A realistic modelling framework to characterize individual- and population- level effects of chemicals on *Daphnia magna*. Implications for ecological risk assessment

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SUMMARY

The current approach for environmental risk assessment (ERA) of chemicals suffers several limitations. For instance, environmental protection goals often target populations of species whereas ERA relies on standardized laboratory tests in which toxicity is measured on individual endpoints. From these tests, the threshold concentration of a chemical, below which no population-level effects should occur, is derived. Such procedure is not based on sound science and its effectiveness in capturing effects on individuals and populations is highly disputed. In addition, these laboratory tests are conducted under optimal conditions whereas in the field, populations have to cope with varying environmental conditions and natural stressors as well. Accounting for the environmental context remains, however, only marginally considered in ERA. A realistic estimation of population-level effects of chemicals calls for the use of comprehensive methodologies that allow extrapolating effects on individuals to the population level, as well as accounting for multiple stress effects.

In this context, the potential of ecological models in improving the accuracy of ERA of chemicals has been increasingly advocated. In the present thesis, I contribute to demonstrating the power of this tool in providing a more accurate and comprehensive ERA of chemicals. I used an established individual-based model (IBM) for *Daphnia magna* to explore different research questions that limit the accuracy of the current methodology. For each addressed question, I refined the model accordingly by implementing toxicity submodels, additional individual traits or further environmental processes.

Summary

I was first interested in a toxicant for which adverse effects were detected at the determined threshold concentration and thus challenged the conservationism of the current ERA approach. Multiple effects of this toxicant were reported on exposed daphnids, in addition to the commonly measured effects on reproduction and survival. I applied a multi-modelling approach to understand individuals' responses and extrapolate them to the population level. Thereby, I combined the IBM with different toxicity submodels describing individual effects on reproduction and survival. Using the IBM, I ran simulations to extrapolate these effects to the population level. Simulation results confronted to population experiments revealed that these endpoints did not fully capture effects on populations. To this end, additional individual-level effects had to be integrated and were thus behind the failure of risk assessment to be conservative.

Second, I explored the influence of ecological interactions on population sensitivity to chemicals with different modes-of-action on individuals, by using the IBM as a virtual laboratory. Thereby, I tested multiple stress exposure scenarios, by combining different chemical and non-chemical stressors' effects on individuals. In the model, chemical toxicity targeted different vital individual-level processes. As for species interactions, predatory and competition effects were implemented following a worst-case approach. Population dynamics were simulated at different food levels and exposure scenarios. Results revealed that population responses to chemicals are highly sensitive to the environmental stressor (predator or competitor). Additionally, important ecological features like density dependence, availability of food resources or the Allee effect caused by predatory behaviour were identified as prominent drivers of population sensitivity to chemicals. This study demonstrates that population resilience cannot be attributed to chemical stress only and that accounting for relevant chemical and non-

chemical interactions would reduce uncertainties when extrapolating chemicals' effects to the population level.

Finally, I used the same modelling framework to assess the impact of the environmental scenario on population recovery from lethal effects. Population recovery is still mainly determined from mesocosm experiments, which provide only limited information related to the adopted experimental design. Simulation experiments were performed for chemicals with different lethality levels at different food and temperature conditions, with and without species interactions (predation or competition). Results revealed that recovery of populations strongly depended on the environmental scenario. This dependency was expressed in the highly heterogeneous responses to the same chemical when the environmental conditions changed. In addition, no specific role could be attributed to any environmental variable in isolation. Only the complex interactive mechanisms between the different factors constituting the full environmental scenario determine their mutual roles in controlling the recovery of populations. Unless these combinations of factors and effects are simultaneously taken into account in ERA, we cannot achieve a complete understanding of the mechanisms controlling population recovery from chemical exposure.

In conclusion, the findings of the present thesis demonstrate that ecological modelling holds a great potential in assisting risk assessment of chemicals in the future by i) providing a mechanistic explanation of toxicity effects on individuals and their consequences on populations, ii) identifying the modes of action triggering population-level effects, and iii) integrating the necessary environmental complexity related to the species and its environment for a more realistic estimation of population-level effects.

ZUSAMMENFASSUNG

Der aktuelle Ansatz der Umweltrisikoeinschätzung von Chemikalien zeigt bei näherer Betrachtung einige Schwächen: Schutzziele werden oft auf Populationsebene einer Spezies definiert, wohingegen die Risikobewertung auf standardisierten Laborstudien basiert, bei denen die Toxizität einer Substanz auf einzeine Endpunkte der Organismenebene berichtet wird. Auf Basis dieser Laborstudien wird der Grenzwert einer chemischen Substanz in der Umwelt festgelegt unterhalb dessen keine Wirkung auf Populationsebene zu erwarten ist. Durch eine solche Herangehensweise ist es fraglich, ob Effekte auf Individuen- oder Populationsebene korrekt beschrieben werden können. Des Weiteren werden diese Laborstudien unter optimalen Bedingungen durchgeführt, während eine Population im Feld variierenden Umweltbedingungen und anderen natürlichen Stressbelastungen ausgesetzt ist. Dieser ökologische Kontext wird in der Risikobewertung jedoch nur am Rande betrachtet.

Um eine realistische Abschätzung der Effekte von Chemikalien auf Populationsebene geben zu können, benötigt man umfassende Methoden, die sowohl eine Extrapolation von solchen Effekten von der Individuen- zur Populationsebene ermöglichen als auch den Effekten von multiplen Stresseinflüssen Rechnung tragen. In diesem Zusammenhang wird das Potential von ökologischen Modelle zur Verbesserung der Präzision der Risikobewertung der Umwelt zunehmend herausgestellt. In dieser Doktorarbeit zeige ich das Potential dieser Methode für eine präzise und umfassende Risikobewertung. Dazu wendete ich ein bereits etabliertes individuen-basiertes Modell (IBM) für *Daphnia magna* an, um verschiedene Fragestellungen zu

untersuchen und die heutige Methodik der Risikobewertung zu hinterfragen. Für jede Fragestellung hatte ich das Modell durch Implementierung eines Toxizitätsmodells, zusätzlichen individuellen Charakteristiken oder zusätzlichen Umweltprozessen modifiziert.

Zunächst befasste ich mich mit einer Substanz, für die bei aus Standardtests abgeleiteten Schwellenwertkonzentrationen Effekte auf Populationen Festgestellt worden waren und die dadurch den ausreichenden Konservatismus der aktuellen Risikobewertung in Frage stellt. Zusätzlich zu den üblich gemessenen Auswirkungen auf die Reproduktion und das Überleben, wurden weitere multiple Effekte bei exponierten Daphnien beschrieben. Ich habe einen Multi-Modellansatz angewendet, um das Verhalten der Individuen zunächst zu verstehen und anschließend auf die Populationsebene zu extrapolieren. Hierzu habe ich das bestehende DaphnienModell mit verschiedenen Toxizitätsmodellen kombiniert, die jeweils die Effekte der Substanz auf die Reproduktion und das Überleben der Individuen beschreiben. Mit Hilfe des individuen-basierten Modells habe ich dann die auftretenden Effekte auf die Populationsebene extrapoliert. Vergleicht man die Ergebnisse dieser Simulationsläufe mit Ergebnissen von Populationsstudien, stellt sich heraus, dass die mässig ermittelten Endpunkte (Reproduktion und Mortalität) nicht ausreichen, um die beobachteten Effekte auf Populationsebene zu erklären. Weitere Endpunkte auf der Organismenebene waren daher notwendig, um eine protective Risikoabschätzung Für die Population zu erhalten.

Anschließend habe ich das Daphnien Modell als virtuelles Labor genutzt, um den Einfluss von ökologischen Wechselwirkungen auf die Populationssensitivität gegenüber Chemikalien mit verschiedenen Wirkmechanismen zu untersuchen. Hierzu habe ich multiple Stressszenarien getestet, in dem ich chemische mit natürlichen Stressfaktoren kombiniert habe: Die chemische Toxizität richtete sich gegen verschiedene lebenswichtige Prozesse auf

Individuenebene, während biotische Wechselwirkungen durch Räuber oder Konkurrenzdruck in Form eines worst-case Ansatzes implementiert worden sind. Populationsdynamiken wurden bei unterschiedlichen Stufen der Nahrungsverfügbarkeit und Expositionsszenarien simuliert. Die Ergebnisse zeigten, dass der Effekt der toxische Substanz auf Population eine stark von dem herrschenden Umweltstress abhängig ist (Räuber oder Konkurrenz). Zusätzlich wurden auch wichtige ökologische Faktoren, wie die Dichtabhängigkeit, die Verfügbarkeit von Nahrungsressourcen oder der Allee-Effekt, hervorgerufen durch das Verhalten der Räuber, als treibende Faktoren der Populationssensitivität gegenüber einer toxischen Substanz identifiziert. Somit zeigte die Studie, dass die Resilienz einer Population nicht ausnahmslos von chemisch induziertem Stress abhängig ist. Durch die Berücksichtigung von relevanten chemischen und ökologischen Interaktionen können die Unsicherheiten bei einer Extrapolation von chemisch induzierten Effekten auf die Populationsebene reduziert werden.

Im letzten Teil der Arbeit habe ich den oben beschriebenen Modellierungsansatz genutzt, um den Einfluss der Umweltbedingungen auf die Erholung der Population von letalen Effekten abzuschätzen. Dieser Endpunkt wird immer noch hauptsächlich mit Hilfe von Mesokosmos-Experimenten bestimmt, die jedoch nur begrenzte Informationen zu dem angewandten experimentellen Design bieten. Modellsimulationen wurden bei verschiedenen Bedingungen durchgeführt: Substanzen mit unterschiedlich starker wirkung auf Mortalität bei verschiedenen Nahrungsangebot- und Temperaturbedingungen jeweils mit und ohne durch Räuber oder Konkurrenzdruck. Die Ergebnisse zeigen, dass die Erholung der Population stark vom jeweiligen Umweltszenario abhängig ist und einzelnen Umweltparametern keine spezifische Rolle zugewiesen werden konnte. Ausschließlich die komplexen Wirkungsmechanismen zwischen den verschiedenen Umweltfaktoren bestimmen ihre

jeweiligen Rollen bei der Erholung von Populationen. Solang diese Faktoren und Effekte nicht gemeinsam für die Risikobewertung der Umwelt in Betracht gezogen werden, sind wir nicht in der Lage ein vollständiges Verständnis der Mechanismen der Populationserholung nach einer Schadstoffexposition zu entwickeln.

