping. Simulating intermediate percentages of contaminated area with the two models, instead, did not give the same results, as RAMAS predicted a much lower decrease of population size with increasing percentages of contamination.

Table 5.3. Recommendations for choosing a model type: fit to specific purposes.

	Individual-based model	**RAMAS model**
Are subpopulations properties time-dependent?	X	
Does any behavior other than dispersal matter?	X	
Are you interested in the effects of more than one stressor?	X	
Does fine-scale spatial resolution matter?	X	
Is the modeled system best described as a discontinuous set of subpopulations within a matrix of unsuitable habitat?		X
Do you want to perform rigorous statistical analysis on model outcomes, such as population viability analyses?		X

This is a critical point when a model is supposed to be used to answer questions related to spatial heterogeneity of exposure.

The main motivation of this study was that MEMs are not yet widely used in regulatory risk assessment because most stakeholders involved do not know how and when to trust such models. Hunka et al. (2013) report that this lack of trust is largely due to the lack of transparency in the way models are presented and, most importantly, the lack of guidance on what type of models to use for what kind of questions. A major bottleneck in establishing trust in models is thus to provide tools for standardized testing and documentation of ecological models, following good modeling practice. Examples are the ODD (Overview, Design concepts, Details) protocol (Grimm et al., 2006; 2010) and the framework for transparent and comprehensive ecological modeling (TRACE) documentation (Schmolke et al., 2010a).

Our study shows a possible way to increase trust in mechanistic effect models with regard to

model choice and transparency. Our point of departure was the ongoing debate on whether simple or complex models should be favored for supporting environmental risk assessment of chemicals. Similar debates exist in Conservation Biology (Beissinger and Westphal, 1998) and other fields of ecological application (Pagel et al., 2008). Our main conclusion is that the either/or question is not meaningful. Every model type has its own pros and cons (Schmolke et al., 2010a); it is impossible to combine all pros in one single model. We therefore recommend, whenever possible, to develop and use both types of models for the same question and data set (Grimm et al., 2009). This requires additional resources for model development, testing, and analysis, but has a number of important benefits.

First, "canned" models like RAMAS are black boxes to users which have no training or experience in modeling. There is a high risk that model assumptions are not fully understood, or default settings uncritically used (Grimm et al., 2004). Trying to make the output of an IBM and a simpler, canned model match for appropriate simple scenarios means that the canned model has to be understood and parameterized in all detail. This detailed understanding can then be communicated using the ODD protocol, a standard format for describing individual-based models (Grimm et al., 2006; 2010). Here, for the first time a spatially explicit matrix model was described using this protocol. This allows to better compare the simple and complex models, and it makes all assumptions of the "canned" more simple model transparent. On the other hand, detailed IBMs, which are developed from scratch, have their own limitations. They are more complex and their output can thus be hard to test and understand. How can we be sure that model results indeed emerge from reasonable model assumptions and not from undetected bugs in the code and wrong assumptions? One way to build up trust in IBMs is to develop, like we did here, a simpler matrix model and demonstrate that results are the same for scenarios which can be represented with both models.

Ideally, one would develop both a simple and complex model for all models which are supposed to support environmental decision making. In practice, however, resources are limited and one still has to decide with which model type to start with. Here, the obvious advice is that the choice of model type should depend on the questions one wants to address. For instance, if any behavior, other than dispersal, matters, an IBM approach should be chosen. In Table 5.3 we listed a series of questions a hypothetical model user should try to answer about which model approach should be chosen first. In fact, as Stephens et al. (2002) pointed out, how much ecology is included in an ecological model matters for some questions we want a model to answer, but not for others. In their study they compared the ability of different models to reproduce observed, for the Alpine marmot, population abundance, its variations, and behavioral responses. They concluded that any attempt to prove that model predictions are true or false is misguided. Independent data sets are needed, and even when they are available, the levels of variance inherent in each model make it dif-

ficult to establish accurate patterns: only qualitative judgments are possible (Stephens et al., 2002).

The main conclusion from the present study is that when a well-tested IBM exists, it can be worthwhile to develop a simplified matrix population or, if spatially explicit, metapopulation model. This can be done using "canned" models like RAMAS, or simple models developed from scratch. The effort for this far below that required for the original IBM. On the other hand, if a matrix model was developed first, describing it using the ODD protocol helps to understand that matrix models are, if they are used for projection, just simulation models with a certain structure which can easily be summarized by using matrices. Starting from the ODD description, developing a corresponding IBM and then exploring the effects of explicitly representing individual life cycles and behavior requires some effort but is straightforward. Facing the benefits of using "two pairs of eyes" rather than only one, it is not unlikely that combining simple and complex model will be recommended in guidance documents for regulatory risk assessment.

5.5 Acknowledgment

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Chapter 6

Synthesis and general discussion

As a way to control and limit the impacts of human activities, such as the use of chemical products like pesticides, on the environment, an increasing number of regulations have been enforced in the EU. Especially for what concerns the production and use of agrochemicals, human and environmental risk assessment procedures represent primary instruments to answer the requirements of chemicals control laws. Still, the capability of traditional ERA procedures for predicting actual consequences on biological communities is poor, since they generally do not consider the complex compensatory mechanisms and the interactions between biotic and abiotic components that control natural ecosystems. Moreover, it must be considered that non target organisms are not exposed to a single harmful chemical only, but often to mixtures of toxicants and to different stressors, both anthropogenic and natural.

With my thesis I provide evidence in support of the inclusion of ecological effect models in risk assessment of chemicals. For this purpose I developed population models: this type of modelling approach has been recognized as a good tool for supporting environmental decision-making. This thesis therefore contributes to the application of mechanistic effect models in environmental risk assessment, and the findings could be useful to develop more ecologically relevant ERA procedures. The thesis is focused on the collembolan *Folsomia candida*, which is a species routinely used in standard toxicity tests (Organisation for Economic Co-operation and Development, 2009), and I have concentrated on environmental risk assessment for terrestrial ecosystems.

In all the experimental work conducted and reported in this thesis, copper has been used as the model toxicant. It is widely used as a pesticide and, for this reason, is the most widely distributed pollutant among all metals. It is thus not only a representative of the important group of pollutants formed by metals, but also highly relevant with respect to the ecological risk assessment of pesticides under Regulation EC 1107/2009 (European Commission, 2009).

6.1 Terrestrial risk assessment: adding ecological relevance with mechanistic effect models

From the description given in **Chapter 1**, it is apparent that the risk assessment scheme for soil invertebrates is rather minimal, despite their role in the delivery of ecosystem services by soils at plot and landscape scales, which is important but largely ignored (Lavelle et al., 2006). Invertebrate abundance and species richness are used in different soil quality indices. Their presence alters the rate and extent of the physical, chemical and biological processes that develop in soil, as well as the physical and chemical structure of the soil (Lavelle et al., 2006). Collembola contribute functionally to the terrestrial food web at several trophic levels, and soil-dwelling springtails decompose plant residues. Therefore, soil invertebrates are key drivers of important ecosystem

services such as nutrient cycling and soil formation (Lavelle et al., 2006). In this light, it can be argued that risk assessment schemes for this group of organisms should be more thorough. Nevertheless, it has often been recognized that assessment of the effects and risks of chemicals for the terrestrial environment is a complex matter. As reported by Tarazona et al. (2002), in the past ecotoxicologists have for various reasons focused on aquatic systems, so terrestrial risk assessments have been forced simply to apply the aquatic model to soils, or have focused on specific targets such as risk posed by agrochemical pesticides to birds, bees and beneficial arthropods. This has generated inconsistencies in estimates of risk for different uses of the same active substance, and it is not clear whether they originate from uncertainty, cost/benefit considerations, or the lack of scientific knowledge when the guidelines were set (Tarazona et al., 2002).

Ecological effect models can be an important instrument to add ecological relevance to terrestrial risk assessment. These models are primarily envisioned to be used in current ERA schemes as an alternative refinement option at higher tiers. Population modelling has in fact already been included in the new guidance document on risk assessment for birds and mammals among the options, to be chosen on a case-by-case basis, for higher tier refinement (European Food Safety Authority, 2009). This use of ecological models can be particularly helpful for terrestrial ecosystems, as the current refinement options, primarily ex situ bioassays, Terrestrial Ecosystem Models (TME) and field surveys, have obvious limitations. The main drawbacks they share are the difficulty to link the observed effects to a specific toxic component in the soil and to find a proper reference site or soil (Jensen and Pedersen, 2006). Both these issues could be solved using ecological effect models, which mechanistically link effects at individual or sub-individual level to the population or community level, and easily allow comparison of different contaminated scenarios to a reference or control situation. In particular, a major strength of population models is the possibility to start simple, and progressively include further complexity if the need arises. In a regulatory context, this means that a simpler model, simulating constant temperature and optimal food conditions can be used as a first step to answer more basic questions. Only where it is necessary to refine the estimate of risk to more realistic conditions, such as variable climatic regimes or more complex exposure scenarios, further parameters or submodels can be implemented. Unlike in field survey, this strategy allows to interpret the results distinguishing between effects of the different factors. To apply it, either two types of models have to be developed, as shown in **Chapter 5**, or a flexible modelling approach, which allows to easily add more submodels, needs to be used. An example of the latter approach is given in **Chapter 4**, where three extra submodels have been integrated in the IBM (see Section 4.2) in order for the model to be capable of testing different hypotheses. First, simulations with constant temperature and no disturbances were run (Figs. 4.3 and 4.4), then only a natural disturbance was added (Fig. 4.5), and finally simulations with different patterns of both natural and anthropogenic disturbance factors were run (Fig. 4.6). In this way it was

possible to better understand the effects of the different factors, like avoidance behaviour, habitat fragmentation and disturbances, or temperature.

As these results show, spatial heterogeneity in exposure is an important ecological complexity, but is mostly overlooked in environmental risk assessment, especially for terrestrial ecosystems. In fact, it is well known that in soil both natural properties, such as moisture and organic matter concentrations, and abiotic factors, such as chemical contamination, are heterogeneously distributed (Lavelle and Spain, 2001; Becker et al., 2006), and that this influences the distribution and functioning of soil populations (Hoy and Hall, 1998). From the results of the studies conducted in **Chapters 3, 4** and **5** it is apparent that a more realistic exposure assessment can significantly influence estimates of risk for soil organisms. Exposure in soil is currently defined by a PEC which is assumed to be homogeneous; however, model results show that assuming homogeneous concentrations of a toxicant in soil might lead to overestimation of risk for collembolan populations, if the actual application method is unlikely to cause homogeneous contamination. For instance, the comparison of homogeneous and heterogeneous scenarios in Fig. 3.6 shows that, while decreased in size with respect to the control, a population can still survive in heterogeneously contaminated environments, even at very high concentrations. Also when using a metapopulation modelling approach (Fig. 5.6), the same results are obtained: for the tested species homogeneous exposure is more harmful, and represents a worst case scenario, although this might not be the case for all species or contaminants (see e.g. Palmqvist and Forbes, 2008).

The influence of spatial heterogeneity of exposure in soil is confirmed by the findings of a number of empirical studies on populations and communities of soil invertebrates. In experiments with microcosms, Salminen and Sulkava (1996) showed that soil animals populating a defaunated and patchily polluted soil area proved to concentrate in sites with the lowest pollution level. Results of a field study (Salminen and Haimi, 1999) suggest enchytraeids may have population dynamics connected to patches (sources–sinks) caused by uneven distribution of metals, and this can mitigate the effects of metals on their population densities. Results of yet another field survey (Gongalsky et al., 2009) indicate that the patchiness of soil pollution may act as a leading factor of belowground soil invertebrate distribution.

Furthermore, as a way of improving the link between exposure and effects, and thus to obtain more precise estimates of risk, avoidance behaviour should be included as a standard endpoint, considering the great influence that its inclusion or exclusion had on model results. For instance, Fig. 5.5 shows that, as concentration and percentage of contaminated area increase, so does the difference between the outputs of simulations with and without avoidance. This suggests that ignoring whether the chemical of concern is avoided or not, may lead to over- or undestimations of risk. Furthermore, Fig. 4.4 shows that variability among replicates is much higher when the contaminant is not avoided: data on avoidance behaviour could therefore be relevant to better

inform on the precision of risk estimates. The implementation of avoidance behaviour tests as screening tools in ERA has already been supported by several studies (e.g. da Luz et al., 2004; Loureiro and Nogueira, 2005). The use of avoidance behaviour of soil invertebrates as an indicator of unfavourable conditions allows a preliminary assessment of contaminated soils in a short period of time, with a high degree of sensitivity (Aldaya et al., 2006). Moreover, from an ecological point of view, avoidance is a relevant endpoint, and avoidance tests can be more sensitive to within-species population differences (Aldaya et al., 2006). A combination of both types of tests could provide more detailed information on the impact of pesticides and other harmful substances on Collembola (Heupel, 2002).

Population models clearly cannot substitute for low tier short-term laboratory tests on individuals, which are necessary as a first screening tool, and also to produce the effects data necessary to parameterize the models. In fact, in order to get realistic estimates of effects of a model contaminant at the population level it is necessary to implement the relevant toxicity data (e.g. concentration-response equations) at the individual or sub-individual level. An example of the importance of using accurate toxicity data in a mechanistic effect model is represented by the simulation results produced by the IBM, and especially the tests conducted within the pattern-oriented framework to verify the model (**Chapter 3**, Fig. 3.5). In this regard, in order to harmonize the use of laboratory tests and mechanistic effect models in risk assessment procedures, modifications to the test endpoints currently reported in ERA dossiers are recommended. In fact, it is often the case that the data necessary to incorporate toxic effects in a population model are recorded during a low-tier test, but not reported in the final dossier if this is not required, whilst the information provided (e.g. NOEC, LOEC or ECx) is not ideal in terms of model parameterization. The NOEC, LOEC and ECx as expressions of the toxicity of a chemical compound on an endpoint of interest have been already heavily criticized, for different reasons. Laskowski (1995) and Jager (2011), for instance, provide "Some good reasons to ban the use of NOEC, LOEC, ECx and related concepts in ecotoxicology". Among them are the facts that NOEC and LOEC depend heavily in on the concentrations tested, and disregard the intrinsic variability of life, as they imply a threshold value below which no effect can be found, while there is always some fraction of a population that is affected by any level of a toxicant. The criticisms to the ECx concept stem from its dependence on exposure time, on the tested endpoint and on how it is expressed, and on environmental conditions. Furthermore, ECx is not meaningful for nonconstant exposure, and it is purely descriptive. Despite these criticisms, which are based on both biological and statistical reasons, NOEC and ECx still feature prominently, not only in regulatory contexts, but also in scientific publications. Therefore, if ecological models are to be used in regulatory risk assessment, it is desirable that these measures are abandoned in favour of other solutions, such as regression analysis (Bruce and Versteeg, 1992; Stephan and Rogers, 1985; Hope, 2005), that allow deriving

the concentration-effect relationship, which can be implemented in ecological models.

Finally, the insight, provided by the mechanistic models described in **Chapters 3-5**, into the population dynamics of *Folsomia candida* and the long-term effects of copper sulphate, suggests that *F. candida* does not seem to be a particularly vulnerable species. For instance, EC50 values for copper sulphate on *F. candida* reproduction are generally within the range 500-750 mg Cu kg^{-1}, but even when only a small percentage of clean habitat is available, populations can survive despite lethally high concentrations (e.g. Fig. 5.5). This is especially relevant in the perspective of ERA, where the species tested are chosen to represent entire groups of organisms. *F. candida* is widely used in risk assessment because it is generally considered to be sensitive to the effects of a wide range of chemicals and is easy to rear in the laboratory (Fountain and Hopkin, 2005). However, due to the parthenogenetic mode of reproduction and exceptionally high population growth rate (Gregoire-Wibo and Snider, 1977), populations of this species are not particularly vulnerable even when different stress factors frequently reduce them to very low numbers. Therefore, to ensure that risk assessment covers more vulnerable soil invertebrate species it may be useful to look for a substitute species. Krogh (2008) recommends, especially for chemicals that are suspected to interfere with the reproductive biology of sexually reproducing species, that another species such as Folsomia fimetaria is used in combination with, or instead of, *F. candida*.

6.2 Model type comparison: exercise on model aggregation

Comparison of different model types and their ability to correctly predict ecological processes has been tried for several ecological applications, especially during the past decade, when computer-based simulation models became increasingly popular. Much of the debate around the choice of model type for specific purposes has focused on individual-based versus matrix models (see e.g., Stephens et al., 2002; Topping et al., 2005; Hilker et al., 2006; Sable and Rose, 2008), and a series of arguments are traditionally raised in favour or against the two types of models. Among the most popular arguments in support of IBMs are the facts that the individual-based approach can simulate thousands of individuals, keeping track of their traits such as size, age, sex, and location. The equations and rules that define the behaviour of individuals in the model depend on the state of the individual itself, other nearby individuals, and environmental conditions (Grimm and Railsback, 2005). Individuals can differ from one another in their state variables, interact locally with each other, and, in spatially explicit applications, they move within the model arena (Tyler and Rose, 1994). Density-dependent growth, mortality, and reproduction emerge from the collective outcome of individual processes, rather than having to be explicitly defined a priori by the model developer (Sable and Rose, 2008). Disadvantages of IBMs are that they often require large

amounts of data, need customized computer coding, and produce large amounts of multivariate output that is often hard to validate and interpret (Grimm, 1999). They are also considered less "transparent" than more traditional modelling approaches. In contrast, matrix models track the numbers of individuals in a series of age or stage classes that comprise the life cycle of the population of interest, treating individuals in the same class as identical average individuals (Caswell, 2001). Some other traditional arguments that support matrix models are that they are relatively easy to construct, make use of readily available demographic data on survival, growth, and reproductive rates, and have been widely used in ecology because they are mathematically tractable and can be easily solved numerically (Dixon et al., 1997). Equilibrium (eigenvalue) analysis of matrix models generates many useful metrics of population dynamics, such as population growth rate, stable age or stage distribution, and elasticities of life-cycle traits (Forbes et al., 2001). The disadvantages to matrix projection models are that they do not easily permit to record different conditions experienced by individuals during their life history, focus on population dynamics, thereby not allowing to implement community and food web effects, and density-dependent relationships must be defined as part of the model development (Sable and Rose, 2008).

Drawing from my experience developing the ecological models presented in this thesis, I found that, for these specific models, some of the above-mentioned arguments are not true. For instance, the types of data needed to parameterize the IBM were as easy and in some case easier to produce compared to what was needed for a full parameterization of a matrix model that included the same processes. In general one can say that IBMs require more data because they often include more complexity, whereas comparing two equally complex models, the gap in data needs is not very wide. Furthermore, the type of demographic data necessary to parameterize a matrix model are not abundantly available in the literature with a sufficient degree of detail when it comes to effects of chemicals. Also, when available, there is often a general bias towards small and short-lived species that can be cultured in a laboratory. A similar view on the parameterization of simple and complex ecological models has been reached by Topping et al. (2005). In this study they concluded that, while their IBM is very data intensive in respect to description of landscape, agronomy and wildlife behaviour, mortality, fecundity, density dependence and stochasticity are all emergent properties. The life-history model they used, on the other hand, required to specify the vital rates appropriate to the new scenario and therefore made different demands on data, not necessarily easier to fulfil.

The issue of choosing the correct model type, however, is especially important when a model is developed for regulatory purposes. In this case, the model has to be understood and run by a number of users that are not modellers themselves: therefore it is essential to understand how much complexity is necessary to answer a regulatory question and to find a trade-off between standardization and flexibility of model structure.

As reported in **Chapter 2**, stakeholders involved in ERA of chemicals have contradicting expectations about ecological models in support of the decision-making process. Models are supposed to be simple and user-friendly enough to be easily understood, parameterized, and used in a standardized way. At the same time, however, they should be complex enough to be realistic and capable of capturing a wide range of ecological scenarios. Thus, in order to clarify what can be expected from a specific type of model in terms of its contribution for improving estimates of risk, the costs and benefits of additional complexity for ERA procedures need to be demonstrated by contrasting simple and complex models more often than it is currently done,.

In **Chapter 5**, the comparison of a matrix metapopulation model and the IBM presented in **Chapter 3** showed that, if the endpoint of interest is population-level effects of homogeneous soil contamination, the added complexity of the IBM is not necessary, as its predictions are very close to the simpler metapopulation model projections. Nevertheless, simulations with heterogeneous contamination showed a lack of consistency between the RAMAS model and the IBM predictions. In particular, at lower concentrations and percentages of contaminated area, the RAMAS model results are close to outcomes of the IBM with avoidance behaviour, whereas at higher concentrations and percentages of contaminated area, they are closer to IBM results without avoidance. Avoidance behaviour is not a standard, routinely measured endpoint for collembolans, and therefore for most chemicals it is not known whether they are avoided or not. This decreases the confidence in the RAMAS model predictions of population-level toxic effects, because in some cases the model overestimates (when the compound is avoided) and in other cases it underestimates (when the compound is not avoided) the risk. The flexibility of an IBM, which allows exploration of both scenarios, gives a better overview of how populations in the field are likely to be affected by a contaminant under different conditions. Furthermore, the RAMAS model was found to be less sensitive than the IBM in detecting population-level effects of different spatial patterns of exposure.

A number of other studies dealing with the issue of simplification and aggregation of complex models in various ecological applications, such as conservation biology (Akçakaya, 2000) and invasive species management (Nehrbass and Winkler, 2007), are available in the scientific literature. Findings of these studies suggest, as do the results presented in **Chapter 5**, that when individual variability and behavioural responses are likely to influence the outcome of a model, it is better to use an individual-based approach over a matrix one. For instance, Nehrbass and Winkler (2007) developed an IBM based on the same data set as a matrix model previously developed. They found that the two models had opposite outcomes in predicting the spread of an invasive plant species. The authors identified individual variability as the main cause of these results. Stephens et al. (2002) compared the ability of different matrix and individual-based model implementations of alpine marmot populations to reproduce observed behavioural responses and

population abundances and variations. One of these models, a spatially-explicit individual-based model that ignores behavioural aspects, proved to be highly unrealistic, as it predicted equilibrium densities significantly different from observed values. All models were also used to predict potential density-dependent effects on alpine marmot population growth, with very different results. The authors concluded that different models were useful for different purposes. While the simplest matrix model was adequate to predict equilibrium population sizes or densities, for predictions requiring an understanding of transient dynamics only the behavioural model was adequate. As Stephens et al. (2002) point out, how much ecology is included in an ecological model matters for some questions we want a model to answer, but not for others.

Therefore, based on the results presented in **Chapter 5**, I argue that the choice of model type to be used in risk assessment of chemicals should be based on the specific regulatory question, rather than on generic issues of model complexity. In fact, the study presented in **Chapter 5** showed that by describing the two models following the same template makes them equally transparent and understandable, despite their different complexity and structure.

6.3 Ecological models and risk assessment

In recent years, great interest has been shown towards the use of ecological models, and several publications have promoted its use (Forbes et al., 2008; Schmolke et al., 2010a; Schmolke et al., 2010b; Thorbek et al., 2009).

The main advantage and selling point of using ecological models is the meaningful extrapolation of laboratory toxicity data, which are usually generated under constant and optimal conditions, to different exposure regimes, and to longer temporal scales than it is possible to test empirically. In **Chapters 3** and **4**, for example, I showed how it is possible to extrapolate effects of different spatial distributions of the toxicant (Fig. 3.6) and of different level of heterogeneity in the concentrations (Fig. 3.8) to the population-level under constant conditions (food and temperature). Predictions of population-level effects under a more complex exposure scenario are instead shown in Fig. 4.6, where recovery after different series of disturbance events is investigated over two consecutive years of simulations.

Furthermore, the inclusion of more ecological processes into standard ERA procedures has often been recommended (Van Straalen, 2003; Van den Brink, 2008), and ecological models are among the tools that are mostly mentioned as capable of achieving this goal. The ecosystems services framework has especially gained momentum in recent years as a basis for environmental management and offers promise as a valuable tool for setting meaningful ecological protection goals (Millennium Ecosystem Assessment, 2005; Nienstedt et al., 2012). Ecological models are

the only practical tool currently available to link measurement endpoints, obtained from standard laboratory tests, to relevant protection goals defined within this framework (Galic et al., 2012; Forbes and Calow, 2012).

Ecological models are also very suitable for comparing outcomes of alternative scenarios (Galic et al., 2012). A way to exploit this resource within the decision-making process for pesticide authorization is to use ecological effect models as a management tool, to explore the effects of different prospective risk mitigation options. For instance, ecological models with a highly flexible structure, such as IBMs, allow testing of the effects of different scenarios in terms of number and modes of application, or different buffer zone (i.e. unsprayed) areas.

An example of such a test is presented in **Chapter 4**, where the IBM was used as a hypothesis testing tool to simulate long-term effects on *F. candida* populations of different combinations of theoretical disturbance events and patterns of spatial aggregation of the model contaminant. In this exercise, each disturbance event is assumed to represent the application of a pesticide that has a strong acute effect but short degradation time. Looking at the model results one can argue that, as a measure of risk mitigation, controlling the spatial aggregation of the resulting soil contamination is more effective that reducing the application area without controlling for spatial aggregation (**Chapter 4**, Fig. 4.6b and c). The disturbance patterns tested in this modelling exercise are very generic, but scenarios could easily be refined to be more realistic if necessary for regulatory application.

Despite the increasing recognition of their potential, mechanistic effect models are not yet widely used in regulatory risk assessment because most stakeholders involved do not know how and when to trust such models. As reported in **Chapter 2**, this lack of trust is largely due to the lack of transparency in the way models are presented and, most importantly, the lack of guidance on what type of models to use for different kinds of questions. A major bottleneck in establishing trust in models is thus to provide tools for standardized testing and documentation of ecological models, following good modelling practice. Examples are the ODD (Overview, Design concepts, Details) protocol (Grimm et al., 2006; 2010) and the framework for transparent and comprehensive ecological modelling (TRACE) documentation (Schmolke et al., 2010a).

The use of ecological models to support environmental decision making processes has not always been successful, as some studies demonstrate (Hall, 1988; Comiskey et al., 2004; Gross, 2005; Pilkey and Pilkey-Jarvis, 2007). Failures in previous attempts in using ecological models for environmental decision making are probably the result of too much reliance on predictive abilities of the models and of flawed assumptions or incorrect parameters, which the lack of transparency in model descriptions did not allow to detect. These failures have likely contributed to the current lack of confidence shown by stakeholders involved in chemicals risk assessment.

The evidence provided in **Chapter 5** shows a possible way to increase trust in mechanistic

effect models with regard to model choice and transparency. As every model type has its own pros and cons (Schmolke et al., 2010a), and it is impossible to combine all pros in one single model, developing and using two types of models for the same question can increase confidence in predictions of risk generated by the models and understanding of the factors that influence the system. Referring to the case-study presented in **Chapter 5**, the simpler model helps to verify the more complex one and can be used for more homogeneous exposure patterns. The more complex model helps to detect and understand the limitations of the simpler model and is needed to ensure ecological realism for more complex exposure scenarios.

6.4 Final conclusions and outlook

Ecological risk assessment has been going through significant changes during the last decade (Van den Brink, 2008). As human pressures on the environment increases and new and more sensitive measurement tools are developed to detect chemicals in the environment, the risk assessment of these chemicals is adopting novel methods, including ecological effect models, for estimating risks.

The present thesis, as a case study for the application of ecological effect modelling to ERA, dealt mostly with the issue of spatial heterogeneity in soil exposure for collembolan populations. Results presented in **Chapters 3, 4** and **5** showed that disregarding spatial heterogeneity, as is the case in current ERA procedures for terrestrial ecosystems, may lead to an overestimation of risk if homogeneous contamination is assumed when this is not the case. More generally, these results suggest that a more realistic exposure assessment can significantly influence estimates of risk for soil organisms. As a way of improving the link between exposure and effects, and thus obtain more precise estimates of risk, avoidance behaviour should be included as a standard endpoint, considered the great influence its inclusion or exclusion had on model results. The implementation of avoidance behaviour tests as screening tools in ERA has already been supported by several studies (e.g. da Luz et al., 2004; Loureiro and Nogueira, 2005), and in combination with classical ecotoxicological tests can increase the ecological relevance of effect characterization (Aldaya et al., 2006).

Another observation that can be made from the results produced by the models I developed, and which can be of particular relevance for the risk assessment for soil invertebrates, is that *Folsomia candida* does not seem to be a particularly vulnerable species. This is mainly due to its fast population growth rate. Therefore it might be worth using another species in combination with *F. candida*, or developing ecological models not specifically parameterized for *F. candida*, but instead simulating a generic collembolan species with lower reproductive and growth rates than *F. candida*, to make sure that the more vulnerable species are actually covered by the assessment of risk.

In conclusion, time seems to be ripe for the stakeholders to get together and develop a strategy to include ecological models in ERA, as well as guidance document on how to use them, as this seems the only way to proceed (**Chapter 2**). Ecological models have proven to be a useful tool to extrapolate effects of chemicals from the individual- to the population-level, and to add ecological relevance to ERA; therefore it seems only reasonable to now put them to good use.

6.5 References

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Appendices

Appendix 1

Interview guide. All interviews were semi-structured and kept as open as possible. The guide was used as a framework for each conversation.

1. Introduction/opening questions
   - What is your involvement in risk assessment/management?
   - What are your responsibilities?
2. Networking/relationship with other stakeholders
   - Who are your main partners?
3. Risk assessment/management
   - What do you see as the biggest strength and the biggest weakness of the current risk management practice?
   - What are the most important consequences of overestimating and underestimating the pesticide risk?
4. Protection goals
   - What is it that risk assessors are trying to protect?
   - What are the protection goals?
5. Ecosystem services [1]
   - Are you familiar with the ecosystem services concept?
   - Is it useful for the development of protection goals?
6. Models' familiarity
   - Do you use models in your work?
   - Do you use models to predict effects?
7. Attitude towards models and modeling
   - What do you think of model's use in risk assessment?
8. Criteria – expectations
   - What criteria does a model need to fulfill to be used?

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1 This theme has been added later during the course of the study, hence used only in 30 interviews.

- What prevents people from using ecological models?

9. Policy/regulation change
   - What changes in pesticide risk policies have you observed in recent (10) years?
10. Societal trends behind policy changes
   - What is, in your opinion, a general drive for these changes?
   - What values, in your opinion, influence pesticide risk policy?
11. Relevance/sufficiency of regulations
   - Are you satisfied with current pesticide risk regulations?
   - Had you an opportunity, what would you change in risk regulations?
12. Stakeholder involvement
   - Who, in your opinion, should be given a voice?
13. Risk communication
   - Any room for improvements?
14. Transparency of risk assessment
   - Do you think there is anything in the current risk assessment that is not transparent enough?
15. Risk perception: public vs. experts
   - What is your perception of pesticide risk?
   - What does "general public" think of pesticides in your opinion?

Appendix 2

TRACE documentation for the individual-based model

1 Model development

1.1 Problem formulation

The model is designed to estimate the effects of toxicants on collembolans at the population level, and will be used for hypothesis-testing and for evaluating and improving standard ecotoxicological tests based on the modelled species, *Folsomia candida* (Willem 1902).

The purpose of the model is to investigate the effects of spatial heterogeneity in soil contamination on the population dynamics of *Folsomia candida*. *F. candida* has been used extensively as a model arthropod in many ecological and evolutionary studies. Moreover, it is used as a standard test organism for toxicity tests: a 28-day reproduction test (ISO 11267, 1999; OECD 232, 2009) is included in the refinement options for ecological risk assessment of plant protection products to soil organisms. However, one of the limitations of virtually all standard toxicity tests with soil organisms is that soil contamination is assumed to be homogeneous, whereas the heterogeneous nature of soil is well known. Spatial heterogeneity in soils occurs at widely different scales, from continental and regional to micro aggregates within specific soil horizons. Moreover, contamination of soils is heterogeneous as well because the distribution of chemicals in soil depends on the source of contamination (i.e., point vs. non-point source) and on specific soil properties that result in different interactions between chemicals and soil particles. The ability of *F. candida* to sense and avoid contamination in soil is known and currently being used to develop a guideline to establish a standardized avoidance test. This model simulates the avoidance behavior of *F. candida* and the effect of heterogeneously contaminated soil on population dynamics.To obtain a more comprehensive understanding of how behavioral responses such as avoidance affect population dynamics, population structure, and distribution of individuals in soils with heterogeneous contamination, population models can help to overcome the logistical constraints of short-term laboratory experiments.