Zusammenfassend zeigen die Ergebnisse der vorliegenden Studie, dass die Einbeziehung von ökologischen Modellen in die Risikobewertung von Chemikalien eine weitaus realistischere Einschätzung der toxischen Wirkungen auf Nicht-Zielorganismen im Feld schaffen kann. Dies geschieht zum einen durch die Bereitstellung einer mechanistischen Erklärung der toxischen Wirkungen auf Individuen und ihrer Folgen auf die Population, zum anderen durch die Ermittlung der relevanten Endpunkte, die auf Populationsebene Effekte auslösen, und durch die Einbeziehung der erforderlichen ökologischen Komplexität der Art und seiner Umwelt, für eine realistische Einschätzung der Auswirkungen auf Populationsebene.

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CHAPTER 1

General introduction

1.1. Human well-being and ecosystem welfare

Our modern society is strongly marked with an increasing motivation to satisfy human comfort and well-being. We witness a spectacular development of all industrial activities, either in the sectors devoted to serving our daily needs (e.g. food, household, textile or transport industries) or those that directly target our health (e.g. pharmaceuticals, healthcare products, etc.). Chemicals have undoubtedly become an integral part of our everyday life and new products with a higher efficiency are being daily manufactured and placed into the market.

Such extensive use of chemicals unfortunately results in considerable amounts of anthropogenic substances which are directly spilled into the aquatic environment, or reaching the water bodies via various pathways like drift, drainage, run-off or erosion (mainly for the plant protection products). Water contamination is one of the main threat sources to global freshwater biodiversity, along with over-exploitation, degradation of habitat and invasion by exotic species (Dudgeon et al. 2006). A loss in freshwater biodiversity severely impairs the balance and good functioning of aquatic ecosystems (Sala et al. 2000) which would in turn significantly affect the human well-being (WHO, 2005; Dudgeon et al. 2006). In fact, aquatic ecosystems support the economic development by sustaining fisheries and fishing industries

(Tilman et al. 2002). Additionally, they provide freshwater for irrigation, domestic use and power (Postel et al. 1997). Furthermore, an aquatic ecosystem in a good status provides important socio-cultural services and recreational activities to mankind like tourism, sports or education (Harwell et al. 1992; Costanza et al. 1997; De Groot et al. 2002). Most important, human health directly depends upon ecosystem products and services, several of which are provided by aquatic ecosystems like provisioning good-quality food as well as potable freshwater (Postel et al. 1997; Tilman et al. 2002; WHO, 2005). Hence, maintaining the human well-being stems for preventing the loss of ecosystem resilience (Walker, 1995; Tilman et al. 2002).

1.2. Risk assessment of chemicals in Europe

The preservation of ecosystems and living communities from effects of chemicals has become a topic of global concern. The need for chemicals is inevitable, but an appropriate use can prevent many of their negative impacts while maintaining their multiple benefits for the human society (Forbes, 2010). In this context, the European Commission imposes strict regulation rules whereby the impacts of newly manufactured chemicals are quantified. These regulations come in two parts: the human health risk assessment and the ecological risk assessment (ERA) (EFSA, 2013; SANCO, 2013). Unlike in the first regulation where the human being is the only entity to be protected, in ERA, preventing chemicals from disrupting the good functioning of an ecosystem presupposes defining measures that ensure the protection of all entities living within that ecosystem (SANCO, 2013). Since it is neither possible nor ethical to test every single organism within each exposed ecosystem, ERA addresses the use of test species as surrogates to establish protective measures for all non-target organisms (European Commission, 2003; SANCO, 2013).

1.3. *Daphnia magna* as a surrogate species in ecological risk assessment

The water flea *Daphnia magna* is extensively used in ERA as a surrogate species for freshwater aquatic invertebrates and marine arthropods (e.g. ostracods, copepods). The adoption of *D. magna* in ERA stems from several reasons. First, the physiology and biology of *Daphnia* are well-studied (De Marchi, 1987) due to its ease of handling and culturing in the laboratory. Secondly, its parthenogenetic reproduction as well as its short life span allow to easily conducting chronic toxicity tests (De Marchi, 1987; OECD, 2008). Hence, its sensitivity to a wide range of chemicals including pesticides, insecticides or industrial chemicals is supported by a substantial amount of literature (Wogram and Liess, 2001; OECD, 2004, 2008). Finally, its cosmopolitan distribution (from acidic swamps to freshwater lakes, ponds and rivers) in addition to its important position in the aquatic food chain as a filter-feeder of phytoplankton and a prey for several insect (e.g. *Chaoborus* larvae) and fish species makes it more ecologically relevant than other species (De Marchi, 1987).

1.4. Current risk assessment practice for aquatic invertebrates

In ERA of aquatic invertebrates, the response of *Daphnia* to toxicity is derived from the standard *Daphnia* immobilization (OECD 202, 2004) and *Daphnia* reproduction (OECD 211, 2008) tests, whereby effects are reported on two main individual endpoints, survival and reproduction. Occasionally, effects on growth and development are also determined. Unlike vertebrates whose individual mortality has to be prevented, for aquatic invertebrates, the aim is usually to protect populations (EFSA, 2010). Thus, toxicity effects measured on individuals have to be extrapolated to the population level. Nonetheless, individual responses to chemical exposure do not directly translate into population-level effects (Hammers-Wirtz and Ratte,

2000; Thorbek et al. 2010; Preuss et al. 2010) but other important factors also control the population dynamics under natural and stressed conditions.

1.5. Factors influencing population dynamics

1.5.1. Life-history traits of the species

Individual life-cycle properties such as lifespan, time to first reproduction, fecundity and generation time control important population-level aspects related to abundance and population structure and subsequently determine the population's resilience to natural (La Montagne and McCauley, 2001) and anthropogenic (Stark et al. 2004 a; Solomon et al. 2008; Preuss et al. 2009; Thorbek et al. 2010) stressors. In fact, species exhibiting differences in these key life history traits respond differently to equal levels of mortality or inhibition of reproduction (Stark et al. 2004 a).

1.5.2. Density dependence

Important biological processes regulating population dynamics of several invertebrate (daphnids, copepods, springtails; see Preuss et al. 2009; Sibly et al. 2000; Ferguson and Joly, 2002; respectively) and vertebrate (fish, wood mice, birds, see Hazlerigg et al. 2012; Stenseth et al. 2002; Rodenhouse et al. 2003; respectively) species are highly density-dependent. These processes can include the reproductive strategy, feeding behavior, growth and/ or survival of individuals. Density-dependence is very important as it influences the adaptive mechanisms of populations to different stress sources like population resistance to starvation (Preuss et al. 2009), resilience to exploitation (Hazlerigg et al. 2012) but also its sensitivity to chemical stress exposure (Linke-Gamenick et al. 1999; Solomon et al. 2008; Preuss et al. 2010; Hazlerigg et al. 2012). In the latter case, it has been shown that density-dependence can alleviate the severity of the chemical stressor impact (Solomon et al. 2008; Hazlerigg, 2011) or conversely, to increase it

(Klüttgen and Ratte, 1994; Forbes et al. 2003; Knillmann et al. 2012 a). Accounting for density-dependence is therefore crucial for assessing the real impact of toxicants on population dynamics.

1.5.3. Natural inter-individual variability

Organisms of the same species, even those originating from the same mother and belonging to the same clone or brood (in the case of *Daphnia*; Boersma, 1997) are not identical (Grimm and Uchmański, 2002; Bolnick et al. 2011; Jager, 2013). This inherent heterogeneity is expressed through different behaviors towards abiotic conditions, resource use, anti-predatory defenses or competitive ability (Bolnick et al. 2011). This, in turn leads to different physiological parameters related to the feeding, growth, development, reproduction or survival of individuals. Natural inter-individual variability also generates different intrinsic sensitivities to chemicals (Naylor et al. 1990; Jager, 2013). In comparison, toxicity tests are conducted under optimal conditions in which all efforts are deployed to reduce this natural variability (Sakwinska, 2004). For instance, in *Daphnia* reproduction tests, all test individuals should belong to the same clone, originate from the same culture and be the same age (≤ 24 hours, OECD, 211). In these tests, differences among individuals are considered by means of replicates which are often reduced to a minimum for practicability (10 and 20 animals at least for each tested concentration in the reproduction and acute toxicity tests, respectively; OECD, 211; OECD, 202). Results generate a general response of the species to a certain treatment (Jager, 2013). In comparison, the overall population dynamics and its response to chemical exposure are the result of the sensitivity of each individual within that population (Preuss et al. 2010; Thorbek et al. 2010).

1.5.4. Multiple stress exposure and biological interactions

In natural systems, abiotic (temperature) and biological factors (e.g. food, predation and competition) and their interactions control population dynamics and very likely influence their resilience to chemical stress exposure (Heugens et al. 2006; Coors et al. 2008; Solomon et al. 2008). These co-occurring factors may act additively, synergistically, or antagonistically and alter the population sensitivity to the chemical. Nonetheless, biological interactions are only marginally considered in ERA, i.e. via mesocosm experiments, which are costly, time consuming and are subsequently exclusively used for higher tier risk assessment (Bednarska et al. 2013). In addition, these experiments can only provide information on the specific types of biological and chemicals interactions (EFSA, 2013), which were taken into account in the experimental design, whereas field situations are characterized by a wide range of possible scenarios (Hommen et al. 2010). Thus, complementary methods are needed in environmental risk assessment to account for multiple stress exposure and the variable environmental conditions in the field.

1.5.5. Understanding the mode of action of the toxicant

In most standard toxicity tests, chemical toxicity is evaluated from the negative effects on reproduction, growth or survival of individuals. The mechanisms that lead to such effects are overlooked. In reality, similar inhibition levels of reproduction or survival would lead to different impacts on populations depending on the individual process that was targeted by the toxicant (Martin, 2013) and which provoked the observed magnitude of effect on reproduction or survival. In other cases, capturing how the toxicant acts on individuals can sometimes be crucial for identifying the relevant population-level endpoint. In fact, according to the current methodology in chemicals' risk assessment, unacceptable effects occur when a reduction in the

population abundance is observed (or, if the ecological recovery option is used, when the population abundance cannot recover within a given time frame). In reality, adverse effects of chemicals on populations might not be expressed via a reduction in the population size but other important population endpoints can be altered as well. For instance, in many organisms, not all developmental stages are equally sensitive to toxicant exposure as observed for instance in daphnids exposed to p353-nonylphenol (Preuss et al. 2008; Gergs et al. 2013) or copepods to triphenyltin (Kulkarni et al. 2013). Size distribution is a very important response endpoint that regulates population dynamics and controls their resilience from exposure to natural (La Montagne and McCauley, 2001) and chemical (Stark and Banken, 1999; Gergs et al. 2013) stressors. More importantly, an alteration in the size structure is not necessarily accompanied with a reduction in the population abundance (Gergs et al. 2013). In such cases, size- (stage) dependent toxicity would induce negative drawbacks on the dynamics of the populations, which might not be perceived if we only look at the total abundance (Stark and Banken, 1999; Gergs et al. 2013). Thus, attention should be paid to the mode of action of the toxicant, and on the consequently affected population endpoints.