The model is built using data related to the effects of copper sulphate, and therefore model predictions can be considered valid to gain insight into the population dynamics of springtails only for heavy metals. To extend its validity to other classes of compounds with different environmental behaviour, it would be necessary to implement degradation processes, and make the individuals' exposure dependent on the varying toxic concentration.

1.2 Design and formulation

The modelling approach that has been chosen is Individual-Based (IBM): the question the model is intended to address involves heterogeneity in space, which is easier to implement in an IBM formulation

than in other modelling approaches. The model described in this document is spatially explicit and, although not too simplistic, is rather simple. It does not include a comprehensive simulation of all the dynamics of collembolan populations, but only the most important processes which contribute to explain the avoidance behavior of *F. candida* and the consequences of a heterogeneous contamination of soil at the population level are represented. The main assumptions about the described system apply to some of the population dynamics: in order to keep the model simple, behaviours not strictly related to avoidance, such as inter-specific competition, predation, etc., have not been implemented.

However, the model, incorporating realistic information on behaviour and life history, is designed to represent *F. candida* realistically enough to be used for evaluating and improving standard ecotoxicological tests based on this species.

1.3 Model description

This model description follows the ODD (Overview, Design concepts, Details) protocol for describing individual-based models (Grimm et al., 2006; Grimm et al., 2010). The model was implemented in NetLogo5.0 (Wilensky, 1999), a free software platform for implementing individual-based models.

Entities, state variables and scales.

The model includes three kinds of entities: eggs, female springtails (juveniles and adults), and grid cells they live on. Eggs are immobile and are characterized by age and position. Springtails are represented as mobile individuals with state variables for their age (in days), position (continuous coordinates), direction for movement, energetic status (days-to-death), cumulative distance walked in each hourly time-step (which affects the energy used for the movement), and time spent on contaminated grid cells.

Grid cells are characterized by the following state variables: food level and concentration of toxicant (mg/kg soil). The model world is two-dimensional. Each cell of a 100x100 square grid represents a square patch of soil of 1 cm^2. All processes proceed on daily time steps, except for foraging including movement, which proceeds on time steps of one hour.

The global environment is characterized by six "seasons" (spring and fall are divided into "early" and "late"), which determine the temperature-dependent life-cycle parameters of the springtails: four different parameters sets are implemented, reflecting the temperature ranges 0-5°C (winter), 12-15°C (early spring and late fall), 19-21°C (late spring and early fall) and 24-26°C (summer).

Process overview and scheduling.

Each of the following processes are run, in the given order and by the entities given in parentheses, once per day, except for the foraging procedure, which is executed at hourly time-steps. The order in which the model entities are processed is randomized each time step; state variables are updated immediately. If no executing entity is given, the process is run by the program, or "observer" (Wilensky, 1999). The submodels

representing the processes are described in all detail in Section "Submodels".

Seasons: At the begin of a new season, individuals get a new set of life-cycle parameters, whose values reflect the change in the temperature range.

Foraging (springtails): This process includes rules that individuals follow to move to look for food, but also to avoid contaminated patches of soil.

Re-growth of food (grid cells): When the amount of resource on a food cell is depleted, it is restored at the beginning of the next day.

Aging/growth (springtails): Age is increased by one day. Based on the age, the hatching time and the maturation time, springtails are divided into three stages: eggs, juveniles and adults. When an egg hatches, its age is set to 0.

Reproduction (springtails): Springtails may reproduce when they reach maturity, and afterwards reproduce according to the values of the parameters "time between broods" and "number of broods".

Hatching (eggs): Eggs hatch according to their viability when they reach an age equal to the hatching time. Hatching success depends also on the concentration of toxicant of the grid cell on which the eggs are laid.

Density-dependence and starvation effects (springtails): Fecundity of springtails is reduced when they experience high population density on their grid cell, due to jostling effects. If they do not feed, their energetic status decreases, with consequences on fecundity and survival. Because reproduction needs energy, and *F. candida* do not lay eggs while they are feeding, this procedure is scheduled so that first they look for food and only afterwards check for local population density.

Mortality (springtails): Two different rules, based on survival parameters, are implemented for juveniles and adults. Besides a background rate of mortality, survival depends also on the concentration of toxicant and the amount of time the organism spends on polluted patches.

Update output: The last action executed at daily intervals is an update of model outputs, i.e. plots are updated as well as summary statistics.

Design concepts.

Emergence. Population dynamics and the spatial arrangement of individuals emerge from the behaviour of single organisms, their interactions with each other and their habitat: population dynamics are regulated by the number of reproducing individuals, which themselves depend on population density and the amount of food resources. Life cycle, reproduction, and survival rates are partly imposed via empirical rules and parameters, partly they emerge from the movement path taken by an individual, which will differ among individuals and in terms of contamination, density and resource availability experienced.

Stochasticity.Values of almost all parameters are drawn from uniform or normal probability distributions, in order to reflect heterogeneity among individuals (Table 1). Stochasticity is also used for initializing springtails' starting positions, as well as causing individual behaviour of individuals (movement, reproduction, hatching, mortality) to occur with a specified frequency.

Sensing. Individuals sense the amount of food and the presence of other individuals within a defined distance, as well as whether or the grid cell they are on, or which is ahead in their direction of movement, is contaminated.

Adaptation. Individuals try to optimise their fitness by preferentially selecting cells with high-food resources and by avoiding both cells occupied by too many other individuals and too high contamination levels.

Interaction. Individuals compete for food and space; competition is assumed to be of scramble type.

Observation. Size and structure of the population and spatial distribution of the individuals for different concentrations of toxicant, food resource amounts and distributions are compared.

Table 1. Parameters and values used in the Folsomia candida *model.*

Parameter	Units	Temperature (°C)	Distribution	Value	References
Maturation time: time to reach adulthood (matur_ time)	Days	12-15	Uniform	30-40	Milne, 1960
		19-21		13-29	Snider, 1973
		24-26		11-30	Marshall and Kevan, 1962
Hatching time: time needed for the eggs to develop and hatch to juveniles (hatch_time)	Days	5	Uniform	90	Milne, 1960
		12-15		13-19	Milne, 1960; Fountain and Hopkin, 2005
		19-21		7-15	Marshall and Kevan, 1962
		24-26		7-9	Milne, 1960
Number of eggs per brood, general value for the season (nr_eggs_season)	Number	12-15	Uniform	19-98	Milne, 1960;Snider, 1973; Grimnes and Snider, 1981
		19-21		30-50	Fountain and Hopkin, 2005
		24-26		26-68	Snider, 1973; Green, 1964b
Nr of broods per female: max number of reproductive events (max_num_repr)	Number	12-15	Uniform	9-16	Milne, 1960;Snider, 1973; Grimnes and Snider,1981
		19-21		3-20	Snider, 1973
		24-26		4-6	Snider, 1973; Green, 1964b
Time between broods (repr_interv)	Days	12-15	Uniform	13-15	Snider and Butcher, 1973
		19-21		6-16	Marshall and Kevan, 1962
		24-26		11-13	Marshall and Kevan, 1962
Egg viability: percentage of eggs that successfully hatch (egg_viab)	Number	12-15	Normal	Mean 94.50% S.D 5%	Snider and Butcher, 1973
		19-21		Mean 92% SD 5%	Snider and Butcher, 1973
		24-26		Mean 81% SD 9%	Snider and Butcher, 1973
Juvenile survival., expressed as probability to survive until age at maturity (j_surv)	Number	12-15	Normal	Mean 98% SD 2%	No reference for this temperature; value has been derived from other temperatures
		19-21		Mean 95% SD 2%	Marshall and Kevan, 1962
		24-26		Mean 83.30% SD 2%	Snider, 1973

Parameter	Units	Temperature (°C)	Distribution	Value	References
Adult survival., expressed as the age of death of the individual (a_surv)	Days	12-15	Normal	Mean 241 SD 50	Snider and Butcher, 1973
		19-21		Mean 140 SD 25	Snider and Butcher, 1973
		24-26		Mean 73 SD 26	Snider and Butcher, 1973
Probability to reproduce at every reproductive instar (repr_probab)	Number	12-15	Uniform	96 - 100%	Milne, 1960; Snider, 1973; Grimnes and Snider, 1981
		19-21		95 - 99%	Milne, 1960; Snider, 1973; Grimnes and Snider, 1981
		24-26		94 - 98 %	No reference for this temperature; value has been derived from other temperatures
Distance within which food and conspecifics are sensed	Cm	Independent from temperature	Constant	2.5	Auclerc et al., 2010
Energy level (energy)	Days-to-death	Independent from temperature	Constant	Initial values Max: 30 Min: 0	Final values determined by calibration
Energy reduction per time-step (en_reduce_hour)	Days-to-death	Independent from temperature	Constant	Initial value 0.042	Final value determined by calibration
Energy gained by food intake (food)	Days-to-death	Independent from temperature	Constant	Initial value 0.5	
Energy reduction per step moved (en_reduce_mov)	Days-to-death	Independent from temperature	Constant	Initial value 0.01	
Probability to move at each time-step (probab_mov)	Number	Independent from temperature	Constant	Initial value 0.1	Final value determined by calibration
Maximum energy spent for foraging at each time-step (tradeoff_mov)	Days-to-death	Independent from temperature	Constant	Initial value 0.2	
Tradeoff between energy and reproduction (tradeoff_repr)	Days-to-death	Independent from temperature	Constant	Initial value 20	
Maximum energy spent for avoiding high density at each time-step (tradeoff_dens)	Days-to-death	Independent from temperature	Constant	Initial value 0.1	

Initialization.

A simulation starts the first day of the year, and therefore in the winter season. Usually, 5% of the grid cells, which are randomly chosen, are made food cells. Simulations start with 10 randomly distributed juvenile springtails; values for their state variables are drawn from the distributions reported in Table 1.

Input data.

This model has no time-series inputs or external environmental drivers.

Submodels.

All parameters, their meaning, range of possible value, and source for parameterization are listed in Table 1.

Seasons: At the begin of a new season, individuals get a new set of life-cycle parameters, whose values

reflect the change in the temperature range. Individual variability is represented by independently drawing, for each individual, parameter values from a certain interval (Table 1). When the temperature is too low, springtails are inactive. Joosse and Testerink (1977) observe that below 10°C the percentage of *Orchesella cincta* individuals in a fed state is significantly lower, while Takeda (1984) report that in a population of *Folsomia octoculata* overwintering adults are in an immature state, and they become mature with the stimulation of increasing temperature. Verhoef (1996) notes that during the winter period nearly all the adults of the collembolan *Anurida maritima* die, and it appears that this is due to starvation caused by low locomotor activity in situations of low temperature. Therefore during the time interval corresponding to winter, individuals in the model do not execute any actions except for aging and mortality.

Foraging: This submodel includes rules that individuals follow to move to look for food, but also to avoid contaminated patches of soil. This submodel is comprised of two parts:

First, organisms check whether they are on a contaminated grid cell. If one of the neighbouring cell's concentration is lower, than they move on it with a chance equal to their avoidance probability, which is proportional to the toxicant's concentration (Table 2).

The second part of the submodel contains rules for feeding, and it is executed with a frequency determined by a probability of movement, which is proportional to the olfactory stimulus (amount of food present within the range of perception), and takes into account periods of inactivity. The reason is that movement is triggered by the reduction of the energy level: moving can be dangerous (for example it increases the risk of predation), but on the other hand if the organism does not look for food it will starve. From experimental observations reported in the literature, it is known that collembolans go through periods of inactivity (i.e. they do not move and do not feed), for instance during the moulting process (Joosse and Testerink, 1977; Marshall and Kevan, 1962). Therefore, in order to account for these periods of inactivity, individuals in the model do not move at each time-step, but accordingly to a given probability (probab_mov), which is proportional to the amount of food sensed by the individual (i.e. to the strength of the attractive olfactory stimulus), with a minimum value when the organism does not sense any food; the maximum value for probab_mov is twice the minimum. The value for minimum probab_mov was determined via sensitivity analysis and pattern-oriented parameterization, but has initially been set to 0.1. While springtails forage, they decrease the stock on the food cell on which they are feeding by one food item. The probability of movement is calculated as:

$$probab\_mov = 0.1 * \left(1 + \frac{\text{mean } amount\ of food\ on\ food\ cells\ within\ sensing\ range}{\text{maximum } amount\ of\ food\ initialized\ on\ a\ food\ cell} \right)$$

The foraging submodel is described below using pseudo-code. The rationale for each part of the code, the values of parameters involved and the equations used are explained below (references in square brackets).

Pseudo-code:

```
for all springtails
   if current cell is contaminated and concentration  on one of the neighbouring
   cells is lower
```

```
      move towards it according to p_avoid
   if current energy reserve is below energy_max – 24*en_reduce_hour
      if any food patches in a 2.5 cm radius and if total food in a 2.5 cm
      radius is at least 1 food item
         Set movement probability dependent on average food in 2.5 cm radius [1]
      else
         Set movement probability to minimum movement probability
      While no food found and energy spent for foraging (nr steps moved *
      en_reduce_step) is below threshold (tradeoff_mov) [2]
         if food on current patch is at least 1
            Eat
         if no food on current patch and food on one of the grid cells in the
         semicircle of radius 2.5 cm the individual is facing to contains more
         food than 1
            Turn towards one of these grid-cells [3]
         else
            Turn randomly by 0-359°
         if cell ahead 1 cm is contaminated
            Move towards it according to p_avoid [4]
            Update exposure counter
         else
            Move towards one of the uncontaminated neighbour cells
         Calculate energy loss due to movement
   Update energy reserves: old value plus food intake minus energy loss
Update grid cell variable "local_density" for all grid cells.
```

[1] The energetic level of every individual after hatching is maximum, and at every tick this value is reduced, to take into account the energy expenditure for all the vital functions. Values for the parameters related to the energetic status of the individuals have been indirectly estimated from the literature, and are expressed in terms of number of days an individual could survive without feeding. These parameters have then been refined via sensitivity analysis and pattern-oriented parameterization.

Tully and Ferriere (2008) observe that survival of *F. candida* offspring is affected by dietary and crowding conditions: the mortality rate is multiplied by 12 under high density and starvation, and that during periods with low food conditions the reproductive investment is low. Booth and Anderson (1979) observe that after 10 weeks of starvation, about 50 % of the organisms in the cultures are still alive. They however also note that the culture dishes could not be kept perfectly sterile, and small fungal growths were occasionally observed which could be grazed. Furthermore, Smit et al. (1998) report that although a natural soil was used during their experiment, in the treatments where no food was added, food naturally present in soil (fungi

and nematodes) was insufficient for *F. candida* to reach maturity. Therefore the initial values of energy levels and living costs have been chosen so that organisms could theoretically survive 30 days without food, which it has been assumed to be a good estimation of real conditions.

Besides survival, the energetic status influences also the fecundity of an individual. It is known from the literature that if the organisms are starved the size of egg clutches is reduced (Usher et al., 1971; Booth and Anderson, 1979). In the model the initial assumption is that they stop reproducing if they do not feed for 10 days, and the number of eggs laid decreases exponentially with the energy level.

[2] Individuals keep repeating these actions until the two conditions are met: when they find food or the energy spent for moving during the current time-step passes the threshold, they exit the foraging procedure. Initial values of the parameters involved in this process are: 0.5 for the energy gained by food intake (en_gain_food), 0.2 for the maximum energy an individual can spend during one time-step to look for food (tradeoff_mov), while for every step moved, the cost in terms of energy has initially been set to 0.01 (en_reduce_step).