1.6. Ecological models for a more realistic risk assessment of chemicals

Accounting for the features summarized in the previous section goes far beyond the standard toxicity tests. To compensate for these multiple sources of uncertainty, ERA considers the use of safety factors which are assigned to measured toxicity endpoints in laboratory tests, and the resulting concentration is considered safe for populations in natural systems. For example, a regulatory acceptable concentration is derived from a *Daphnia* reproduction test by dividing the NOEC for the inhibition of reproductive output produced by a female over 21 days by an assessment factor which is determined based on data availability. This approach clearly

lacks a scientifically sound background and results in arbitrary estimations of toxic effects on field populations (Forbes et al. 2008). The need for more robust scientific tools has been clearly evoked in the latest EFSA opinion (2013).

Ecological models are effective tools for realistically predicting toxicity effects on populations, and their potential to address important ecotoxicological issues has been recognized for more than 30 years (O'Neill et al. 1982). They allow extrapolations which are impossible to fully address experimentally such as extrapolations to higher organizational levels (from individual to population, from mesocosm to the field, etc.), between exposure scenarios (Forbes et al. 2008) or to other species (Hommen et al. 2010). Their greatest advantage remains their ability to integrate the required ecological complexity by accounting for interactive effects of the different factors stated in the previous section, leading to more realistic predictions of population level effects of toxicants (Forbes et al. 2008; Grimm et al. 2010; Hommen et al. 2010; EFSA, 2013). This issue has been advocated (European Commission, 2012; EFSA, 2013) as the highest priority task which should be addressed by the scientific community to improve the effectiveness of ERA, and ecological models are mentioned as the only tool that allows preventing adverse toxicity effects on the environment.

1.7. Aims and structure of the thesis

In this thesis, I test the power of ecological modelling to improve the effectiveness of the current ERA of chemicals. I apply an established individual-based population model (IBM) for *Daphnia magna* (IDamP, Preuss et al. 2009) to answer different research questions of relevance to ERA. Pertinent to each research question addressed in the different chapters, the IDamP model is refined accordingly, by implementing different toxicity submodels, additional

individual properties or/and by incorporating further environmental realism. This refinement of the IDamP model is explained in detail in the subsequent chapters.

First, an overview of the IDamP model's purpose, concept, validation and applications is provided in **Chapter 2**. In the subsequent chapters, the IDamP model is only outlined briefly to avoid repetitions.

In **Chapter 3**, I employ a multi-modelling approach, by coupling the IBM to different toxicity submodels (regression models and toxicokinetic/toxicodynamic models), to understand individuals' responses to chemical exposure and extrapolate the effects to the population level. I select a toxicant for which adverse effects on laboratory populations were detected at the threshold concentration derived from standard toxicity tests and thus challenged the conservatism of the current risk assessment method. In addition to the toxicant effects on survival and reproduction, I identify further modes of action triggering population-level effects and which were the reason behind the failure of the current risk assessment to be protective at the population level.

In **Chapter 4**, I adapt the IDamP model to predict *Daphnia* individual life-cycle and population dynamics in a more ecologically relevant manner, by implementing a submodel describing the body size of neonates with different maternal traits and environmental variables. The importance of this parameter in determining several processes in *Daphnia* populations has been broadly acknowledged; yet, almost never addressed in ecological models. I identify the most potent maternal traits and environmental factors controlling the variation in the newborn body size of daphnids, and validate the model against an independent dataset obtained with different *Daphnia* clones and a different food source.

In **Chapter 5**, I apply the virtual ecologist approach by using the IDamP model as a virtual laboratory to explore the influence of different environmental factors and species interactions on population sensitivity to chemicals with different modes-of-action on individuals. Species interactions include worst-case scenarios for competition and predatory effects. The purpose of this study is to investigate the changes caused by the presence of different (combinations of) environmental stressors in population sensitivity to chemicals and to address the implications for the current risk assessment.

In **Chapter 6**, I investigate the importance of the environmental scenario in affecting population recovery from exposure to lethal toxicity. Understanding recovery processes is one of the main priorities (defined by SANCO, 2013) towards improving ERA of chemicals. To this date, this concept is used as an option in the evaluation of microcosm or mesocosm experiments, which are very expensive and time demanding but at the same time, investigate a limited range of exposure scenarios. In this chapter, and using the same modelling framework, the potential of the environmental scenario in altering the recovery of *Daphnia* populations is evidenced in several exposure scenarios.

Finally, in **Chapter 7**, I summarize the important findings from the different chapters and explain how they can assist in achieving a more realistic decision making.

CHAPTER 2

The individual-based model IDamP: General overview

IDamP (Preuss et al. 2009) is an individual-based population model that predicts the population dynamics of *Daphnia magna* based on individual life cycles including feeding, somatic growth, development, reproduction and survival processes (Fig. 2.1). The main drivers of these processes are the food conditions and, via crowding effects, the density of the population. IDamP addresses a laboratory scale of vessels maintained at 20 °C and with the algae *Desmodesmus subspicatus* as a food source.

Population dynamics including population capacity and size structure emerge from the interactions of the individuals with each other (intra-specific competition) and with their environment (food concentration).

IDamP was implemented in Delphi using Embarcadero 2010 RAD studio XE2. It is documented using the ODD (Overview, Design concepts, Detail; Grimm et al. 2006) protocol for describing individual-based models (Preuss et al. 2009). In addition, a detailed TRACE documentation (Transparent and Comprehensive Ecological modelling documentation) of the IDamP model was compiled by Gabsi et al. (2014) following Schmolke et al. (2010).

In the following paragraphs, I briefly describe the different individual processes (submodels) included in IDamP. In addition, I provide an overview on the different forcing

functions of the model which influence the life cycle of individual daphnids. The full details about these functions and processes can be found in Preuss et al. (2009).

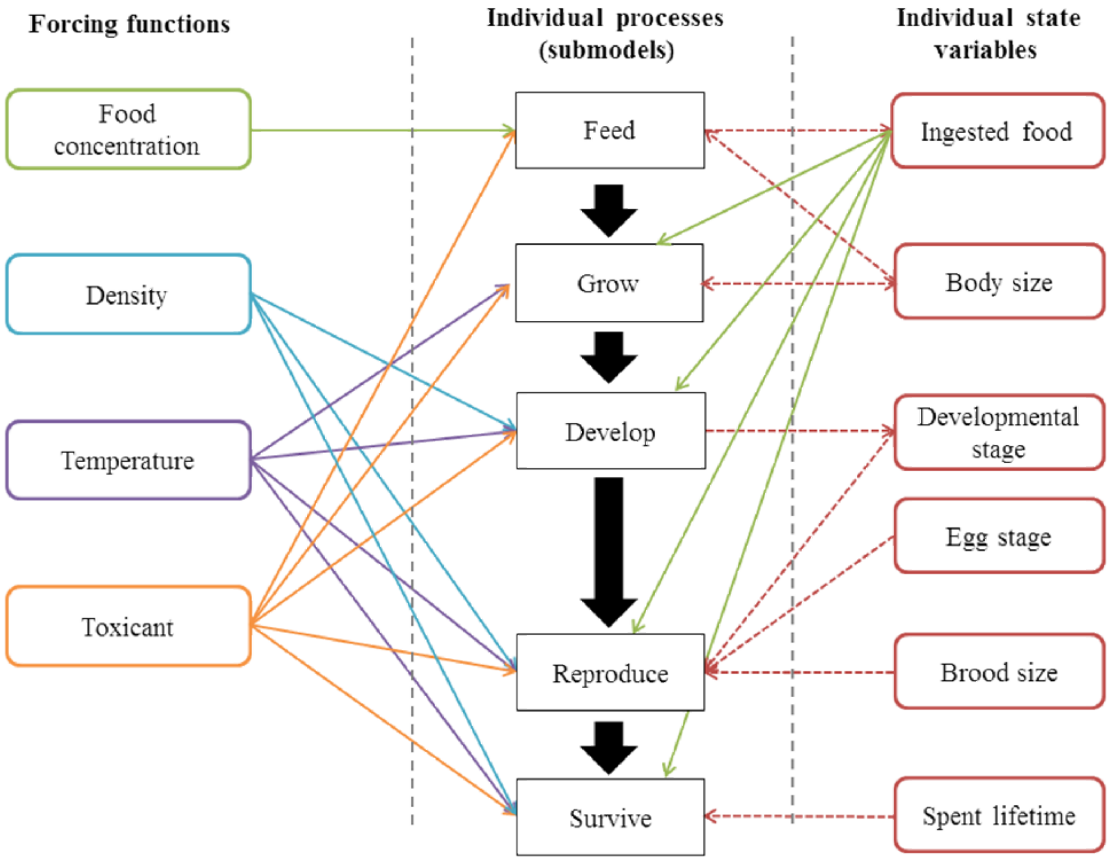

Fig. 2.1. Conceptual diagram of the IDamP model describing the asexual life cycle of *Daphnia magna* including the different individual processes. Full arrows indicate the processes which are influenced by the forcing functions considered in the model. Dashed arrows indicate the relationships between the individual state variables and the life cycle processes within the model.

2.1. Description of the different submodels

All submodels of IDamP representing the life-cycle processes are descriptive regression models, which are based on a large dataset from different life cycle tests. In addition to the average life cycle parameters for different food levels, stochasticity is considered in all

submodels (processes) whereby an individual random parameter derived from different statistical distributions (determined based on results of the life table experiments used for calibration, Preuss et al. 2009) is attributed for each individual daphnid and each process.

2.1.1. Feeding rate

The feeding rate of a daphnid is calculated using the maximum filtration rate (depending on the length of the daphnid), a half-saturation constant and the incipient limiting level. The filtration rate is constant below the incipient limiting level and then it decreases with increasing food concentration.

2.1.2. Somatic growth rate

The individual daphnid's growth is calculated using the Von Bertalanffy growth equation. In this equation, the maximum length depends on the feeding rate. The daily increase in length and thus the growth rate are calculated. The somatic growth rate depends therefore on the amount of ingested food.