[3] If organisms sense food they move towards it, otherwise they move randomly; according to Auclerc et al, 2010, the average maximum distance at which *F. candida* can detect food is 2.5 cm. Organisms move 1 cm at a time; movement costs energy, and they keep moving until they find food or as long as their energy balance allows it. This balance, for the hourly time-step t, is calculated as:

$$energy_t = energy_{t-1} + food - energy\ loss$$

Where energy loss is proportional to the distance the organism has moved.

[4] While the organism is looking for food, before it moves, it also checks if the patch towards which it is directed is contaminated: in this case, according to its probability of avoidance (p_avoid), it can turn in another direction or walk on the contaminated patch. The probability to avoid different copper concentrations has been calculated from Boiteau et al. (2011) data (Table 2). If the organism walks on a contaminated grid cell, a toxicity counter is increased; it is assumed that the whole time step (1 hour) is spent on the polluted patch.

Reproduction, density dependence and starvation effects: Springtails reproduce when they reach maturity, and afterwards reproduce according to the values drawn for the parameters determining the time between broods and number of broods. Individuals have a certain probability to reproduce at every reproductive instar (Table 1), which is drawn from a specified distribution for every season, and lay a predetermined number of eggs, which depends not only on the temperature but also on the local density (i.e. number of organisms on the same patch) and the energy level of the organism. As shown by Green (1964a), fecundity of springtails is reduced when they experience high population density, due to jostling effects: the effect of crowding upon fecundity has been calculated as an exponential function (Table 2) that interpolates Green (1964a) data. The same type of mathematical relationship has been assumed to exist between energetic status of the organism and number of eggs laid (Table 2). Besides fecundity, the energetic status affects also the survival of an organism: if energy level is below the minimum (energy_min), the individual dies.

The number of eggs laid also depends on the contamination experienced by the organism, in terms of concentration and time spent on a polluted patch: from literature data (Sandifer and Hopkin, 1996), a linear regression (Table 2) between concentration and reduction of fecundity has been calculated, and then a coefficient that takes into account the amount of time spent on a contaminated patch was applied.

F. candida can sense the presence of conspecifics (Leonard and Bradbury, 1984) and therefore they move to look for a less crowded area: in the model it is assumed that the range within which the olfactory stimulus of other organisms is perceived is the same as for food. This process - described using pseudo-code - and the rationale for it, are presented below.

```
for springtails with energy above tradeoff_repr and age above matur_time [5]
   if local_density on any cell in a radius of 2.5 cm is lower than local_density
   of current cell

      while local_density on any cell in the semicircle of radius 2.5 cm
      the individual is facing is lower than local_density of current cell,
      and energy spent for moving (nr steps moved * en_reduce_step) is below
      tradeoff_dens [6]

            Turn towards one of these grid-cells
            if cell ahead 1 cm is contaminated
               Move towards it according to p_avoid
               Update exposure counter
            else
               Move towards one of the uncontaminated neighbour cells
            Calculate energy loss due to movement
   Update energy reserves: old value minus energy loss
Update grid cell variable "local_density" for all grid cells.
```

[5] Stress caused by the presence of other conspecifics at the moment of oviposition influences the fecundity of *F. candida*, reducing the number of eggs laid. Organisms, therefore, try to look for less crowded cells. They do not reproduce if their energy level is not high enough.

[6] Individuals look for less crowded cells the same way as they look for food: if they can sense a lower density on another cell they move towards it, one cell at a time, until they do not sense any better cell or until the energy consumption reaches the trade-off value (initially set to 0.1). In the meantime, before they move, they also check if the patch towards which they are directed is contaminated.

Hatching (eggs): Eggs hatch according to their viability when they reach an age equal to the hatching time. Hatching success depends also on the concentration of toxicant of the grid cell on which the eggs are laid. From the data reported in Xu et al (2009), the concentration-effect relationship for the reduction of egg viability caused by copper has been derived (Table 2). When an egg hatches, it changes its status to "springtail"; age is set to 0, and energy level is set to maximum.

Mortality: Two different rules, based on survival parameters, are implemented for juveniles and adults.

Juvenile survival is implemented as the probability to survive each day until maturation:

$$probability\ to\ survive = \left(juvenile\ survival \right)^{1/maturation\ time}$$

Adult survival is implemented via the age of death: every organism, when it hatches and again when the season changes, draws a value for this parameter from a normal distribution, which is different for every season of the year, and every day it checks if its own age is still below this value, otherwise it dies.

Survival is reduced by exposure the toxicant: from literature data (Sandifer and Hopkin, 1996), a linear regression between the logarithm of the concentration and reduction of survival (where 0 equals no reduction, 1 equals no surviving organisms) has been calculated (Table 2) and applied to both juveniles and adults. To account for the fact that the toxicity data used for this regression are the result of 28 days exposure to homogeneous contamination, it has been corrected by the ratio of the toxicity counter (number of hours spent on contaminated patches) and the number of hours in 28 days, i.e. 672 hours. When the toxicity counter of an individual is greater than 672, this coefficient is set to one.

Table 2. Equations for the linear regressions used in the model.

Independent variable	Dependent variable	Regression	R^2	References
ln concentration	Reduction of survival	y = 0.0824x – 0.1366	0.847	Sandifer and Hopkin, 1996
ln concentration	Reduction of fecundity	y = 0.2189x – 0.8743	0.919	Sandifer and Hopkin, 1996
ln concentration	Nr of hatched eggs (Normalized to the control)	y = -0.2243x +1.8893	0.932	Xu et al., 2009
ln concentration	Percentage of avoidance	y = 5.7475x – 1.4235	0.926	Boiteau et al., 2011
Local density	Normalized nr of eggs	y = 1.0637e-0.305x	0.942	Green, 1964a
Energy	Normalized nr of eggs	y = 0.01e4.6052x	1	Assumed

1.4 Parameterization

Parameter values used in the model(see Table 1) were taken directly from the literature or determined inversely by making the model reproduce several patterns observed in laboratory populations at different scales and levels of biological organization ("pattern-oriented modelling", Grimm et al., 2005; see Section 1.5). Calculations used to implement toxicant's effects in the model:

- *Avoidance behaviour*. For calculation of the percentage effect per concentration of the tested substance or per soil dilution (in case of contaminated natural soil), the number of springtails in the test soil is compared with the number of springtails in the control soil:

$$x = \left(\frac{n_c - n_t}{N} \right) * 100$$

where:

x is avoidance, expressed as a percentage;

n_c is the number of springtails in the control soil (either per vessel or in the control soil of all replicates);
n_t is the number of springtails in the test soil (either per vessel or in the test soil of all replicates);
N is the total number of springtails (either per vessel or in the control soil of all replicates).
This equation has been applied to the raw data reported in Boiteau et al. (2011) to calculate % avoidance at different concentrations (Table 3), which have then been used to calculate the linear regression used in the model (Table 2).

Table 3. Avoidance data for Folsomia candida *and copper sulphate (Boiteau et al., 2011)*

Concentration (mg Cu kg^{-1})	% animals in control soil	Nr animals in control soil (60 animals per concentration in total)	Nr animals in test soil (60 animals per concentration in total)	% avoidance (according to ISO method)
0	51	31	29	2
150	60	36	24	20
200	63	38	22	26
800	70	42	18	40
1600	70	42	18	40
3200	75	45	15	50

- *Reduction of survival and reproduction*. Sandifer and Hopkin (1996) measured the survival and reproductive output of 10 *F. candida* individuals exposed to increasing copper concentrations for 28 days. We normalized the numbers of surviving individuals and offsprings produced to the control (Table 4) and calculated linear regressions (Table 2).

Table 4. Experimental data for the effects of copper sulfate on reproduction and survival of Folsomia candida. *From Sandifer and Hopkin (1996) (pH=6, temperature=20°C).*

Concentration (mg Cu kg^{-1})	Survival			Reproduction		
	mean	std dev	Reduction of survival	mean	std dev	Reduction of fecundity
0	6,5	2,4	0	797	190,36	0
10	8,8	1,2	-0.060	1032	338	-0.295
40	7,5	2,4	0.096	801	92	-0.005
200	6	1,2	0.277	774	54	0.029
1000	5	1,6	0.398	291	92	0.635
3000	3	3	0.639	0	0	1

- *Reduction of hatching success*. Xu et al. (2009) measured the number of *F. candida* eggs (out of 20) that hatched after 10 days exposure to copper in soil (mean and standard error of mean foor four replicates). We normalized the numbers of eggs to the control (Table 5) and calculated a linear regression (Table 2). The number of eggs hatched at 100 mg kg^{-1} is not significantly different than the control; therefore for concentrations below 100 mg kg^{-1} in the model is assumed that eggs hatch with the normal viability.

Table 5. Experimental data for the effects of copper on hatching of 20 Folsomia candida *eggs exposed to different concentrations of toxicant. From Xu et al. (2009).*

Concentration (mg Cu kg^{-1})	Eggs hatched (mean)	Std error	Eggs hatched (normalized to the control)
0	19	0,71	1
100	17,5	0,65	0,921
200	13,8	1,31	0,726
400	7,5	1,71	0,395
800	7,75	1,65	0,408
1600	4,25	1,8	0,224
3200	2,5	1,04	0,132

1.5 Calibration

As shown in Table 1, for some parameters it was not possible to find values in the literature. These are all related to the energy level of individuals and their movement. Initial values have been indirectly estimated from observations reported in the literature. A sensitivity analysis (described in Section 2.2) was used to identify those parameters having the strongest effect on model output, and these were selected for calibration.

According to Wiegand et al. (2003), we used different patterns to determine unknown parameters using an inverse modelling approach. The central idea of pattern-oriented parameterization is to make the model produce multiple patterns simultaneously, so that the structural realism of the model is increased, i.e., the internal organization of the modelled system is more likely to be captured sufficiently for the intended purpose of the model. As an indicator of structural realism, model output is checked for secondary, independent predictions, i.e., system-level patterns observed in reality that were not used for model design or parameterization.

The following patterns have been used for model design and parameterization:

Pattern 1: Food-dependence (Usher et al., 1971). Three different observations describe this pattern: population growth with excess food, with marginally limiting food and with limiting food supply. Usher et al. (1971) observed that when food is not a limiting factor or is only marginally limiting, being supplied in proportion to population density, the establishment of an equilibrium population size is achieved, but the speed of establishment is proportional to the rate at which food is supplied, and population densities approach those reached with excess food. When the food supply is independent of density and limiting, equilibrium population size is reduced.

Pattern 2: Population growth rate and density dependent population size (Seifert et al., 1979).Microcosm experiments on *F. candida* run by Seifert et al. (1979) showed that population growth rates had decreased in all cultures before the termination of the experiments after 43 days, which indicated density-dependent effects. Estimates of exponential rates of increase were based on population increases from the 7th through the 31st day from the beginning of experiment.

The three observations that comprise the first pattern were used as filters to progressively exclude combi-

nations of parameter values: 10 replicate simulations with every combination of the relevant parameters within a range of ±20% around the initial value were run and then compared to the first observation (population growth with excess food) using chi-square statistics. The 20 best combinations were chosen, and the same procedure repeated for the other two observations (population growth with limiting and slightly limiting food). Sets of values that met all of the three observations were then used to simulate Pattern 2. Simulated ranges of final population size and exponential growth rate were compared to the observation from Seifert et al. (1979), and the parameter set which gave the best fit, in terms of overlapping ranges, was chosen. The resulting final parameter set was used in all subsequent simulations.

The final parameter set, after using patterns 1 and 2 as filters, was: energy_max = 30, energy_min = 4, en_reduce_hour = 0.0462, tradeoff_mov = 0.18 and probab_mov = 0.12. The outputs of simulations run with the best parameter set are compared to the data sets that comprise Pattern 1 and 2 in Tables 6 and 7 respectively.

Table 6. Pattern-oriented parameterization results: outputs of simulations with the best parameter set (average and 95% confidence limits: 10 replicates) are compared to the three observations in Pattern 1 (data from Usher et al., 1971).

Time (days)	Excess food				Slightly limiting food				Limiting food			
	Observed	Simulated			Observed	Simulated			Observed	Simulated		
		Mean	95% LCL	95% UCL		Mean	95% LCL	95% UCL		Mean	95% LCL	95% UCL
0	6	6	6	6	6	6	6	6	6	6	6	6
5	6	6	6	6	6	6	6	6	6	6	6	6
10	6	6	6	6	6	6	6	6	6	6	6	7
15	13	6	6	7	8	8	6	10	8	11	6	15
20	45	13	10	17	26	26	13	39	18	33	18	48
25	75	27	18	37	50	35	17	53	40	56	31	81
30	130	49	33	65	85	35	17	52	70	67	39	94
35	220	83	62	104	120	36	19	54	120	69	40	97
40	310	142	111	173	140	43	26	59	125	73	43	103
45	410	236	189	282	155	64	38	90	127	81	48	114
50	550	400	313	487	166	106	47	164	128	93	56	129
55	690	644	513	775	175	150	69	232	122	115	69	161
60	870	929	768	1090	185	177	80	275	140	135	77	192
65	1090	1211	1054	1367	195	185	93	277	170	156	91	221
70	1380	1430	1288	1572	210	203	115	292	200	186	112	260
75	1450	1552	1446	1657	230	264	171	357	230	217	143	290
80	1485	1579	1494	1665	260	337	236	438	260	235	167	304
85	1490	1579	1502	1657	290	383	292	475	285	256	193	319
90	1500	1577	1499	1654	340	423	355	492	305	268	212	325

95	1500	1577	1499	1654	400	445	388	502	325	270	210	329
100	1500	1577	1499	1654	430	453	394	513	340	275	208	342
105	1500	1577	1499	1654	440	456	394	518	365	279	207	351
110					460	461	402	520	390	293	216	370
115					500	466	408	523	420	299	222	376
120					550	466	409	523	455	296	227	366
125					610	468	409	523	500	289	228	350

Table 7. Pattern-oriented parameterization results: outputs of simulations with the best parameter set are compared to the observations in Pattern 2 (data from Seifert et al., 1979). The area of the simulation arena is the same as the vessels used in the microcosm experiment by Seifert et al. (1979). .

	Final population density (individuals/culture)		**Population growth rate (r)**	
	Mean	**Range**	**Mean**	**Range**
Observed	463,21	207,62 – 774,67	0,178	0,166 – 0,199
Simulated	548,6	442 – 670	0,163	0,158 – 0,175

2 Model testing and analysis

2.1 Verification

The structural realism of the model, i.e. its ability to make valid independent predictions, was tested.

The tests executed to verify the implementation of the model ranged from very simple checks using the instrument provided by the software platform, to more in-depth analyses. Tests included:

- syntax checking of the code
- visual testing through NetLogo interface
- print statements, i.e. inserting statements that write information out to the display or to a file so it is possible to see what is going on. Common use of print statements is to output the value of key variables at different times to help diagnose why a model behaves unexpectedly (Railsback and Grimm, 2012)
- spot tests with "agent monitors", i.e. opening a few NetLogo"agent monitors" and manually recording the value of the variables, calculating by hand how they should change, and then stepping the model through one iteration of its schedule and seeing if the change reported by the agent monitor matches the expectation (Railsback and Grimm, 2011)
- stress tests with extreme parameters values to expose errors that may be hidden under normal conditions
- test procedures, i.e. adding new procedures to the code just to produce intermediate output, used only for testing
- test programs, i.e. writing a separate short program that serves only to test aparticular algorithm or

procedure. This test has been executed on most of the submodels: for instance, to test the procedure for background mortality and toxicity-dependent survival, a test program has been written, where individuals do not do anything else but grow old and die. This makes it easy to record the proportion of individuals surviving during the simulation, and confront it with the theoretical survival curves. In the full model this would not be possible, as the organisms are reproducing and the number of entities in the model depends on both births and deaths.

- code reviews. The program has been checked by a reviewer to check for logical errors and other mistakes, and compare it to the model formulation.

2.2 Sensitivity analysis

As reported in Section 1.5, a sensitivity analysis was performed in order to explore the behaviour of the model in response to variations in the values of parameters that were not directly determined from the literature.

The initial values assigned to these parameters were used as a central condition. Subsequently, analysis was carried out by running multiple replicates of input parameter sets varied around this central condition. Parameters were adjusted independently to ±10, ±20, ±30, ±40, ±50 % of their central values. Linear and second order polynomial regressions were calculated between the relative changes in each parameter value and the two model outputs, final population size and average weekly population growth rate. For this analysis, 40 replicate simulations of 120 days were run for each parameter value, and, in order to simplify interpretation of the results, all simulations were run at a constant temperature interval of 19-21°C. All statistical analyses were performed using Systat ver. 13.0.

Among the parameters included in the sensitivity analysis, only those for which the regressions were statistically significant ($p < 0.01$) for both dependent variables were selected for calibration, i.e., energy maximum and minimum, metabolic rate, maximum energy spent to forage at each time-step and probability to move at each time-step (Table 8).

Table 8. Sensitivity analysis results.

	Final population size				Growth rate			
Parameter	**Adjusted R^2**	**First order coeff.**	**Second order coeffi.**	**Regression p-value**	**Adjusted R^2**	**First order coeff.**	**Second order coeff.**	**Regression p-value**
Energy_max	0.787	-534.84	-1,825.77	0.000	0.788	-0.015	-0.044	0.000
Energy_min	0.545	410.15	864.53	0.000	0.026	-0.006	-0.007	0.000
En_reduce_hour	0.816	-1,803.18	-1,778.81	0.000	0.772	-0.069	-0.150	0.000
Tradeoff_repr	0.045	85.71	-104.31	0.000	0.000	0.002	0.004	0.510
En_reduce_step	0.013	-25.22	-7.57	0.022	0.006	-0.001	0.001	0.109
Tradeoff_mov	0.494	-205.48	-230.54	0.000	0.387	-0.006	-0.007	0.000
Tradeoof_dens	0.273	-115.35	222.02	0.000	0.012	-0.001	-0.003	0.025
Food	0.000	-11.81	-14.89	0.227	0.000	0.000	0.002	0.293
Probab_mov	0.621	-282.91	-227.11	0.000	0.420	-0.008	-0.008	0.000

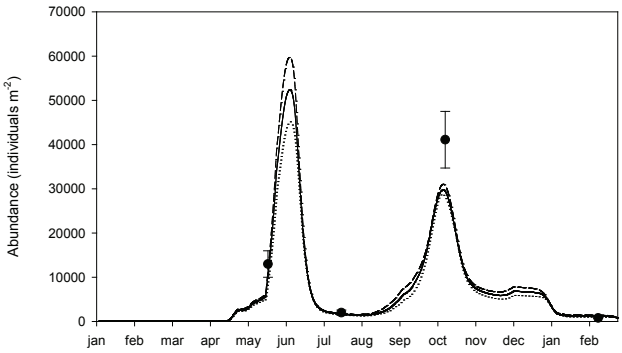

Fig. 1. Pattern 4: population abundance of F. candida in different seasons. Solid and dashed lines represent respectively mean and range of model simulations: dots represent Klironomos and Kendrick (1995) data.

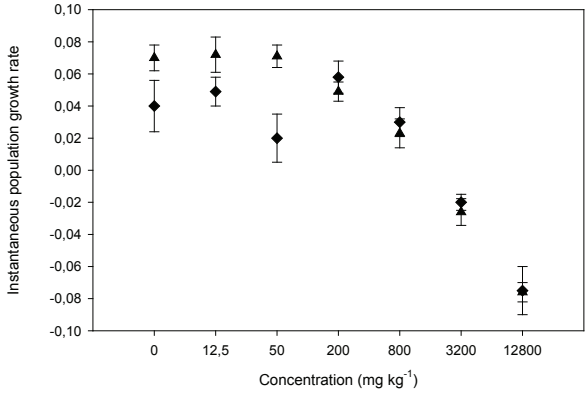

Fig. 2. Pattern 5: mean (±SEM, four replicates) instantaneous rate of population increase of F. candida exposed to different copper concentrations. Simulation results represented with ( ), Herbert et al. (2004) data with (♦).

2.3 Validation

For the comparison of model outputs with empirical data that were not used for parameterization or calibration three patterns have been identified from the literature, which have been numbered 3-5 to distinguish from the patterns used for calibration (1-2):

Pattern 3: Number of generations per year (Marshall and Kevan, 1962). The authors observed that in a greenhouse (constant temperature 22° C) *F. candida* can have as many as 12 generations per year.

Pattern 4: Seasonal variation in population size in the soil of a temperate forest (Klironomos and Kendrick, 1995). In this study, a 100 m^2 plot was set up in a sugar maple forest in Canada. The soil profile was divided into layers (i.e. litter (forest floor), 0-10 cm, 10-20 cm and 20-30 cm) and sampling was carried out four times throughout the year (May 1991, July 1991, October 1991 and February 1992) to account for seasonal variation. For comparison with the model, data for the litter layer were considered. Results of this survey showed that the highest population density was reached in October, with a relatively high peak also in May,

while in July and February population abundance was very low.

Pattern 5: Instantaneous rate of population increase (r_i) under copper contamination (Herbert et al., 2004). Soil concentrations of copper up to 12,800 µg g^{-1} were tested. Calculated ri values ranged from -0.086 (extinction) to 0.077 (in one replicate at 200 µg g^{-1}). The mean control ri was calculated as 0.041, although the authors noted that adult survival and juvenile production in the controls were lower than specified in the ISO guidelines. Copper significantly affected r_i with significant differences found between the control and treatment at concentrations of 3,200 µg g^{-1} and higher.

For Pattern 3, the mean number of generations produced during model simulations lasting one year at constant temperature range (19-21°C) was compared to the number of generations obtained in a greenhouse (Marshall and Kevan, 1962), also at constant temperature (22°C). The model output ranged from 11 to 13 generations per year, with an average of 11.6 compared to the 12 generations found by Marshall and Kevan (1962).

A comparison of the population abundance (individuals/m^2) predicted by the model with the data reported by Klironomos and Kendrick (1995) (Pattern 4, Fig. 1), shows a good fit for the data for spring, summer and winter, whereas the fall peak predicted by the model was lower. The highest peak in the simulated population abundance occurred in June, but since there were no data points for this month in Klironomos and Kendrick (1995), it is not possible to compare this model prediction with a field observation.

Finally, we tested the performance of the IBM in predicting population-level effects of copper on *F. candida* (Pattern 5). Toxic effects were implemented using only individual-level data (Table 2), with end-points on fecundity, survival, hatching success and avoidance; therefore we compared model output to the data presented in Herbert et al. (2004), where the authors measured the instantaneous rate of population increase (r_i) after exposure to different copper concentrations. Results are shown in Fig. 2. There was a higher simulated growth rate for the control and the two lowest concentrations, however for higher toxicant levels the model output and data matched well.

3 Model application

3.1 Results

Simulations with homogeneous contamination and with two different heterogeneous scenarios were conducted (Fig. 3), using the parameter set chosen using POM. In these scenarios the spatial arrangement and connectivity among contaminated cells is different, but the percentage of contaminated area is the same, 80%, while the remaining 20% is either uncontaminated or has a lower Cu concentration (Fig. 3). Two different combinations of concentrations, named combination a and combination b, were tested (Table 3). The total amount of toxicant was equal in both combinations, and the average contamination was the same

as in the homogeneous scenario. The level of heterogeneity (i.e., the difference among patches with high and low concentration) decreased from combination a to b. The total amount of food available was kept constant among simulations, but the distribution of food resources on the grid cells (Fig. 4) was randomized at the beginning of every model run.

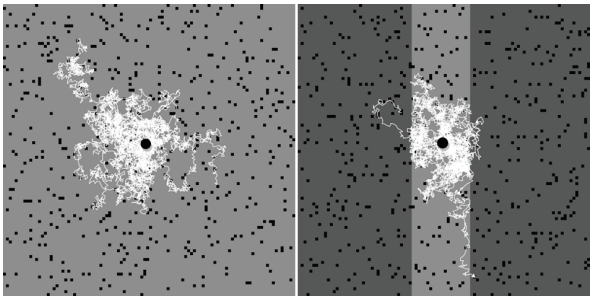

Fig. 3. Movement paths of 10 springtails after 20 days of simulation in absence (left panel) and presence (right panel) of toxicant on 80% of the area (dark grey), with a concentration of 3200 mg kg^{-1}. Five percent of the grid cells of 1 cm^2 are food cells (in black). Initially, the springtails are released in the uncontaminated region (light grey), in the centre of the simulation grid (black dot).

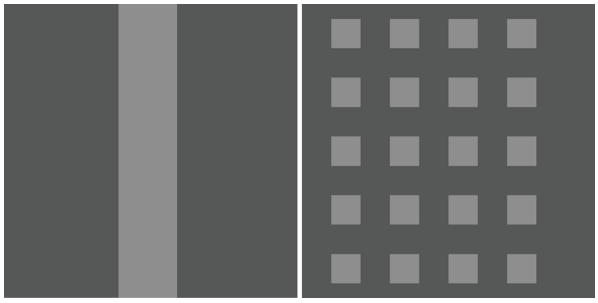

Fig. 4. Spatial arrangement of contaminated patches (dark grey) in Scenario 1 (left) and 2 (right).

Two sets of experiments were performed. In the first set, the temperature was kept constant, in order to compare growth rates and carrying capacity in the different scenarios and with increasing concentrations of toxicant. The length of these simulations was 200 days. In the second set of experiments, temperature was changed with season as described in Section 1.3, and the initial position of the organisms was randomized at the beginning of each model run. The length of these simulations was 365 days, starting the 1st of January.

In all simulation experiments, five replicate runs were performed both for the control (no toxicant) and for all treatments.

Results of the first set of simulation experiments are shown in Figs. 5a-c. Fluctuations in population abundance were much more evident in the control, because of the explosive growth that leads to food limitation. Longer simulations with constant temperature (not shown) indicated that abundance tended to stabilize after a few, dampened oscillations. At the lowest simulated contaminant concentration (125 mg kg^{-1}; Fig. 5a), the final population size reached in the three different scenarios was in the same range, although the

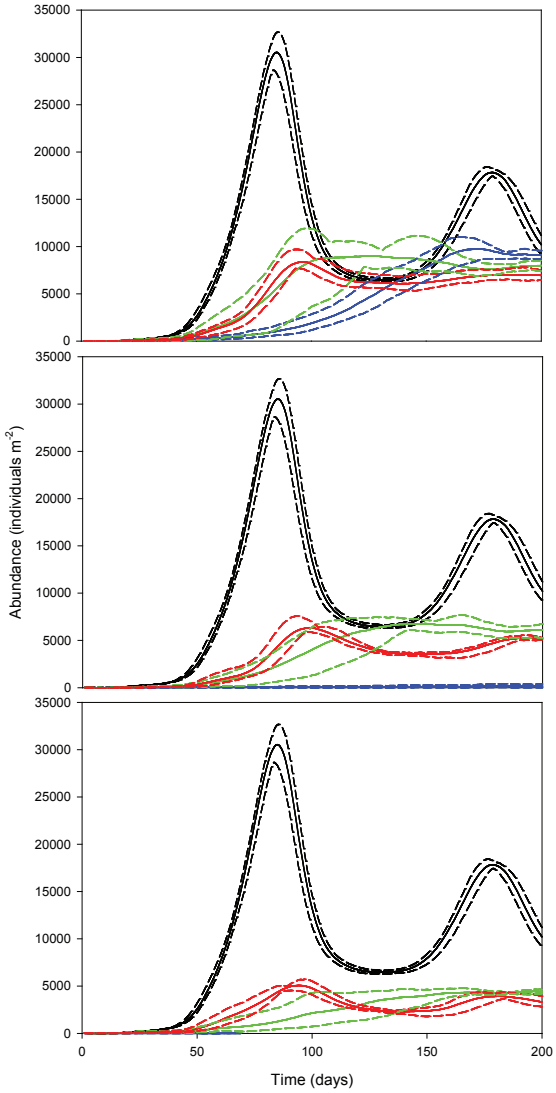

Fig. 5. (a–c) Population abundance in the control (black line), and under homogeneous (blue line) and heterogeneous contamination, combination A (green line Scenario 1, red line Scenario 2) at constant temperature range. Average concentrations are 125 mg Cu kg^{-1} (a), 500 mg kg^{-1} (b) and 2500 mg kg^{-1} (c). Solid lines represent averages, dashed lines minimum and maximum simulated values.

time to reach equilibrium population size was longer under homogeneous contamination. In all scenarios, initial growth rates were smaller than in the control, as were the final population sizes.

At the average concentration of 500 mg kg^{-1} (Fig 5b), the population exposed to homogeneous contamination survived until the end of the simulation, but was barely growing (mean final size 160 individuals), whereas in the heterogeneous scenarios abundance was around 5000 individuals/m^2.

At the highest concentration (2500 mg kg^{-1}; Fig 5c) the population exposed to homogeneous contamination went extinct, while in both heterogeneous scenarios a population of almost 5000 individuals/m^2 was sustained.

Fig. 6 shows the results of the second set of simulation experiments for the lowest concentration used: seasonal fluctuations were less evident in the treatments than in the control, and the spring peak was completely missed under homogeneous contamination, due to the slow initial population growth rate.

Finally, Fig. 7 shows a comparison of population growth in Scenario 1 with average concentration of 500 mg kg^{-1} at constant temperature, as exposed to the two different combinations of concentrations: combination A where 20 % of the grid cells are uncontaminated, and combination B, where they have a lower concentration of toxicant but are nonetheless contaminated.

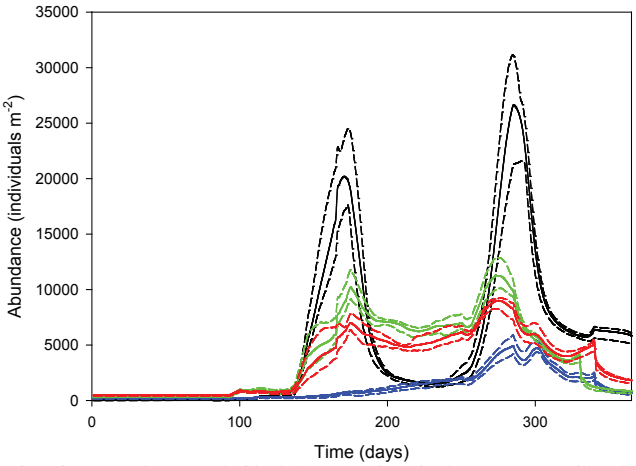

Fig. 6. Population abundance in the control (black line), and under homogeneous (blue line) and heterogeneous contamination, combination A (green line Scenario 1, red line Scenario 2) at varying temperature ranges. The average concentration is 125 mg Cu kg^{-1}. Solid lines represent averages, dashed lines minimum and maximum simulated values.

3.2 Uncertainty analysis

Uncertainty analysis has not yet been performed.

3.3 Recommendation

Simulation results show that, for the two heterogeneous scenarios used, the spatial arrangement of contaminated patches of soil is not particularly important for the growth and maintenance of metapopulations of *F. candida*, given the same percentage of contaminated area. Much more important seems to be the level of heterogeneity among patches of soil. At the same average concentration of 500 mg Cu kg^{-1} soil, which is close to the EC50 for reproduction (i.e. concentration that causes 50% effect, in this case reduction of oviposition), in a homogeneously contaminated scenario the population goes extinct, while in both hete-

rogeneous scenarios and both combinations of concentrations, viable metapopulations are formed in the more suitable soil patches. Looking at Fig. 7, it is obvious that if no clean habitat is offered to the individuals (combination b), the growth is substantially reduced, but it is anyway an important difference if compared to the homogeneous scenario, where, given the same average concentration, the population went extinct in few weeks. Therefore, since this species is used in standard ecotoxicological tests, and its ability to sense and avoid toxicants has been demonstrated (Boiteau et al., 2011; Greenslade and Vaughan, 2003; Boitaud and Ponge, 2006; Filser and Holscher, 1997), particular attention should be paid to the spatial distribution of chemicals when assessing risk.