2.1.3. Development

The juvenile development rate (the reciprocal value of the time to reach maturity) is proportional to the log of the ingestion rate. The actual proportion of the juvenile development finished is calculated by summation of daily development rates and maturity is reached if the spent juvenile development exceeds 1.

2.1.4. Reproduction

The reproductive potential is determined by the brood size, which is proportional to the length of the daphnid. The reproductive rate is positively correlated to the feeding rate.

2.1.5. Survival

Survival probability over time is described by the Weibull function whose time and shape factors are related to the ingested food. Death occurs when the spent lifetime exceeds the age at death, which is assigned from a random uniform distribution for each daphnid at birth.

2.2. Driving forces of individual processes in IDamP

2.2.1. Food concentration

All individual processes directly depend on the ingested amount of food (as described in the previous section and in Fig. 2.1), which is determined by the food concentration administered in the test medium.

2.2.2. Density dependence

Density-dependence is included in IDamP and is expressed via crowding. Crowding occurs when the density of the population becomes high enough that the individuals sense the limitation in the available space and influence each other by releasing metabolic substances or by physical contact (Goser and Ratte, 1994). Crowding corresponds to an available volume per individual daphnid of lower than 50 ml (Goser and Ratte, 1994). In IDamP, reproduction is a highly density-dependent process: under crowding conditions, the individual daphnids shift their reproductive strategy towards producing larger (following an exponential pattern, as discussed later in **Chapter 4**) and fewer offspring. Crowding also slows down both the embryo- and the juvenile- (reciprocal value to reach maturity) development rates, leading to a delay in the maturity process. Finally, the survival of neonates and juveniles (but not adult survival) is affected with increasing crowding conditions.

2.2.3. Temperature

The influence of temperature on the different individual processes was not described in Preuss et al. (2009). Because the variability in temperature conditions and its consequences on populations are considered in the present thesis (**Chapter 6**), the implementation of the effects of this environmental parameter on individual processes is described in the subsequent paragraphs.

For a species-specific range of temperature, the variation of a certain process (F (t)) with temperature is usually well described by the Arrhenius equation (1889; Eq. 1) (Kooijman, 2000; Rinke and Vijverberg, 2005).

$$F(T) = \exp\left(\frac{T_A}{T_{ref}} - \frac{T_A}{T}\right) \quad (1)$$

with T being the absolute temperature, T_{ref} the chosen reference temperature and T_A the Arrhenius temperature.

Kooijman et al. (1989) proposed an Arrhenius temperature (T_A) of 6400 K at a reference temperature (T_{ref}) of 293 K for *D. magna*. This proposed fit was tested against a large literature dataset for *D. magna* for the different processes within IDamP, including the filtration rate, embryo- and juvenile development rates, somatic growth rate as well as reproduction and survival. Data from literature were normalized to 20 °C by dividing the rate value at a specific temperature with that at 20 °C. If no data were reported at 20 °C, either the average of the temperature below and above 20 °C, or the temperature with a deviation of 1 °C was used.

In the following paragraphs, the calibration of individual processes with temperature is described. When no deviation to the literature data was found, the Arrhenius function was used without any further calibration. Conversely, when this model did not explain the data, other functions were fitted into the transformed data (using SigmaPlot 11.0 SPSS Inc.) using a best fit

approach. This was especially the case when temperatures rising above an optimum for the organism induced a decline in a specific rate.

2.2.3.1. Influence of temperature on the filtration rate

Five dataset were used to calibrate temperature effects on the filtration rate whereby temperature ranged between 5 and 35 °C (McMahon, 1965; Burns, 1969; Plath, 1998). A clear optimum curve (Fig. 2.2) was depicted from the data which could not be described by the Arrhenius equation. Therefore the following four-parameter peak curve was used (Eq. 2).

$$F(t) = a \times \frac{c-1^{\frac{1-c}{c}}}{c} \times \left|\left(\frac{T-T_{opt}}{b}+\frac{c-1^{\frac{1}{c}}}{c}\right)^{c-1}\right| \times e^{-1\times\left|\left(\frac{T-T_{opt}}{b}+\frac{c-1^{\frac{1}{c}}}{c}\right)^{c-1}+\frac{c-1}{c}\right|} r^2 = 0.85 \quad (2)$$

with a = 1.265; b = 6475.8; T_{opt} (°C) = 26.02; c = 911.5.

The optimum temperature calibrated (26 °C) was near the optimum of 25 °C observed by Mitchell and Lampert (2000).

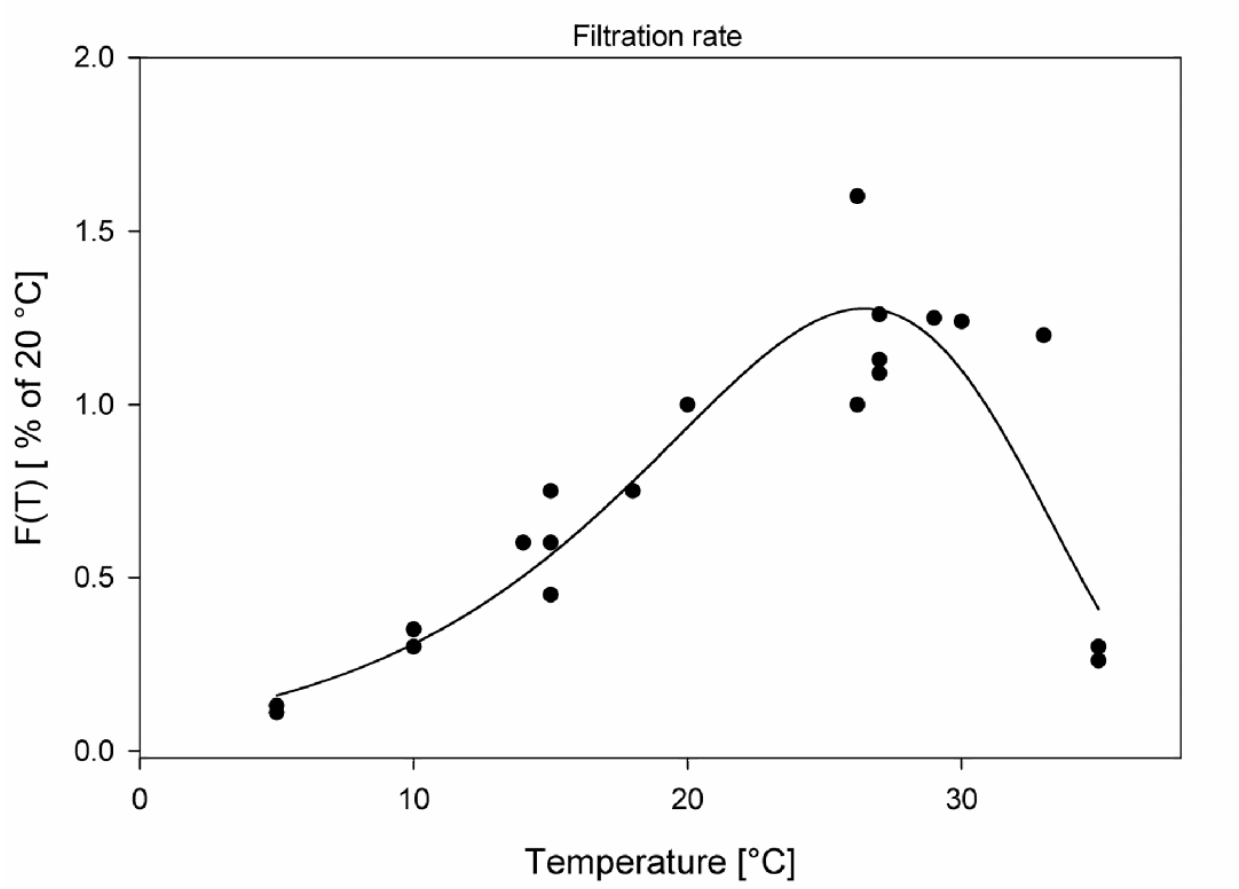

Fig. 2.2. Dependency of the filtration rate on temperature. The filtration rates of *D. magna* were normalized to 20 °C, resulting in the temperature function F (T), and are shown as black dots. The solid line represents the fitted regression function.

2.2.3.2. Influence of temperature on the survival time and the somatic growth rate

In the dataset used to calibrate effects on survival, temperature ranged between 14 and 29 °C (Orcutt and Porter, 1984; Korpelainen, 1986; Fitsch, 1990) whereas for the somatic growth rate dataset, a range of 10 to 27 °C was reported (Foran, 1986; Fitsch, 1990; Reichwaldt et al. 2004). For both processes, the Arrhenius equation fitted well into the literature data (Fig. 2.3 a, b) and was therefore used without further calibration

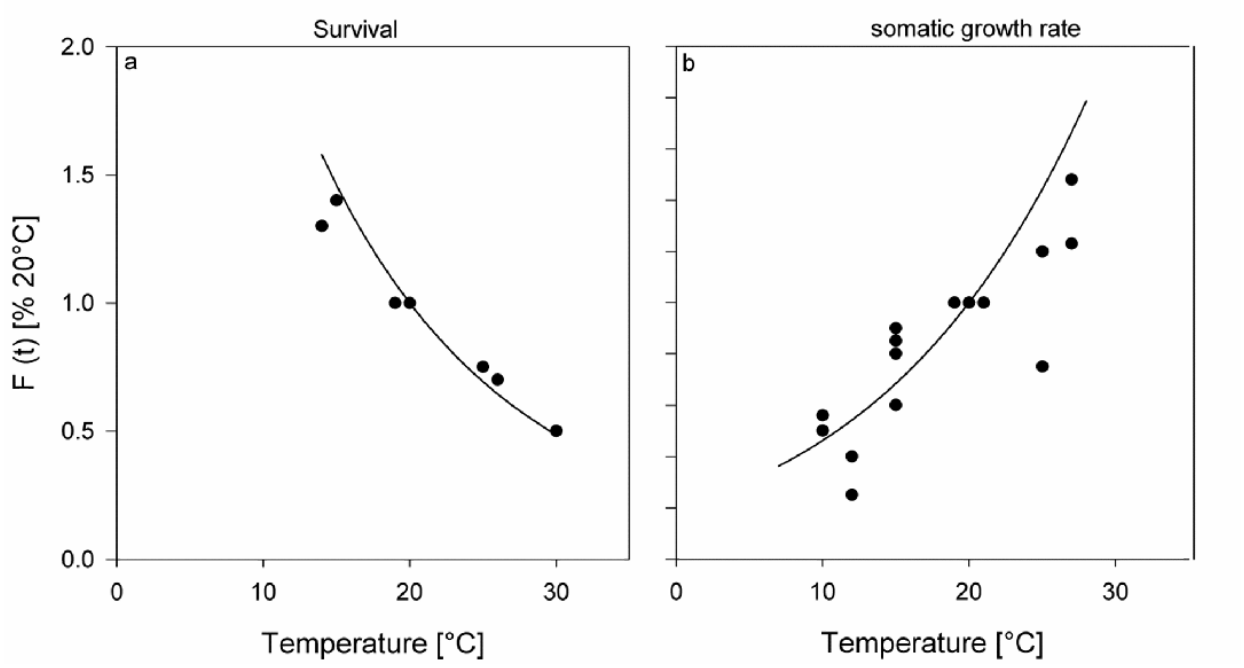

Fig. 2.3. Dependency of survival (a) and somatic growth rate (b) of *D. magna* on temperature. Data were normalized to 20 °C, resulting in the temperature function F (T), and are shown as black dots. Solid lines represent the fitted Arrhenius function.

2.2.3.3. Influence of temperature on the developmental rates

Four datasets were available for calibrating temperature effects on the embryo-development rate (Orcutt and Porter, 1984; Stephenson and Watts, 1984; Fitsch, 1990; Dülmer, 1998) and temperature ranged between 10 and 25 °C. For the juvenile development rate, six datasets were found between 5 and 30 °C (Goss and Bunting, 1983; Orcutt and Porter, 1984; Stephenson and Watts, 1984; Sakwinska, 1998; Giebelhausen and Lampert, 2001).

For both processes, a plateau, but not a decrease, was observed in the data above 20 °C, leading to a deviation from the Arrhenius equation at higher temperatures. Additionally, at temperatures below 15 °C, a deviation to the Arrhenius equation was found. The variation of the embryo- and juvenile development rates with temperature (Fig. 2.4 a, b) was successfully described by the following sigmoidal function (Eq. 3):

$$F(t) = \frac{a}{1 + e^{-1 \times \frac{T - X0}{b}}} \quad (3)$$

with a = 1.504; b = 6.018; X_0 = 16.211 for the embryo-development rate (r^2 = 0.93).

and a = 1.232; b = 3.366; X_0 = 15.09 for the juvenile-development rate (r^2 = 0.73).

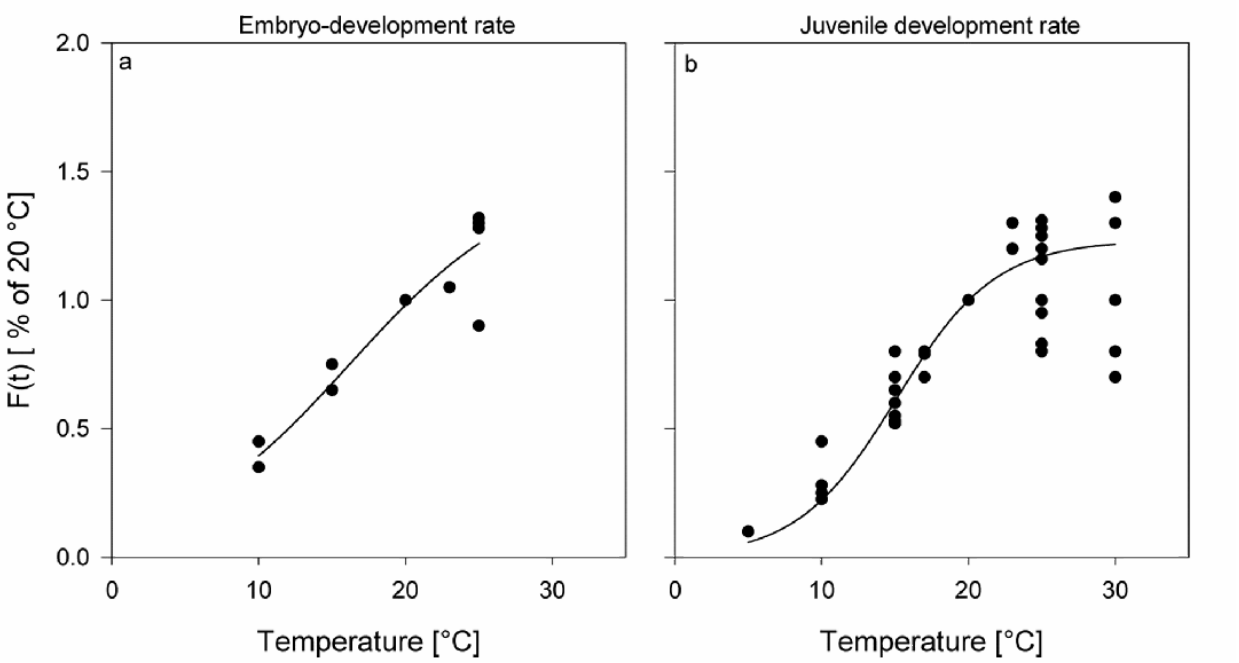

Fig. 2.4. Dependency of the embryo (a) and juvenile (b) development rates on temperature. The developmental rates of *D. magna* were normalized to 20 °C, resulting in the temperature function F (T), and are shown as black dots. The solid lines represent the fitted regression functions.

2.2.3.4. Influence of temperature on reproduction

Three datasets were used to calibrate temperature effects on the brood size. Temperature ranged between 5 and 30 °C (Goss and Bunting, 1983; Orcutt and Porter, 1983; Stephenson and Watts, 1984). Data showed an optimal response (Fig. 2.5) which could not be described by the Arrhenius equation. Therefore, a four-parameter peak function was fitted to the data (Eq. 4).

$$F(T) = \frac{1}{1 + e^{\frac{tal}{T} - \frac{tal}{tl}} + e^{\frac{tah}{th} - \frac{tah}{T}}} \qquad r^2 = 0.85 \ (4)$$

with tl = 281.2; th = 300.3; tal = 18435; tah = 54079.

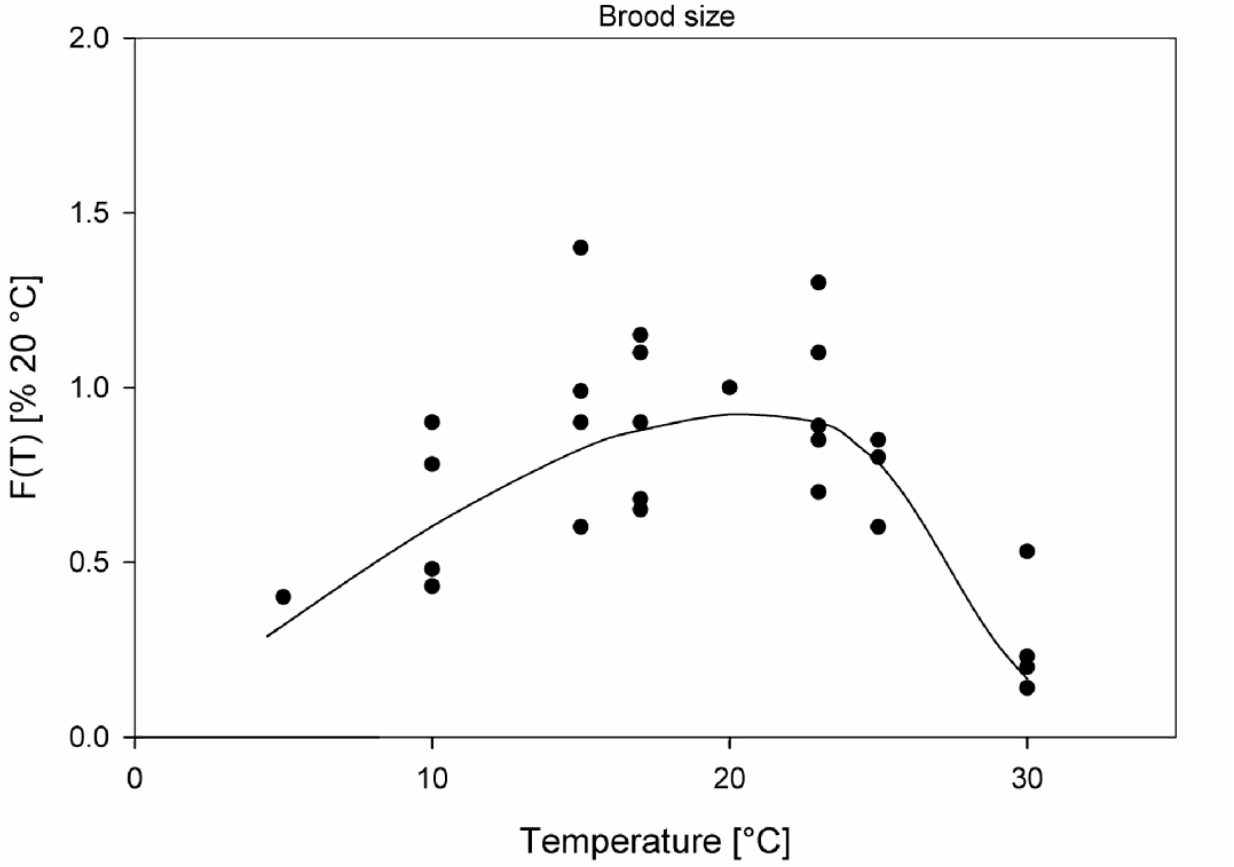

Fig. 2.5. Dependency of the brood size on temperature. The brood sizes of *D. magna* were normalized to 20 °C, resulting in the temperature function F (T), and are shown as black dots. The solid line represents the fitted regression.

2.2.4. Toxicity

Whether an individual-level process is potentially affected by chemical exposure is highly depending on the toxicant compound and on its chemical properties. The influence of toxicity on the life cycle processes of individual daphnids is described in detail in the corresponding chapters.

2.3. Validation and application of IDamP

Predictions of the IDamP model regarding the population size and size structure were successfully validated against population tests with different feeding scenarios (flow-through or semi-static), different food supplies including starvation, crowding conditions and initial population size and size structure (Preuss et al. 2009). Furthermore, IDamP predicted the correct patterns of population responses to chemicals with different modes of action. Examples include 3,4-dichloroaniline (Preuss et al. 2010), Imidacloprid (Agatz et al. 2013) or Nonylphenol (Gergs et al. 2013).