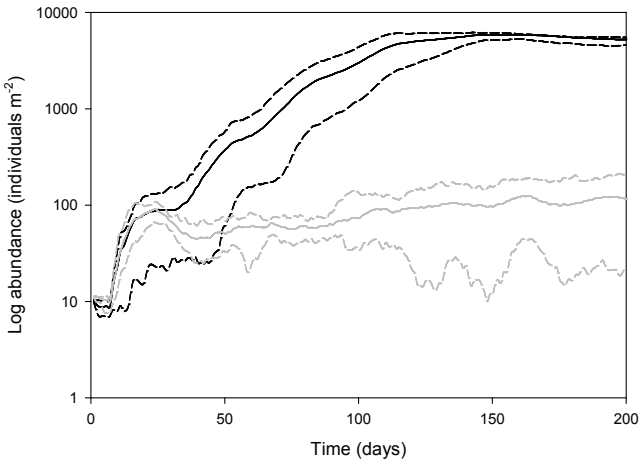

Fig. 7. Logarithm of population abundance under heterogeneous contamination with average concentration of 500 mg Cu kg^{-1} in Scenario 2, at constant temperature range and different combinations of concentrations, i.e. combination A (black line) and B (grey line, see text). Solid lines represent averages, dashed lines minimum and maximum simulated values.

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Appendix 3

NetLogo implementation of the individual-based model

```
globals [day_count season year]


patches-own

 [

   p-conc  ; concentration of toxicant

   p-food  ; number of food items currently present on the cell

   p-regrow  ; variable p-regrow is 0 if the cell is not initialized to be a food source,
1 otherwise


   local_density  ; number of individuals on each cell

 ]


turtles-own [age]


breed [eggs egg]


breed [springtails springtail]


springtails-own

 [

   energy  ; energy level of the organism: it triggers the individual to look for food,
and it has consequences on reproduction and survival


  p_avoid  ; probability to avoid a contaminated cell each time an individual encounters one

   ticktox  ; toxicity counter: it keeps track of the amount of time an individual spent
on containated cells


   repr_interv  ; time between broods

   repr_count  ; counter of the days passed since last reproductive event

   repr_prob  ; probability to reproduce at every reproductive instar

   nr_eggs_season  ; number of eggs per brood (general value for the season)

   nr_eggs_ltd  ; number of eggs per brood as influenced by the physiology and history of
the organism


   num_repr  ; counter of the number of reproductive events of an individual

   max_num_repr  ; max number of reproductive events per female

   matur_time  ; maturation time, i.e. time to reach adulthood
```

```
   j_surv  ; probability to survive until maturing age
   a_surv  ; age of death of the individual
   food  ; variable food is 0 if no food has been found during the current time-step, 1
otherwise
   mov_count  ; movement counter
   tox_count  ; exposure counter
   energy_loss  ; energy spent for moving during one time-step
   en_reduce_mov  ; Energy reduction per step moved
   tradeoff_repr  ; Tradeoff between energy and reproduction
   tradeoff_dens  ; Maximum energy spent for avoiding high density at each time-step
 ]

eggs-own
 [
  hatch_time  ; Hatching time: time needed for the eggs to develop and hatch to juveniles
   egg_viab  ; Egg viability: percentage of eggs that successfully hatch
 ]

;-------------------------------------------------------------------------

to setup

 __clear-all-and-reset-ticks
 reset-timer
 import-pcolors filename
 setup_patches
 setup_turtles
 update-plot
 display-labels

end

;-------------------------------------------------------------------------

to setup_patches
```

```
 set season 6
 set year 1
 set day_count 0
 ask patches
  [
   set p-food 0
   if random 100 < resource-density
    [
     set p-food food-value ; put a food item on patches according to resource density
    set p-regrow 1 ; only patches with p-regrow = 1 (i.e. initialized to be food sources)
will regrow the food

    ]
   ifelse pcolor > 13 and pcolor < 15
    [set p-conc conc]
    [set p-conc 0]
  ]

end

;-------------------------------------------------------------------------

to setup_turtles

 crt num_turtles
  [
   set breed springtails
   set energy energy_max
   set color white
   set age random 100 + 20
   set size 1
   set num_repr random 3
   set repr_count random 15
   set xcor random 100
   set ycor random 100
   set ticktox 0
   set repr_interv random 3 + 13
```

```
   set repr_prob random-float 0.02 + 0.98

   set nr_eggs_season random 80 + 19

   set max_num_repr random 8 + 9

   set matur_time random 11 + 30

   set j_surv random-normal 0.98 0.01

   set a_surv random-normal 241 50

  set p_avoid ((5.7475 * ln(conc + 1)) - 1.4235) / 100  ;avoidance probability is calculated
using the regression line of Boiteau (2011) data


   set en_reduce_mov 0.01

   set tradeoff_repr 20

   set tradeoff_dens 0.1

  ]


end


;-------------------------------------------------------------------------


to go


 tick

 if season != 6  ;springtails are not active in winter, so they do not move and they do
not feed


  [

   ask springtails [set energy energy - en_reduce_hour] ;every hour the level of energy
decreases: this trigger the organism to move and look for food


   forage

  ;the organism can stay 12 hours without feeding: moving is dangerous because it increases
the risk of predation, so some time is required to trigger it -> not sure, deleted (with
[energy < 9.5])

   ;feeding and movement procedures are executed at every tick (i.e. once every hour)

   if ticks mod 24 = 0

   ;all the procedures called within this command are executed only every 24 ticks (i.e.
once a day)


    [

     density-and-food-limitations

     ask springtails with [(age >= matur_time) and ((repr_count = 0) or (repr_count >=
repr_interv))]
```

```
     ;individuals that have reached maturation and have the counter for days since last
reproduction at least equal to the set time between broods can entre the reproduction
procedure

      [

        reproduction

      ]

    ]

  ]

 if ticks mod 24 = 0

  [

   set day_count day_count + 1

   ;1 tick is equal to 1 hour: day_count is a variable that is increased by 1 every 24 ticks

   seasons

   ask patches [regrow]

   ask springtails

    [

     if ticktox > 672 [set ticktox 672]

      mortality  ;mortality procedure does not include eggs, because their survival is
regulated within the hatching procedure


     grow

    ]

   ask eggs

    [

     if day_count = 340 and random 100 < 50 [die]  ; only half of the eggs survive over
winter


     set age age + 1

     if age >= hatch_time [hatching]

    ]

   update-plot

   display-labels

  ]

 if day_count = 365  ;after 1 year the cycle of days and season starts again

  [

   set year year + 1

   reset-ticks

   set day_count 0

  ]
```

```
 if (year > 1 ) or (not any? turtles) [stop]

end

;-----------------------------------------------------------------------------

to seasons

 if day_count = 80
  [
   set season 1  ;season 1 = early spring
   ask springtails
    [
     set repr_interv random 3 + 13
     set repr_prob random-float 0.02 + 0.98
     set nr_eggs_season random 80 + 19
     set max_num_repr random 8 + 9
     set matur_time random 11 + 30
     set j_surv random-normal 0.98 0.01
     set a_surv random-normal 241 50
    ]
   ask eggs
    [
     set egg_viab random-normal 0.94 0.025
     set hatch_time random 7 + 13
    ]
  ]

 if day_count = 130
  [
   set season 2  ;season 2 = late spring
   ask springtails
    [
     set repr_interv random 11 + 6
     set repr_prob random-float 0.02 + 0.97
     set nr_eggs_season random 21 + 30
```

```
    set max_num_repr random 18 + 3
    set matur_time random 17 + 13
    set j_surv random-normal 0.95 0.01
    set a_surv random-normal 140 25
   ]
  ask eggs
   [
    set egg_viab random-normal 0.9 0.05
    set hatch_time random 9 + 7
   ]
 ]

if day_count = 165
 [
  set season 3  ;season 3 = summer
  ask springtails
   [
    set repr_interv random 3 + 11
    set repr_prob random-float 0.02 + 0.96
    set nr_eggs_season random 31 + 14
    set max_num_repr random 3 + 4
    set matur_time random 20 + 11
    set j_surv random-normal 0.833 0.01
    set a_surv random-normal 73 25
   ]
  ask eggs
   [
    set hatch_time random 3 + 7
    set egg_viab random-normal 0.8 0.05
   ]
 ]

if day_count = 250
 [
  set season 4  ;season 4 = early fall
  ask springtails
```

```
    [
     set repr_interv random 11 + 6
     set repr_prob random-float 0.02 + 0.97
     set nr_eggs_season random 21 + 30
     set max_num_repr random 18 + 3
     set matur_time random 17 + 13
     set j_surv random-normal 0.95 0.01
     set a_surv random-normal 140 25
    ]
   ask eggs
    [
     set egg_viab random-normal 0.9 0.05
     set hatch_time random 9 + 7
    ]
  ]

 if day_count = 285
  [
   set season 5  ;season 5 = late fall
   ask springtails
    [
     set repr_interv random 3 + 13
     set repr_prob random-float 0.02 + 0.98
     set nr_eggs_season random 80 + 19
     set max_num_repr random 8 + 9
     set matur_time random 11 + 30
     set j_surv random-normal 0.98 0.01
     set a_surv random-normal 241 50
    ]
   ask eggs
    [
     set hatch_time random 7 + 13
     set egg_viab random-normal 0.94 0.025
    ]
  ]
```

```
 if day_count = 340
  [
   set season 6  ;season 6 = winter (overwintering individuals are not active until spring)
   ask springtails
    [
     set repr_interv 90
     set repr_prob 0
     set nr_eggs_season 0
     set matur_time 90
     set j_surv random-normal 0.83 0.01
     set a_surv 0
    ]
   ask eggs
    [
     set hatch_time 110
     set egg_viab 0
    ]
  ]

end

;-------------------------------------------------------------------------

to grow

 set age age + 1
 if age >= matur_time
  [
   set color white
   set size 1
   set repr_count repr_count + 1
  ]

end

;-------------------------------------------------------------------------
```

```
; this procedure contains all the rules involved in the movement. Movement is controlled
by presence/absence of food, and population density.

; While individuals look for food and for less crowded cells, they also avoid contamination.


to forage


 ask springtails

  [

   set food 0

   set mov_count 0

   set energy_loss 0

   set tox_count 0

   if [p-conc] of patch-here > 0  ;even if the individual does not move for feeding or
crowdness, it checks if the current patch is contaminated


    [

     ifelse (random-float 1 < p_avoid) and ([p-conc] of one-of neighbors = 0)  ;if one of
the neighbouring cell is clean the individuals move to that cell


    [

      face one-of neighbors with [p-conc = 0]

      fd 1

      set mov_count mov_count + 1

      set energy energy - en_reduce_mov

     ]

     [

      set tox_count tox_count + 1

     ]

    ]

   if energy < (energy_max - (24 * en_reduce_hour))

    [

     ifelse (any? patches in-radius 2.5 with [p-regrow = 1]) and (sum [p-food] of patches
in-radius 2.5 / food-value > 1)


     [

      if random-float 1 < probab_mov * (1 + (mean [p-food] of patches in-radius 2.5 with
[p-regrow = 1] / food-value))


      [
```

```
       while [(energy_loss < tradeoff_mov) and (food = 0)]
        [
         ifelse [p-food] of patch-here >= 1
          [
           set food 0.5
           ask patch-here [set p-food p-food - 1]  ;harvest the food
          ]
          [
           ifelse [p-food] of one-of patches in-cone 2.5 180 >= 1  ;organisms can sense
the presence of food within 2.5 cm distance

            [
             face one-of patches in-cone 2.5 180 with [p-food >= 1]  ;if they sense the
food, they turn toward the patch where the food is

             ifelse [p-conc] of patch-ahead 1 > 0
              [
               ifelse (random-float 1 < p_avoid) and ([p-conc] of one-of neighbors = 0)
                [
                 face one-of neighbors with [p-conc = 0]
                 fd 1
                 set mov_count mov_count + 1
                ]
                [
                 fd 1
                 set tox_count tox_count + 1
                 set mov_count mov_count + 1
                ]
              ]
              [
               fd 1
               set mov_count mov_count + 1
              ]
            ]
            [
             rt random 360  ;if they do not sense any food they move randomly
             ifelse [p-conc] of patch-ahead 1 > 0
              [
```

```
              ifelse (random-float 1 < p_avoid) and ([p-conc] of one-of neighbors = 0)
               [
                face one-of neighbors with [p-conc = 0]
                fd 1
                set mov_count mov_count + 1
               ]
               [
                fd 1
                set tox_count tox_count + 1
                set mov_count mov_count + 1
               ]
             ]
             [fd 1 set mov_count mov_count + 1]
           ]
         ]
        set energy_loss mov_count * en_reduce_mov
       ]
      set energy (energy + food - energy_loss)  ;add the net energy return (or loss) to
the forager's total energy

     ]
    ]
     [
     if random-float 1 < probab_mov
      [
      while [(energy_loss < tradeoff_mov) and (food = 0)]
       [
        ifelse [p-food] of patch-here >= 1
         [
          set food 0.5
          ask patch-here [set p-food p-food - 1]  ;harvest the food
         ]
         [
          ifelse [p-food] of one-of patches in-cone 2.5 180 >= 1  ;organisms can sense
the presence of food within 2/3 cm distance

           [
            face one-of patches in-cone 2.5 180 with [p-food >= 1]  ;if they sense the
```

```
food, they turn toward the patch where the food is
              ifelse [p-conc] of patch-ahead 1 > 0
               [
                ifelse (random-float 1 < p_avoid) and ([p-conc] of one-of neighbors = 0)
                 [
                  face one-of neighbors with [p-conc = 0]
                  fd 1
                  set mov_count mov_count + 1
                 ]
                 [
                  fd 1
                  set tox_count tox_count + 1
                  set mov_count mov_count + 1
                 ]
               ]
               [
                fd 1
                set mov_count mov_count + 1
               ]
             ]
             [
              rt random 360  ;if they do not sense any food they move randomly
              ifelse [p-conc] of patch-ahead 1 > 0
               [
                ifelse (random-float 1 < p_avoid) and ([p-conc] of one-of neighbors = 0)
                 [
                  face one-of neighbors with [p-conc = 0]
                  fd 1
                  set mov_count mov_count + 1
                 ]
                 [
                  fd 1
                  set tox_count tox_count + 1
                  set mov_count mov_count + 1
                 ]
               ]
               [fd 1 set mov_count mov_count + 1]
```

```
            ]
          ]
         set energy_loss mov_count * en_reduce_mov
        ]
       set energy (energy + food - energy_loss)  ;add the net energy return (or loss) to
the forager's total energy

      ]
      ]

     ]
     if tox_count > 0 [set ticktox ticktox + 1]
     set mov_count 0
     set food 0
     set energy_loss 0
    ]
 ask patches [set local_density count springtails-here]

end
;-------------------------------------------------------------------------

to regrow

if p-food <= 1 and random 100 < regrow-rate and p-regrow = 1 [set p-food food-value]

end

;-------------------------------------------------------------------------

to reproduction

 if ((random-float 1 < repr_prob) and (num_repr <= max_num_repr) and (energy >= tradeoff_
repr) and (season != 6) )

 ;reproduction is allowed according to a preset reproduction probability, to the number
of reproductive events already undergone

 ;by the individual and its energy level (i.e. if it spends more than a week without
feeding it cannot reproduce)
```

```
  [
   ifelse conc = 0
    [
     hatch-eggs nr_eggs_ltd
      [
       set age 0
       set size 0.5
       set shape "circle"
       if season = 1
        [
       ;these rules are the same ones in the "seasons" procedures, but since the "seasons"
procedures is executed only at the first day of the season,

         ;when new turtles are created their state variables need to be set to the right
values

         set hatch_time random 7 + 13
         set egg_viab random-normal 0.94 0.025
        ]
       if season = 2
        [
         set hatch_time random 9 + 7
         set egg_viab random-normal 0.9 0.05
        ]
       if season = 3
        [
         set hatch_time random 3 + 7
         set egg_viab random-normal 0.8 0.05
        ]
       if season = 4
        [
         set hatch_time random 9 + 7
         set egg_viab random-normal 0.9 0.05
        ]
       if season = 5
        [
         set hatch_time random 7 + 13
         set egg_viab random-normal 0.94 0.025
```

```
        ]
      ]
    ]
    [
    hatch-eggs nr_eggs_ltd - (nr_eggs_ltd * (0.2189 * ln(conc) - 0.8743) * (ticktox / 672))
     ;when the model world is contaminated the fecundity is reduced according to Sandifer
& Hopkin (1996) data

     ;because the organisms do not spend all the time on a contaminated patch, reduction
of fecundity accounts also for the time spent on a contaminated patch

     ;(if they happen to come on a contaminated during 1 tick, it is assumed they spent
1 hour on it) -> 672 is equal to 28 days

      [
       set age 0
       set size 0.5
       set shape "circle"
       if season = 1
        [
         set hatch_time random 7 + 13
         set egg_viab random-normal 0.94 0.025
        ]
       if season = 2
        [
         set hatch_time random 9 + 7
         set egg_viab random-normal 0.9 0.05
        ]
       if season = 3
        [
         set hatch_time random 3 + 7
         set egg_viab random-normal 0.8 0.05
        ]
       if season = 4
        [
         set hatch_time random 9 + 7
         set egg_viab random-normal 0.9 0.05
        ]
       if season = 5
```

```
        [
         set hatch_time random 7 + 13
         set egg_viab random-normal 0.94 0.025
        ]
      ]
    ]
    set repr_count 0
    set num_repr num_repr + 1
   ]

end

;-------------------------------------------------------------------------

to hatching

;hatching of the eggs occurs at a predetermined hatching time, and according to the eggs
viability

 ifelse [p-conc] of patch-here < 100
  [
   ifelse random-float 1 < egg_viab
     [
      set breed springtails  ;when eggs hatch, they obtain values for avoidance prob and
life-cycle parameters that define the breed "springtails". Values change according to the
season
      set age 0
      set energy energy_max
      set color white
      set size 0.5
      set repr_count -1
       set p_avoid ((5.7475 * ln (conc + 1)) - 1.4235) / 100  ;avoidance probability is
calculated using the regression line of Boiteau (2011) data

      set en_reduce_mov 0.01
      set tradeoff_repr 20
      set tradeoff_dens 0.1
      if season = 1
       [
```

```
        set repr_interv random 3 + 13
        set repr_prob random-float 0.02 + 0.98
        set nr_eggs_season random 80 + 19
        set nr_eggs_ltd nr_eggs_season  ;when the egg hatches, the nr of eggs per brood
is set to the general value; as the organism grows, if it experience starvation or
coontamination, this nr will decrease
        set max_num_repr random 8 + 9
        set matur_time random 11 + 30
        set j_surv random-normal 0.98 0.01
        set a_surv random-normal 241 50
       ]
      if season = 2
      [
        set repr_interv random 11 + 6
        set repr_prob random-float 0.02 + 0.97
        set nr_eggs_season random 21 + 30
        set nr_eggs_ltd nr_eggs_season
        set max_num_repr random 18 + 3
        set matur_time random 17 + 13
        set j_surv random-normal 0.95 0.01
        set a_surv random-normal 140 25
      ]
      if season = 3
      [
        set repr_interv random 3 + 11
        set repr_prob random-float 0.02 + 0.96
        set nr_eggs_season random 31 + 14
        set nr_eggs_ltd nr_eggs_season
        set max_num_repr random 3 + 4
        set matur_time random 20 + 11
        set j_surv random-normal 0.833 0.01
        set a_surv random-normal 73 25
      ]
      if season = 4
      [
        set repr_interv random 11 + 6
        set repr_prob random-float 0.02 + 0.97
```

```
        set nr_eggs_season random 21 + 30
        set nr_eggs_ltd nr_eggs_season
        set max_num_repr random 18 + 3
        set matur_time random 17 + 13
        set j_surv random-normal 0.95 0.01
        set a_surv random-normal 140 25
      ]
      if season = 5
      [
        set repr_interv random 3 + 13
        set repr_prob random-float 0.02 + 0.98
        set nr_eggs_season random 80 + 19
        set nr_eggs_ltd nr_eggs_season
        set max_num_repr random 8 + 9
        set matur_time random 11 + 30
        set j_surv random-normal 0.98 0.01
        set a_surv random-normal 241 50
      ]
      if season = 6
      [
        set repr_interv 110
        set repr_prob 0
        set nr_eggs_season 0
        set nr_eggs_ltd nr_eggs_season
        set matur_time 110
        set j_surv random-normal 0.83 0.01
        set a_surv 0
      ]
     ]
     [die]
  ]
  [
   ifelse random-float 1 < egg_viab * (-0.2243 * ln(p-conc) + 1.8893)
     [
      set breed springtails  ;when eggs hatch, they obtain values for avoidance prob and
life-cycle parameters that define the breed "springtails". Values change according to the
season
```

```
      set age 0

      set energy energy_max

      set color white

      set size 0.5

      set repr_count -1

       set p_avoid ((5.7475 * ln (conc + 1)) - 1.4235) / 100  ;avoidance probability is
calculated using the regression line of Boiteau (2011) data


      set en_reduce_mov 0.01

      set tradeoff_repr 20

      set tradeoff_dens 0.1

      if season = 1

       [

        set repr_interv random 3 + 13

        set repr_prob random-float 0.02 + 0.98

        set nr_eggs_season random 80 + 19

         set nr_eggs_ltd nr_eggs_season  ;when the egg hatches, the nr of eggs per brood
is set to the general value; as the organism grows, if it experience starvation or
coontamination, this nr will decrease

        set max_num_repr random 8 + 9

        set matur_time random 11 + 30

        set j_surv random-normal 0.98 0.01

        set a_surv random-normal 241 50

       ]

      if season = 2

      [

        set repr_interv random 11 + 6

        set repr_prob random-float 0.02 + 0.97

        set nr_eggs_season random 21 + 30

        set nr_eggs_ltd nr_eggs_season

        set max_num_repr random 18 + 3

        set matur_time random 17 + 13

        set j_surv random-normal 0.95 0.01

        set a_surv random-normal 140 25

      ]

      if season = 3

      [

        set repr_interv random 3 + 11
```

```
  set repr_prob random-float 0.02 + 0.96
  set nr_eggs_season random 31 + 14
  set nr_eggs_ltd nr_eggs_season
  set max_num_repr random 3 + 4
  set matur_time random 20 + 11
  set j_surv random-normal 0.833 0.01
  set a_surv random-normal 73 25
]
if season = 4
[
  set repr_interv random 11 + 6
  set repr_prob random-float 0.02 + 0.97
  set nr_eggs_season random 21 + 30
  set nr_eggs_ltd nr_eggs_season
  set max_num_repr random 18 + 3
  set matur_time random 17 + 13
  set j_surv random-normal 0.95 0.01
  set a_surv random-normal 140 25
]
if season = 5
[
  set repr_interv random 3 + 13
  set repr_prob random-float 0.02 + 0.98
  set nr_eggs_season random 80 + 19
  set nr_eggs_ltd nr_eggs_season
  set max_num_repr random 8 + 9
  set matur_time random 11 + 30
  set j_surv random-normal 0.98 0.01
  set a_surv random-normal 241 50
]
if season = 6
[
  set repr_interv 110
  set repr_prob 0
  set nr_eggs_season 0
  set nr_eggs_ltd nr_eggs_season
```

```
        set matur_time 110
        set j_surv random-normal 0.83 0.01
        set a_surv 0
      ]
     ]
     [die]
  ]

end

;-------------------------------------------------------------------------

to density-and-food-limitations

 ask springtails
  [
   if energy < (energy_min) [die] ;if an individual spends more than the equivalent of 20
day (if immobile) without feeding, it dies of starvation

   set nr_eggs_ltd nr_eggs_season
   set mov_count 0
   set energy_loss 0
  ]
 ask springtails with [energy >= tradeoff_repr and age >= matur_time]
  [
   ;the nr of eggs is set back to the "default" value every time an individual enter this
procedure '

  ;because this parameter is influenced only by the conditions at the moment of oviposition
(see Green, 1964 (density))

   let tox_count_2 0
   if (count springtails-on one-of patches in-cone 2.5 180 < count springtails-here)
    [
     repeat (round (tradeoff_dens / en_reduce_mov))
      [
       ;organisms can sense the presence of other individuals within 2.5 cm distance
       face min-one-of patches in-cone 2.5 180 [count springtails-here]  ;if they sense
lower density, they turn toward the patch least crowded
```

```
       ifelse [p-conc] of patch-ahead 1 > 0
        [
         ifelse (random-float 1 < p_avoid) and ([p-conc] of one-of neighbors = 0)
          [
           face one-of neighbors with [p-conc = 0]
           fd 1
           set mov_count mov_count + 1
          ]
          [
           fd 1
           set tox_count_2 tox_count_2 + 1
           set mov_count mov_count + 1
          ]
        ]
        [
         fd 1
         set mov_count mov_count + 1
        ]
       set energy_loss mov_count * en_reduce_mov
      ]
    ]
 set energy (energy - energy_loss)  ;subtract the energy lost for the movement to the
forager's total energy

 if (energy <= (energy_max - (24 * en_reduce_hour))  and energy >= tradeoff_repr) [set
nr_eggs_ltd nr_eggs_season * (0.01 * e ^ (4.6052 * ((energy - tradeoff_repr) / (energy_max
- tradeoff_repr))))]  ;if an individual spends more than 1 day without feeding, fecundity
is reduced

]
 ask patches [set local_density count springtails-here]
 ask springtails
  [
   if local_density > 1 ;when they are too crowded they lay less eggs
    [
     set nr_eggs_ltd nr_eggs_ltd * (e ^ (-0.305 * local_density))
    ]
  ]
```

```
end

;-----------------------------------------------------------------------

to mortality



  ifelse conc = 0

  [

   ifelse age < matur_time

    [if random-float 1 > (j_surv ^ (1 / matur_time)) [die]]

   ;j_surv is the cumulative probability to survive until adult age: this is the combination
of a series of independent events (i.e. the probability to survive each day)


    [if age > a_surv [die]]

     ;a_surv is expressed in days: it is a number drawn from a normal distribution and
represents the age of death of any single individual


  ]

  [

   ifelse age < matur_time

    [if random-float 1 > ((j_surv ^ (1 / matur_time)) - (0.0824 * ln(conc) - 0.1366) *
(ticktox / 672)) [die]]


    [if age > a_surv - ((a_surv * 0.0824 * ln(conc) - 0.1366) * (ticktox / 672)) [die]]

   ;adult survival is reduced when the individual spends time on the contaminated patches,
according to Sandifer & Hopkin (1996) data


  ]


end


;-----------------------------------------------------------------------

to update-plot


  set-current-plot "Energy"

  if any? springtails [plot mean [energy] of springtails]


  set-current-plot "Spatial distribution"
```

```
  histogram [local_density] of patches

  set-current-plot "Population size and structure"

  set-current-plot-pen "juv+ad"
  plot count springtails

  set-current-plot-pen "juveniles"
  plot count springtails with [age < matur_time]

  set-current-plot-pen "adults"
  plot count springtails with [age >= matur_time]

  set-current-plot-pen "eggs"
  plot count eggs

end

;-------------------------------------------------------------------------

to display-labels

 ask springtails
  [
   set label ""
   if show-energy? [set label precision energy 2]
  ]
 ask patches
  [
   set plabel ""
   if show-density? [set plabel local_density]
  ]

end
```