CHAPTER 3

Coupling different mechanistic effects models for capturing individual and population level effects of chemicals: lessons from a case where standard risk assessment failed

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3.1. Introduction

Environmental risk assessment (ERA) involves determining the adverse effects that chemicals and other stressors exert on ecological systems. Because it is impossible to eliminate all environmental effects of human activities, decisions were made to define protection goals which strike a compromise between the benefits of using the chemicals and costs in terms of acceptable effects. Protection goals vary among different biological levels of organization. In contrast to vertebrates, where the visible mortality of individuals has to be prevented, the target entity for aquatic invertebrates is the population rather than the individual (Brock et al. 2006; EFSA, 2010; Hommen et al. 2010), which implies that lethal and sublethal effects on individuals are accepted if they do not impair the functioning of the population.

Nevertheless, the standard ERA procedure for aquatic invertebrates still relies on laboratory tests at the individual level (Forbes et al. 2008), testing for effects of chemicals on simple endpoints like survival, growth or reproduction. One of the commonly used approaches in estimating the risk posed by chemicals relies on applying safety factors to the measured EC_x (the x % effective concentration) or NOECs (the no observed effect concentration) of tests with acute or chronic exposure to the chemical, to calculate the PNEC, the predicted no effect concentration (European Commission, 2003).

Such measures of risk have been criticized as they might not always be sufficient to ensure that protection goals are reached, thus limiting the application of risk assessment as a tool for managing environmental resources (Forbes et al. 2010). One example of the limitations of the current standard approach are the laboratory population test results for daphnid populations exposed to Dispersogen A (Hammers-Wirtz and Ratte, 2000), a substance used as an additive in several pesticide formulations as well as in industries such as textile printing

(Kromm, 1995) Dispersogen A has been shown to spread into the aquatic environment (Karl, 1998; Schoenberger and Kaps, 1994).

Dispersogen A has a low acute toxicity for *Daphnia magna* (EC_{50} = 167 mg L^{-1}, 48 h) and a NOEC for reproduction of 10.2 mg L^{-1} (derived from 21-day reproduction tests, Hammers-Wirtz and Ratte, 2000). The PNEC value derived from standard reproduction tests, calculated as the ratio of NOEC to a safety factor of 50 (European Commission Technical Guidance Document, 2003), turned out to be not protective even for laboratory populations (conducted under optimal conditions) as it led to a reduction of population size by almost 20 % (Hammers-Wirtz and Ratte, 2000).

This suggests that, in this case, changes in population properties following exposure did not emerge solely from toxicity effects on the survival of individuals and on the number of living offspring, which are the endpoints considered in the classical risk assessment methodology, but that additional effects of the toxicant were important as well.

In addition to the measured toxicity effects on reproduction and survival, Dispersogen A has further complex effects on individual daphnids. First, a stimulatory (hormetic) response of the reproductive output accompanied by a decrease in the body length of neonates was reported (Hammers-Wirtz and Ratte, 2000). Secondly, the same study showed significant effects on several endpoints in daphnid individuals born in the F1 generation (for details, see the material and methods section below). Neither the stimulatory effects on the individual reproductive output nor the effects on the F1 generation are currently adressed in the risk assessment.

Therefore, here we explored the hypotheses that the risk assessment failed to be protective for populations in the case of Dispersogen A due to ignoring either the stimulatory toxicity effects on reproduction, or the observed effects on the F1 generation, or to ignoring

both of these effects. To explore these different hypotheses and identify the most likely real drivers of effects observed at the population level, we need a tool that enables us to independently capture the toxicant's modes of action at the individual level and to test their effects at the population level. Mechanistic effect models, and particularly individual-based population models (IBMs), are used to overcome the limitations of standard tests. They allow us to test different assumptions about the organism level effects of chemicals (Forbes et al. 2008; Grimm et al. 2009; Preuss et al. 2009) and to explore which of these organism-level endpoints are most predictive of population-level effects (Preston and Snell, 2001). Moreover, IBMs allow the integration of different TK/TD models, which dynamically simulate the processes that lead to toxicity within an organism, and its corresponding effects on survival (Ashauer et al. 2011; Jager et al. 2011).

In this study, we used an existing IBM of daphnids (IDamP, Preuss et al. 2009) combined with a Toxicokinetic/ Toxicodynamic (TK/TD) model for survival (GUTS, Jager et al. 2011) to extrapolate the effects of Dispersogen A from daphnid individuals to the population level. We contrasted different assumptions about individual-level effects of the toxicant and tested how well the resulting population models explained observations from two laboratory population experiments (Hammers-Wirtz and Ratte, 2000). Our main aim was to identify the modes of action triggering the population-level effects in daphnids exposed to Dispersogen A, which were the reason behind the failure of the current risk assessment to be protective.

3.2. Material and methods

3.2.1. Dispersogen A: Properties and modes of action

Dispersogen A is a condensation product of Naphthalene sulfonic acid with formaldehyde (Kromm, 1995). According to *Daphnia* reproduction tests (Coors et al. 2004;

Hammers-Wirtz and Ratte, 2000), adverse effects of Dispersogen A were reported on the reproductive output of daphnids at as low as 0.1 mg L^{-1}. However, and contrary to classical toxicants which induce a reduction in the clutch size, exposure to Dispersogen A increases the clutch size (by as much as 53 % compared to the control) up to a concentration of 10.2 mg L^{-1}, at the expense of decreasing neonate body length (fitness) (lowest observed effect concentration, LOEC = 0.1 mg L^{-1}). It is only at higher concentrations (25.6 mg L^{-1}) that the clutch size is reduced, and at concentrations exceeding 64 mg L^{-1}, reproduction is completely inhibited (Hammers-Wirtz and Ratte, 2000). In addition to effects on reproduction, Dispersogen A causes significant mortality (EC_{50} = 16.5 mg L^{-1}, Hammers-Wirtz and Ratte, 2000) at the individual level.

Furthermore, experiments with neonates from exposed mothers that were grown individually in uncontaminated medium showed that toxic effects of Dispersogen A transmit to the next generation (F1) where they cause even stronger negative effects than in the original generation. Examples include significant decreases in the body and clutch sizes observed at even very low concentrations, e.g. 1.64 mg L^{-1} in the F1 generation, compared to effects observed at a concentration of 25.6 mg L^{-1} in the original generation, or the decrease in neonate survival observed at 0.001 mg L^{-1} in the F1 generation test compared to 1.64 mg L^{-1} in the original generation (Hammers-Wirtz and Ratte, 2000).

3.2.2. The models

3.2.2.1. The *Daphnia* population model IDamP

We used the individual-based population model IDamP **(Chapter 2)** for *D. magna* to simulate the effects of Dispersogen A.

3.2.2.2. Toxicity effects on reproduction: Reprotox model

The toxicant's effects on reproduction were accounted for by calibrating stress functions. We used life history data from chronic tests at the individual level which include effects on clutch size and neonate length. Data originated from three laboratory reproduction tests (Agatz, unpublished diploma thesis, RWTH Aachen University, 2009; Hammers-Wirtz and Ratte, 2000) and comprised six exposure levels to Dispersogen A (0.001, 0.1, 1.64, 4.1, 10.2 and 25.6 mg L^{-1}). In these tests, clutch size increased with increasing exposure concentrations except at the highest concentration where it was reduced to 36 % of the total number of neonates released in the controls. As recommended by the OECD Guidelines (OECD 211, 2008), we applied a hormetic model to account for the stimulatory effects of Dispersogen A observed at low concentrations. Hormetic models derive from a simple log-logistic dose response function but with an additional parameter describing the proportion of the stimulatory response of some process at low toxicant concentrations (Brain and Cousens, 1989). We tested the two models which are most commonly used in the literature, to account for the hormetic effects of Dispersogen A on clutch size. The first one (Eq. 1) was developed by Brain and Cousens (1989):

$$y = c + \frac{d - c + fx}{1 + \exp\left(b\ln(x/e)\right)} \quad (1)$$

with (in our case) y denoting the clutch size (% controls), x the concentration of Dispersogen A (mg L^{-1}), c the clutch size at infinite concentrations, d the clutch size of the untreated control and f the rate of increase in clutch size at low concentrations. Parameters b and e are for calibration and have no biological interpretation (Brain and Cousens, 1989).

Later this model was modified by Cedergreen et al. (2005) who replaced fx by $fexp(-x^{-a})$, with a being an additional calibration parameter. The Cedergreen model (Eq. 2) was shown to yield better fits than the Brain and Cousens model for some substances (Belz and Piepho, 2012):

$$y = c + \frac{d - c + f\exp(-x^{-a})}{1 + \exp\big(b\ln(x/e)\big)} \quad (2)$$

We tested both models with our data and ranked them by (i) comparing the agreement of regressions to the measured data graphically, (ii) taking the highest r^2 value and (iii) the lowest Akaike Information Criterion (AIC; Akaike, 1973) value of both models (Eq. 3).

$$AIC = a\ln(RSS) + 2p \quad (3)$$

with a being the number of experimental observations, RSS the residual sum of squares and p the number of parameters in the model.

Additionally to the measured effects of Dispersogen A on clutch size, exposed daphnids produced neonates of a smaller size at all concentrations (Fig. 3.1 a). These effects were accounted for by fitting an exponential decay function to the measured data (Eq. 4).

$$\text{Neonate length (\% control)} = \frac{100 - 1.32 \times \ln C_{[\text{Dispersogen A}]}}{100} \quad (4)$$

The natural variability of the offspring size is modelled in relation to the relevant maternal traits and environmental factors, and is described in detail in **Chapter 4**.

In the following, we refer to the model accounting for the effects on reproduction (changes in clutch size and neonate length together) as the Reprotox model.

3.2.2.3. Toxicity effects on survival: the GUTS model

Toxicity effects on survival were calibrated using the General Unified Threshold model for Survival, GUTS (Jager et al. 2011). GUTS unifies existing TK/TD models of survival that

can be derived from two main specific assumptions, stochastic death (SD) or individual tolerance (IT) and different dose metrics. SD and IT differ substantially in their underlying assumptions, leading to different predictions of population properties. The dose metrics has to be selected based on knowledge of the mode of action and/or data availability. Since no information could be withdrawn from our data on the uptake rate, the internal concentration could not be determined. We therefore used the scaled internal concentration, C_i as dose metrics (for details, see Jager et al. 2011).

$$dC_i(t)/dt = K_e\left(C_w(t) - C_i(t)\right) \quad (5)$$

with K_e (h^{-1}) being the dominant rate constant and C_w (mg L^{-1}) the external concentration.

SD models assume that when C_i reaches a certain value, the threshold for effects (z, mg L^{-1}), all individuals have an increasing probability of dying. This probability is represented by the hazard rate (h_z), which is proportional to the difference between C_i and the threshold value (z).

$$h_z(t) = K_k \times \max\left(C_i\ (t) - z, 0\right) \quad (6)$$

with K_k (mg $L^{-1}h^{-1}$) being the killing rate.

The hazard rate is then integrated to obtain the cumulative hazard at time t.

$$H_z(t) = \int_0^t h_z(\tau)d\tau \quad (7)$$

with τ representing time from 0 to t.

Survival then decreases with increasing hazard rate

$$S(t) = \exp(-H_z(t)) \quad (8)$$

IT models assume that individuals have different sensitivities to the toxicant, so the threshold level of effects is not fixed but follows a probability distribution function, *F(t)*. The most commonly used probability density functions to describe survival data (exponential, Weibull, log-normal and log-logistic) were tested to calibrate survival effects. The best distribution was chosen according to the graphical accordance between measured and predicted data, and to the r^2 values derived from the predicted-measured plots. Accordingly, the log-logistic probability distribution function yielded the best fit to survival data. The cumulative threshold level (*F(t)*) was therefore calculated following the cumulative distribution function:

$$F(t) = \frac{1}{1 + \left(\frac{\max_{0<\tau<t} C_i(\tau)}{\alpha}\right)^{-\beta}} \quad (9)$$

with α (mg L^{-1}) being the median of the distribution, β (dimensionless) the shape parameter that determines the width of the distribution, and $max\ C_i(\tau)$ (mg L^{-1}) the highest C_i that occurred until time *t*. The survival model was then calculated as:

$$S(t) = (1 - F(t)) \quad (10)$$

Two standard datasets from acute (125, 250 and 500 mg L^{-1}; OECD, 2004) and chronic (0.1, 1.64, 25.6 and 64 mg L^{-1}; OECD, 2008) tests were used to estimate parameters for the two alternative survival models, SD and IT. We used the downhill simplex approach (implemented in Embarcadero 2010 RAD studio Delphi XE2 using TPMath 7.0 program) to maximize the likelihood function (equations 9 and 10 in Jager et al. 2011) and optimize the fit of parameter estimates to the measured data. Selection of the most accurate survival model between SD and IT was based on the regression parameters (r^2, slope and intercept values) of calibrated to measured data (Calculated in Delphi XE2 using Statmaster 3.5; DewResearch).

3.2.2.4. Toxicity effects on the F1 generation: F1 model

The F1 experiments were conducted (Hammers-Wirtz and Ratte, 2000) by transferring neonates from exposed mothers (0.001; 0.1; 1.6 and 10.2 mg L^{-1}) into uncontaminated media and observing their fitness for an additional 21 days. Long lasting effects were reported in F1 generation such as reduced size, and inhibition of reproduction up to 70 %, in addition to mortality which increased up to 40 % (more details can be found in Hammers-Wirtz and Ratte, 2000). Due to the limited data available on this test for the different concentrations, we were unable to parameterize the effects using a concentration response relationship or a TK/TD model for every affected endpoint. Therefore, it is important to emphasize that in this study, the F1 generation effects were only implemented at concentrations equaling or exceeding 4.1 mg L^{-1} and were assumed to be independent of the exposure concentration. Thus, F1 generation effects were not implemented in a realistic but in a worst-case manner, by accounting for the maximum inhibition of reproduction and the maximum observed mortality. IDamP was designed so that toxicity levels could be manually assigned as a percent inhibition from the control. Consequently, the F1 model calculated 40 % higher background mortality and reproduction was inhibited by 70 % in addition to effects on survival and on reproduction calculated with the other effect models for the corresponding concentrations.

3.2.3. Model testing

3.2.3.1. Laboratory population tests

The model was tested against data from two abundance time series tests (first test by Hammers-Wirtz, unpublished, and second test in Hammers-Wirtz and Ratte, 2000) conducted under semi-static conditions. They differed in the start population as well as in the feeding regime.

The first population test (three replicates per treatment) started with 5 neonates and 3 adults, who were fed 0.5 mg C per vessel per day. The test concentrations of Dispersogen A ranged between 0.64 and 25.6 mg L^{-1} and the experiment lasted for 42 days. In the controls, the population grew until reaching a maximum population size of 91 individuals on average, when food became limiting and the population decreased until reaching a plateau (80 individuals at day 18). Population size then oscillated around the carrying capacity (60 to 82), most likely due to competition for food and crowding effects. Overall, the average (± standard deviation) measured control population size was 68 ± 22. All exposed populations showed the same dynamics: after the growing phase, whose duration and magnitude depended on the toxicant concentration, the populations underwent a sharp decline (down to 2 % of the control population at the highest concentration). Except at the highest concentration, populations showed (at day 18) a second series of increase (up to 153 % of the control) followed by a subsequent decrease (down to 52 % of the control). The populations then oscillated around the carrying capacity and did not reach a quasi-stationary equilibrium at any of the exposure levels. In the following, we will refer to the first population test as the capacity experiment.

The second population test (four replicates; Fig. 4 in Hammers-Wirtz and Ratte, 2000) started with 5 neonates who were fed 1.25 mg C per day in the first two weeks and 1.75 mg C per day from the third week till the end of the experiment (45 days). Dispersogen A concentrations ranged between 0.1 and 25.6 mg L^{-1}. In this experiment, the populations grew exponentially and reached a maximum abundance level, which depended on the Dispersogen A concentration. In the following, we will refer to the second population as the exponential growth experiment.

3.2.3.2. Tested scenarios of individual-level effects

In order to distinguish between the different toxicity effects of Dispersogen A, population simulations were run assuming six different scenarios, emerging from three single effect models: effects observed on populations are caused by reproductive toxicity only (Reprotox model) or by survival toxicity only (survival model) with the latter effect being tested either with the SD or with the IT assumption.

The fourth and the fifth scenarios were derived from a combination of Reprotox and survival models, using SD or IT assumptions. These five different alternative scenarios were tested at each exposure level and compared to both datasets. The last tested scenario was the F1 generation model which accounted for the Reprotox model and the survival model in addition to the F1 generation effects. Survival in the F1 model was simulated using only the SD assumption (which provided the best agreement to both population datasets). Therefore, the F1 generation model combined the SD+R+F1 effects. The F1 generation model was tested at 4.1, 10.2 and 25.6 mg L^{-1} and was compared to both datasets.

3.2.3.3. Population predictions

The IDamP model including the toxicity submodels was tested at the population level using the two population experiments (described in paragraph 3.2.3.1) without further calibration. Thus, population-level model results have to be classified as uncalibrated predictions. IDamP was initialized for the different population tests according to the experimental conditions. Exposure was assumed to be constant. Monte-Carlo simulations (1000 simulations) were run over 42 days in the capacity experiment and 45 days in the exponential growth experiment for each effect scenario. To determine extinction risk, 1000 simulations were

run for each scenario for 150 days. This time period was chosen to ensure that all daphnids reached their maximum life time and died at that time point.

3.2.4. Analysis of model outputs

3.2.4.1. Mean population size and size structure

Population size and the 95 % percentiles were used to indicate the effects of the toxicant on population dynamics. To test how well each individual-level effect scenario explained population-level effects, the simulated population size for each toxicant concentration was observed over time (with 95 % confidence levels) for different Dispersogen A concentrations and in comparison to the size of the measured control population. Additionally, the dynamics of the three different size classes of the population (neonates: smaller than 1.4 mm, juveniles: smaller than 2.6 mm, and adults: larger than 2.6 mm) were observed.

3.2.4.2. Extinction probability

Extinction probabilities were calculated for the six toxicity scenarios using IDamP. For each toxicant concentration, the proportion of the population becoming extinct was determined. The concentration that was lethal to 50 % of the population, the $LC_{50\ (pop),}$ was estimated for each scenario.

3.2.4.3. Validation metrics

Different metrics were used to estimate how well the tested individual-level effect models described effects at the population level. First, the deviations from the mean measured data (Eq. 11) and the sum of squared errors (*SSE*; Eq. 12) were calculated using Microsoft Excel 2010 Inc. The smaller these indicators, the more accurately the models describe population dynamics.

$$\text{Deviation } (\%) = \frac{1}{n}\sum_{0}^{n}(|\overline{y_i} - y_i|/y_i) \quad (11)$$

$$\text{SSE} = \sum_{i=1}^{n}(\overline{y_i} - y_i)^2 \quad (12)$$

with n being the number of observations; y_i and $\overline{y_i}$ are respectively the mean measured and predicted population sizes at the i^{th} observation.

Furthermore, following the method of Scholten and Van der Tol (1998), area comparison statistics were determined as additional indicators of goodness of fit. These statistics rely on the comparison of the intervals (delimited by the minimum and maximum values) of both measured '*M'* and predicted '*P'* data, and determining the extent of overlap between the two intervals (Intersection, *I*). If '*I'* is null, there is no overlap between model predictions and the measured observations, and the model is therefore useless. The higher the '*I'* value is, the larger the fraction of measured observations the model predicts, and thus the more useful it gets. Area comparison statistics were estimated in the following way: First, at each time point, the measured area '*M'* was calculated as the difference between the maximum and the minimum (among the replicates) population size. Similarly, the predicted area '*P'* was calculated as the difference between the high (95 %) and low (5 %) confidence intervals. Subsequently, *'I'* was deduced by subtracting the smallest value between the maximum measured and maximum predicted values from the highest value between the minimum measured and minimum predicted values (Eq. 13).

$$I = \big(Min(Max\,P, Max\,M) - Max(Min\,P, Min\,M)\big) \quad (13)$$

The ratio of '*I*' to '*M*' represents the '*adequacy'* of the model, which then describes the proportion of the measured area that is covered by the model, *i.e.* the area that is adequately simulated by the model. The ratio of '*I*' to '*P*' represents the 'reliability' of the model, *i.e.* when

the area covered by the model is high compared to the intersection area, this means that the model has high uncertainty levels and is therefore of low reliability. This method was also used by Preuss et al. (2010) and was recommended in a recent textbook on modelling by Haefner (2005).

Moreover, predicted measured statistics (r^2, intercept and slope values) were calculated for each scenario.

3.3. Results

3.3.1. Calibration results

3.3.1.1. Reproductive toxicity (Reprotox) model

The variation of the neonates body size with Dispersogen A concentrations was successfully predicted by the exponential decay regression model ($r^2 = 0.97$; Fig. 3.1 a).

To simulate the increase in the number of neonates per female at low Dispersogen A concentrations, a suitable hormesis model had to be selected. Model 1 (Brain and Cousens model) and model 2 (Cedergreen model) had r^2 values of 0.96 and 0.91, respectively. The AIC in model 1 was smaller (79.19 compared to 87.46 in model 2). Accordingly, model 1 (Fig. 3.1 b) was more appropriate for describing Dispersogen A effects on clutch size than model 2. All parameters within the model (Eq. 1) significantly contributed to determining the clutch size.