Appendix 4

Supplementary material for the RAMAS metapopulation model

Table 1. Stable-stage distributions used to initialized the models.

Stage	0 mg Cu kg^{-1}	125 mg Cu kg^{-1}	160 mg Cu kg^{-1}	500 mg Cu kg^{-1}	625 mg Cu kg^{-1}	2500 mg Cu kg^{-1}	3125 mg Cu kg^{-1}	Mean (IBM initialization)
Eggs	702	792	798	824	828	751	671	767
Juveniles	242	158	150	110	102	36	22	117
Adults	56	51	52	66	70	213	307	116

Table 2. Resampling estimates of stage and standard deviations matrices elements. Values for the element S3 have not been derived through resampling, but have instead been estimated from IBM simulations.

Concentration	Matrix element	Estimate of mean	Estimate of standard deviation
0 mg Cu kg^{-1}	S1	0.8980	0.0232
	P1	0.0876	0.0199
	S2	0.9481	0.0124
	P2	0.0493	0.0118
	F3	3.8105	1.2220
	S3	0.985	0.015
125 mg Cu kg^{-1}	S1	0.8882	0.0251
	P1	0.0772	0.017
	S2	0.7001	0.0006
	P2	0.0361	0.00003
	F3	3.1231	1.0427
	S3	0.975	0.029
160 mg Cu kg^{-1}	S1	0.8848	0.0258
	P1	0.0741	0.0163
	S2	0.6807	0.0006
	P2	0.0351	0.00003
	F3	2.9119	0.931
	S3	0.974	0.03
500 mg Cu kg^{-1}	S1	0.8641	0.0297
	P1	0.0574	0.0128
	S2	0.5914	0.0006
	P2	0.0305	0.00003
	F3	1.9452	0.6165
	S3	0.968	0.036
625 mg Cu kg^{-1}	S1	0.8586	0.0307
	P1	0.0539	0.0116
	S2	0.574	0.0006
	P2	0.0296	0.00003
	F3	1.7906	0.5769
	S3	0.967	0.0375

Concentration	Matrix element	Estimate of mean	Estimate of standard deviation
2500 mg Cu kg^{-1}	S1	0.7906	0.0424
	P1	0.0240	0.0052
	S2	0.4653	0.0006
	P2	0.0240	0.0000
	F3	0.6112	0.2024
	S3	0.958	0.044
3125 mg Cu kg^{-1}	S1	0.7628	0.0469
	P1	0.0171	0.0034
	S2	0.4478	0.0006
	P2	0.0231	0.00003
	F3	0.4311	0.1349
	S3	0.956	0.045

Model Summary and Assumptions for Fcandida metapop0.mp

Program: RAMAS Metapop version 5.0

Title: Folsomia candida

Replications: 100

Duration: 300 time steps (300,0 days)

Stage structure: there are 3 stages

For all stages:

Relative dispersal=-1

Average weight=-1

- Stage-specific parameters

Stage	Exclude	Basis for Density Dependence
Eggs	True	False
Juveniles	False	True
Adults	False	True

- Stage matrix

NO Cu	Eggs	Juveniles	Adults
Eggs	0,898	0,0	3,8105
Juveniles	0,0876	0,9481	0,0
Adults	0,0	0,0493	0,985

500 mg/kg	Eggs	Juveniles	Adults
Eggs	0,8641	0,0	1,9452
Juveniles	0,0574	0,5914	0,0
Adults	0,0	0,0305	0,968

2500 mg/kg	Eggs	Juveniles	Adults
Eggs	0,7906	0,0	0,6112
Juveniles	0,024	0,4653	0,0
Adults	0,0	0,024	0,958

125 mg/kg	Eggs	Juveniles	Adults
Eggs	0,8882	0,0	3,1231
Juveniles	0,0772	0,7001	0,0
Adults	0,0	0,0361	0,975

160 mg/kg	Eggs	Juveniles	Adults
Eggs	0,8848	0,0	2,9119
Juveniles	0,0741	0,6807	0,0
Adults	0,0	0,0351	0,974

3125 mg/kg	Eggs	Juveniles	Adults
Eggs	0,7628	0,0	0,4311
Juveniles	0,0171	0,4478	0,0
Adults	0,0	0,0231	0,956

- Constraints

  Proportion of each stage matrix element that is survival (as opposed to fecundity)

	Eggs	Juveniles	Adults
Eggs	1,0	0,0	0,0
Juveniles	1,0	1,0	1,0
Adults	1,0	1,0	1,0

- Stochasticity

  Demographic stochasticity is used

  Environmental stochasticity distribution: Lognormal

  Extinction threshold for metapopulation = 1

  Explosion threshold for metapopulation = 0

  When abundance is below local threshold: count in total

  Within-population correlation: All correlated (F, S, K)

  (F = fecundity, S = survival, K = carrying capacity)

- Standard deviations matrix

NO Cu	Eggs	Juveniles	Adults
Eggs	0,0232	0,0	1,222
Juveniles	0,0199	0,0124	0,0

Adults	0,0	0,0493	0,015

500 mg/kg	**Eggs**	**Juveniles**	**Adults**
Eggs	0,0297	0,0	0,6165
Juveniles	1,0128	0,0006	0,0
Adults	0,0	0,00003	0,036

2500 mg/kg	**Eggs**	**Juveniles**	**Adults**
Eggs	0,0424	0,0	0,2024
Juveniles	0,0052	0,0006	0,0
Adults	0,0	0,00003	0,044

125 mg/kg	**Eggs**	**Juveniles**	**Adults**
Eggs	0,0251	0,0	1,0427
Juveniles	0,017	0,0006	0,0
Adults	0,0	0,00003	0,029

160 mg/kg	**Eggs**	**Juveniles**	**Adults**
Eggs	0,0258	0,0	0,931
Juveniles	0,0163	0,0006	0,0
Adults	0,0	0,00003	0,03

3125 mg/kg	**Eggs**	**Juveniles**	**Adults**
Eggs	0,0469	0,0	0,1349
Juveniles	0,0034	0,0006	0,0
Adults	0,0	0,00003	0,045

- Catastrophes

  There are no catastrophes.

- Initial abundances

	Eggs	**Juveniles**	**Adults**
Pop 1	0	1000	0
Pop 2	0	0	0
Pop 3	0	0	0
Pop 4	0	0	0
Pop 5	0	0	0
Pop 6	0	0	0
Pop 7	0	0	0

Pop 8	0	0	0
Pop 9	0	0	0
Pop 10	0	0	0
Pop 11	0	0	0
Pop 12	0	0	0
Pop 13	0	0	0
Pop 14	0	0	0
Pop 15	0	0	0
Pop 16	0	0	0
Pop 17	0	0	0
Pop 18	0	0	0
Pop 19	0	0	0
Pop 20	0	0	0
Pop 21	0	0	0
Pop 22	0	0	0
Pop 23	0	0	0
Pop 24	0	0	0
Pop 25	0	0	0

- Spatial structure

  There are 25 populations (see "Populations" below for coordinates)

- Dispersal

  There are 204 migratory/dispersal connections among the 25 populations

  (34 % of the 600 possible connections).

  The dispersal rates range from 0,0 to 0,06269

  All migration/dispersal rates are symmetric (same in both directions).

- Correlation

  Populations have uncorrelated fluctuations (independent environments).

- Populations

  General

  Relative dispersal is 1,0

  Std. dev. matrix is NO Cu

  Local threshold is 0,0

  All populations are included in the summation

  Density dependence

  Density dependence type is Scramble

  Density dependence is based on the abundances of selected stages

  Density dependence affects all vital rates

Max. growth rate (Rmax) is 1,2019

Carrying capacity (K) is 4000

Standard deviation of K is 0,0

Density-dependent dispersal as a function of source pop. size (slope) is 0,0

Population	X-coordinate	Y-coordinate	Initial abundance	Stage matrix
Pop 1	0,0	0,0	1000	NO Cu
Pop 2	20,0	0,0	0	NO Cu
Pop 3	40,0	0,0	0	NO Cu
Pop 4	60,0	0,0	0	NO Cu
Pop 5	80,0	0,0	0	NO Cu
Pop 6	0,0	20,0	0	NO Cu
Pop 7	20,0	20,0	0	NO Cu
Pop 8	40,0	20,0	0	NO Cu
Pop 9	60,0	20,0	0	NO Cu
Pop 10	80,0	20,0	0	NO Cu
Pop 11	0,0	40,0	0	NO Cu
Pop 12	20,0	40,0	0	NO Cu
Pop 13	40,0	40,0	0	NO Cu
Pop 14	60,0	40,0	0	NO Cu
Pop 15	80,0	40,0	0	NO Cu
Pop 16	0,0	60,0	0	NO Cu
Pop 17	20,0	60,0	0	NO Cu
Pop 18	40,0	60,0	0	NO Cu
Pop 19	60,0	60,0	0	NO Cu
Pop 20	80,0	60,0	0	NO Cu
Pop 21	0,0	80,0	0	NO Cu
Pop 22	20,0	80,0	0	NO Cu
Pop 23	40,0	80,0	0	NO Cu
Pop 24	60,0	80,0	0	NO Cu
Pop 25	80,0	80,0	0	NO Cu
Total			1000	

- Population management

  Population management is not used

R scripts of vital rates resampling

S1:

```
require ("MASS")

# samples viability and stage duration from uniform distributions
```

```
v <- runif (1000, 0.75, 0.97)
d <- runif (1000, 7, 15)

# calculating S1
p <- v ^(1/d)
S1 <- (1-p^(d-1))/(1-p^d)*p

# plotting the sampled distribution
hist (S1, breaks = c(0:100)/100*0.22+0.78, main = "Histogram of S1", xlab = "S1")

# parameter estimates of the normal distribution using MLE
model <- fitdistr(y,"normal")

# generating and plotting the resulting normal distribution
yn <- rnorm(1000, model$estimate[1], model$estimate[2])
ynh <- hist (yn, breaks = c(0:100)/100*0.24+0.77, plot = F)
points (ynh$mids,ynh$counts,type = 'l', col = "blue")
```

S2:

```
require ("MASS")

# samples maturation time from uniform distribution
t <- runif (1000, 13, 29)

# calculating S2
p <- 0.95 ^(1/t)
S2 <- (1-p^(t-1))/(1-p^t)*p

# plotting the sampled distribution
hist (S2, breaks = c(0:100)/100*0.22+0.78, main = "Histogram of S2", xlab = "S2")

# parameter estimates of the normal distribution using MLE
model <- fitdistr(y,"normal")

# generating and plotting the resulting normal distribution
yn <- rnorm(1000, model$estimate[1], model$estimate[2])
```

```
ynh <- hist (yn, breaks = c(0:100)/100*0.24+0.77, plot = F)
points (ynh$mids,ynh$counts,type = 'l', col = "blue")
```

F3:

```
require ("MASS")

# samples time between broods and number of eggs per brood from uniform distributions
t <- runif (1000, 6, 16)
n <- runif(1000, 30, 50)

# calculating F3
F3 <- 0.98*(n/t)

# plotting the sampled distribution
hist (F3, breaks = c(0:100)/100*0.22+0.78, main = "Histogram of F3", xlab = "F3")

# parameter estimates of the normal distribution using MLE
model <- fitdistr(y,"normal")

# generating and plotting the resulting normal distribution
yn <- rnorm(1000, model$estimate[1], model$estimate[2])
ynh <- hist (yn, breaks = c(0:100)/100*0.24+0.77, plot = F)
points (ynh$mids,ynh$counts,type = 'l', col = "blue")
```

Summary

The use of mechanistic effect models in ecological risk assessment of chemicals (ERA), especially plant protection products, has been gaining momentum in recent years. Increasing evidence of their suitability to extrapolate effects from the individual-level, which is usually the object of laboratory testing in current ERA practice, to the population level, which constitutes the protection goal of most EU directives concerning safety of chemical products for environmental and human health, is being provided. At the same time criticism to current extrapolation tools commonly used in ERA, such as the use of fixed safety factors or species sensitivity distributions, is coming from different studies.

Furthermore, another plea for the use of effect models in ERA is related to the possibility they offer to add ecological relevance to the risk assessment of chemicals. For example, effect models allow for incorporating factors that are known to affect growth and post-stress recovery of natural populations but that are not explicitly taken into account in current ERA because they cannot be included in a standardized laboratory test. Among these factors are natural stressors, multiple applications of the same chemical or combined toxicity of a number of compounds.

Despite the increasing recognition of their potential to improve risk estimates, the use of ecological effect models in regulatory decision making is still very limited, unlike environmental fate models. To understand how ecological effect models are perceived by relevant stakeholders and to identify what prevents their inclusion in ERA, findings of a survey conducted among stakeholders from academia, industry, and regulatory authorities involved in ERA are presented. Among the main reasons identified are the lack of official guidance for developing and using mechanistic effect models, and contradicting expectations. According to the study, models are supposed to be simple and user-friendly enough to be easily understood, parameterized, and used in a standardized way. At the same time, though, they are expected to be complex enough to be realistic and capable of capturing a wide range of ecological scenarios.

Therefore, in this thesis I give my contribution to the efforts that are put into clarifying these fears and expectations about ecological models. For this I showed that, through the use of good modelling practice and standardized documentation formats, ecological effect models are a good option to link short-term standard toxicity data to relevant protection goals. More specifically, I developed two population models of the collembolan *Folsomia candida*, and used them to investigate the effects of heterogeneous soil contamination on its population dynamics, thus showing

a possible application of ecological models within the perspective of chemicals risk assessment.

A spatially-explicit individual-based population model (IBM), was developed and tested according to the pattern-oriented modelling theory. Individuals in the model can sense and avoid contaminated habitat with a certain probability, which depends on contamination level. Model rules and parameters are based on previous knowledge of the biology and ecology of the species; for implementation of toxicity, data from standard laboratory tests (survival, reproduction and avoidance) are used. The model was used to test various hypotheses regarding the effects on the growth and recovery of *F. candida* populations of different patterns of habitat fragmentation and disturbance events. These effects can be interpreted as the results of interactions between natural stress factors and different agrochemicals application regimes.

To explore whether all the complexity included in the IBM is necessary to predict risk for a species with a relatively simple life-cycle such as *F. candida*, the IBM was contrasted with a simpler, more standardized model, based on the generic metapopulation matrix model RAMAS. I then explored consequences of model aggregation in terms of assessing population-level effects for different spatial distributions of a toxic chemical. Overall, I found that the RAMAS model was less sensitive than the IBM in detecting population-level effects of different spatial patterns of exposure. I conclude that choosing the right model type for risk assessment of chemicals depends on whether or not population-level effects of small-scale heterogeneity in exposure need to be detected. If in doubt, it is recommendable that both model types should be used and compared.

Finally I discussed some of the model findings in a perspective more broadly related to ecological risk assessment. As the current model results suggest, disregarding spatial heterogeneity in exposure, as it is the case in current ERA procedures for terrestrial ecosystems, may lead to an overestimation of risk if homogeneous contamination is assumed when it is not the case. More generally, these results suggest that a more realistic exposure assessment can significantly influence estimates of risk for soil organisms.

Resumé

Brugen af mekanistiske effekt modeller i økologisk risikovurdering af kemikalier (ERA), særligt plantebeskyttelsesmidler, har vundet momentum i de seneste år. Der har i disse år være en stigende mængde dokumentation for mekanistiske effekt modellers egnethed til at ekstrapolere effekter fra individniveau, som i den nuværende ERA praksis almindeligvis testes ved laboratorieundersøgelser, til bestandsniveau, som udgør målet for beskyttelse i de fleste EU-direktiver om sikkerhed af kemiske produkter til miljøet og menneskers sundhed. Samtidig er der fra forskellige undersøgelser fremkommet kritik af de nuværende ekstrapolerings værktøjer, som almindeligvis anvendes i ERA, såsom brugen af sikkerhedsfaktorer eller såkaldte SSD'er (species sensitivity distributions).

Et andet argument for brugen af effekt modeller i ERA er relateret til de muligheder modellerne åbner op for med hensyn til at indføre mere økologisk relevans i risikovurdering af kemikalier. For eksempel tillader effekt modeller at inkorporere faktorer, som er kendt for at påvirke naturlige populationers vækst og post-stress genopretning, men som der ikke tages eksplicit hensyn til i den nuværende risikovurderings praksis, fordi de ikke er mulige at medtage i standardiserede laboratorietest. Blandt disse faktorer er naturlige stressfaktorer, gentagne udledninger eller påføringer af det samme kemikalie eller kombinerede effekter af flere forskellige kemikalier.

Trods den stigende anerkendelse af deres potentiale til at forbedre risikovurderinger, er brugen af økologiske effekt modeller i den regulatoriske beslutningstagning stadig meget begrænset, i modsætning til brugen af modeller til at beregne og forudsige skæbnen af stoffer i miljøet. For at forstå hvordan mekanistiske effekt modeller bliver opfattet af relevante aktører, og identificere de faktorer som forhindrer modellernes integration i ERA, præsenteres resultaterne af en undersøgelse foretaget blandt interessenter fra den akademiske verden, erhvervslivet og regulerende myndigheder involveret i ERA. Blandt de vigtigste grunde identificeret i studiet er manglen på officielle og standardiserede vejledninger for udvikling og anvendelse af mekaniske effekt modeller, og modstridende forventninger til modellerne. Ifølge undersøgelsen, bør modellerne være enkle og brugervenlige nok til at være letforståelige, lette at parameterisere, og kunne anvendes på en standardiseret måde. Samtidig, forventes modellerne dog også at være komplekse nok til at være realistiske og i stand til at opfange et bredt spektrum af økologiske scenarier.

I denne afhandling giver jeg mit bidrag til de igangværende bestræbelser på atafklare og imødekomme de forventninger der er til brugen af økologiske modeller i risikovurdering. I den forbindelse viser jeg, at ved brug af god modellerings praksis og standardiserede formater til mo-

del dokumentation, er økologiske effektmodeller et egnet redskab til at ekstrapolere fra standard toksicitetsdata til de relevante beskyttelse smål. Mere specifikt har jeg udviklet to populations modeller for springhalen, *Folsomia candida*, og brugt modellerne til at undersøge effekter af heterogen fordeling af jordforurening på populationsdynamik hos springhalen. Studiet demonstrerer dermed en muliganvendelse af økologiske modeller i forbindelse med risikovurdering af kemikalier.

En rumligt eksplicit individ-basere tpopulations model (IBM), blev udviklet og testet i henhold til den såkaldte mønster-orienterede modellerings teori (pattern oriented modelling, POM). Individer i modellen kan sanse og undgå forurenet habitat med en vis sandsynlighed afhængig af forureningsgraden. Regler og parametrei modellen er baseret på forudgående kendskab til artens biologi og økologi. For implementering af toksicitet, anvendtes data for overlevelse, reproduktion og undvigelse af kemikaliet fra standard laboratorieforsøg. Modellen blev brugt til at teste forskellige hypoteser vedrørende effekter af forskellige mønstre af habitatfragmentering og forstyrrende begivenheder på *F. candida* populationsvækst og genopretning efter påvirkning. De pågældende populations effekter kan tolkes som resultater af interaktioner mellem naturlige stressfaktorer og forskellige anvendelser af landbrugskemikalier.

For at undersøge om den kompleksitet der er inkluderet i den pågældende IBM er nødvendig for at forudsige risikoen for en art med en forholdsvis enkel livscyklus såsom *F. candida*, sammenlignedes modellen med en enklere og mere standardiseret modeltype baseret på den generiske metapopulations matrix model RAMAS. Samlet set fandt jeg, at RAMAS modellen var mindre følsom end IBM modellen i forhold til at afsløre populations effekter af forskellige rumlige fordelinger af kemikalier. Jeg konkluderer, at det at vælge den rigtige model type til risikovurdering af kemikalier afhænger af hvorvidt det er nødvendigt og relevant at detektere populations effekter af små-skala heterogenitet i eksponeringen.

Endelig har jeg diskuteret nogle af model resultaterne i et mere bredt risikovurderings perspektiv. De præsenterede modelresultater giver et fingerpeg om at de nuværende ERA procedurer for terrestriske økosystemer, kan føre til en overvurdering af risiko, hvis homogen forurening antages, når det ikke er tilfældet. Derfor peger disse resultater på, at en mere realistisk vurdering af eksponeringen væsentligt kan påvirke den estimerede risiko af kemikalier for jordorganismer.

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