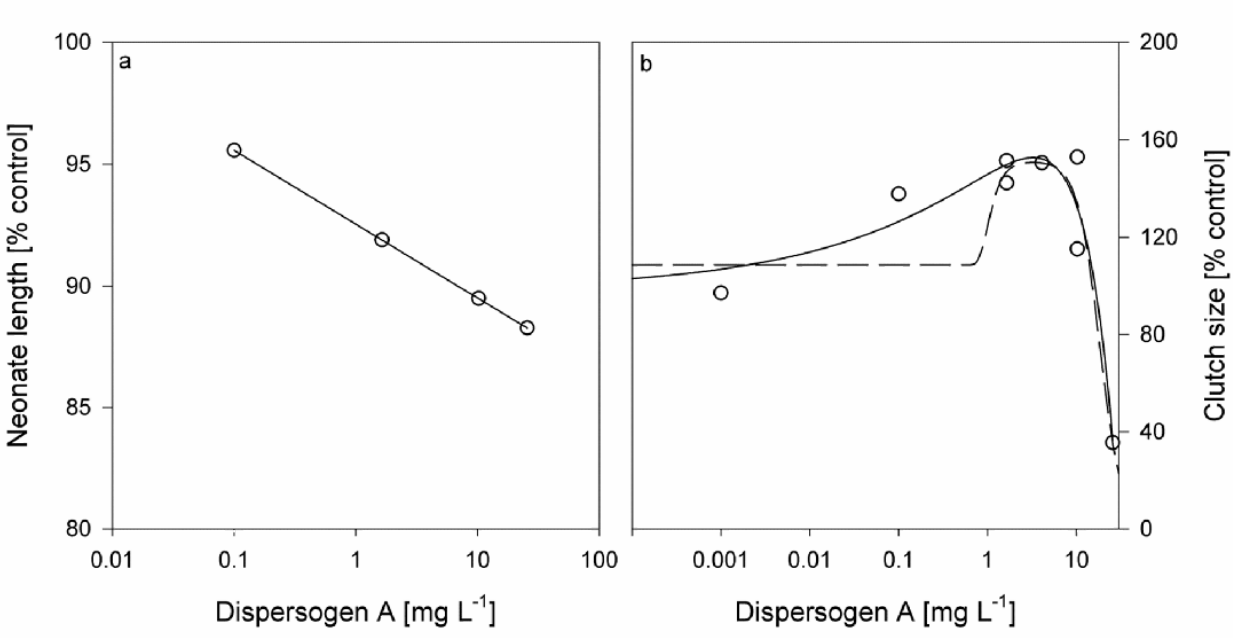

Fig. 3.1. Calibration of toxicity effects on reproduction (Reprotox model): Variation in the neonates' body length (a) and clutch size (b) with Dispersogen A concentrations. Open dots show the data points and the lines represent the fitted regression lines. In Fig. 3.1 b, the fit of model 1 (Brain and Cousens) is indicated by a solid blue line and of model 2 (Cedergreen) by a dashed black line.

3.3.1.2. Survival models

Model calibrations using SD and IT approaches are shown in Fig. 3.2. The corresponding parameters (with the 95 % confidence levels) and estimators of goodness of fit are summarized in Table 3.1. Good fits to data are indicated by r^2 and slope values close to 1 and by intercepts close to 0. Both approaches predicted the variability in the measured data well (r^2 = 0.93). Comparing the slope and the intercepts values, the IT approach was slightly advantageous. A graphic comparison of the goodness of fit at each effect concentration showed that both models fitted the data well at the highest concentrations (125 to 500 mg L^{-1}). At 25.6 mg L^{-1}, IT and SD predicted the decrease in survival but SD overestimated the effect. At 1.6 mg L^{-1} and 64 mg L^{-1}, SD predicted the reduced survival more accurately than IT. From these

results, we were unable to make inferences as to which approach was tangibly more appropriate in describing survival effects. Therefore, in the following simulation experiments, we tested the efficiency of assumptions, SD and IT, for predicting population-level effects.

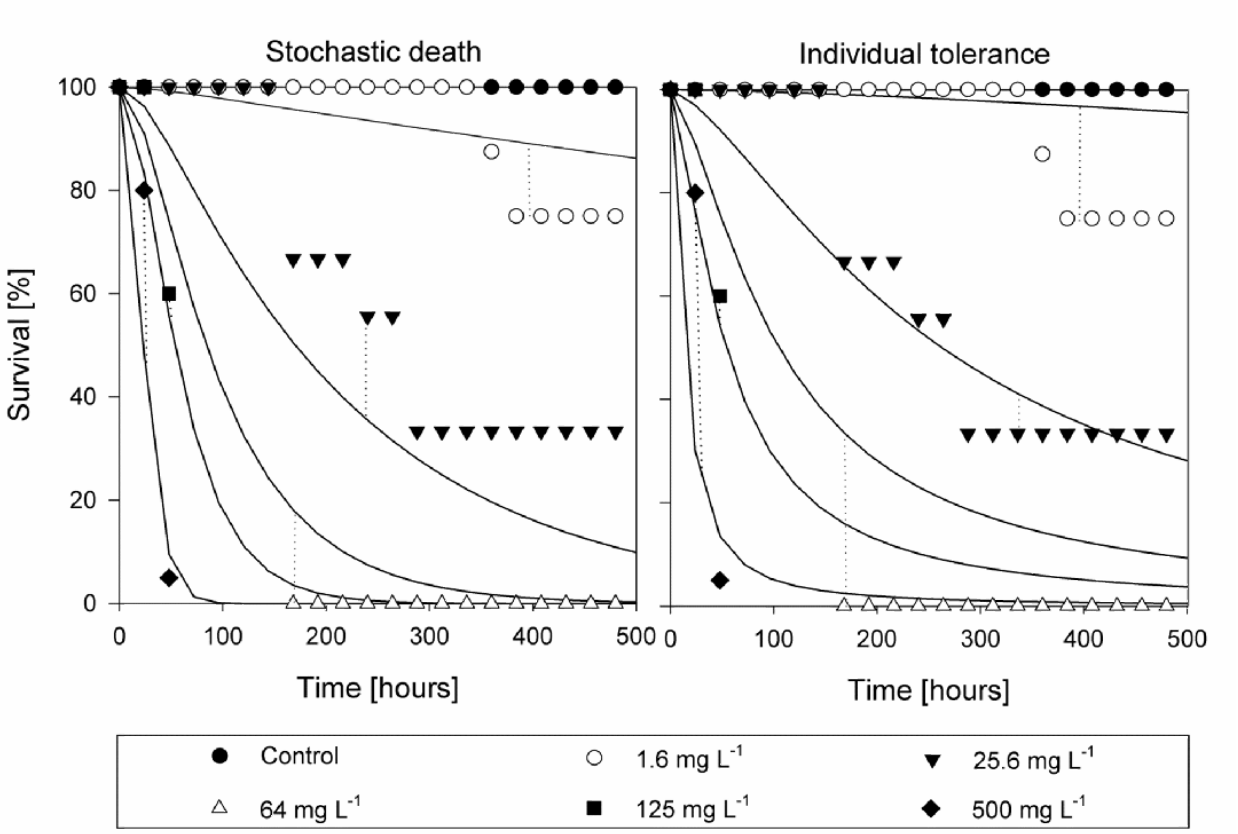

Fig. 3.2. Calibration of toxicity effects on survival (survival models): Two data sets for *Daphnia magna* (acute and chronic tests) analyzed with two reduced TK/TD models for survival applying stochastic death or individual tolerance on the basis of scaled internal concentration. Symbols indicate measured data for the different concentrations, and lines models predictions. Parameter estimates are summarized in Table 3.1.

Table 3.1. Parameter estimates of the survival models and predicted measured statistics. In the stochastic death (SD, Eq. 8) model, the threshold z is a single value, whereas it follows a log logistic distribution in the individual tolerance (IT, Eq. 10) model. Values between brackets are the likelihood based 95 % confidence levels. Empty brackets mean that the value was not estimated

	SD [5 % Cl ; 95 % Cl]	**IT** Log-logistic distribution [5 % Cl ; 95 % Cl]
Models' parameters		
Killing rate K_k (mg L^{-1} h^{-1})	1.92×10^{-04} [1.52×10^{-04}; 2.22×10^{-04}]	[-]
Elimination rate K_e (h^{-1})	0.034 [0.022; 0.041]	6.5×10^{-12} [nd; 10^{-05}]
α (mg L^{-1})	[-]	4.4×10^{-08} [nd; 10^{-05}]
β	[-]	1.46 [1.31; 1.61]
Threshold for effects z (mg L^{-1})	0.018×10^{-04} [nd; 0.032]	[-]
Regression parameters of calibrated to measured data		
Slope	0.9	0.99
Intercept	9.87	-0.42
r^2	0.93	0.93

nd: no lower limit found

3.3.2. Assuming individual-level effects on reproduction and survival

Mean predictions (Monte-Carlo simulations) of population size with different Dispersogen A concentrations (as a percentage of the untreated control data) using SD, IT or Reprotox are shown in Figs 3.3 a and 3.3 b for the capacity experiment and the exponential growth experiment, respectively. The predicted and measured population dynamics over time using these effect models are shown in Fig. 3.4 for the capacity and exponential growth experiments.

In both experiments, the control populations' dynamics and mean population size were accurately predicted by the model with only 15 % and 14 % deviation from the measured data for the capacity and the exponential growth experiments, respectively. However, none of the standard endpoints currently addressed in risk assessment, namely reproduction and survival was able to fully capture, if considered separately, the effects observed at the population level in both experiments. Deviations between model predictions and measured mean abundance were low at lower concentrations and increased with increasing concentrations of Dispersogen A. The deviations were more pronounced in the exponential growth experiment than in the capacity experiment.

The observed increase in mean population size (by almost 4 % of the control) at the two lowest exposure concentrations in the capacity experiment (Fig. 3.3 a) was only predicted by the reproductive toxicity model (with 21 % and 31 % deviation from the data at 0.64 and 1.6 mg L^{-1}, respectively) while with survival models, the population size was reduced (by 2.5 % with SD) or remained constant (with IT). At these concentrations, no increase in the mean population size was observed in the exponential growth experiment, but an increase was predicted by the reproductive toxicity scenario. At 25.6 mg L^{-1}, the mean population size was 16 % and 3 % of

the control population in the capacity and exponential growth experiments, respectively. All single effect models yielded large deviations to the measured data.

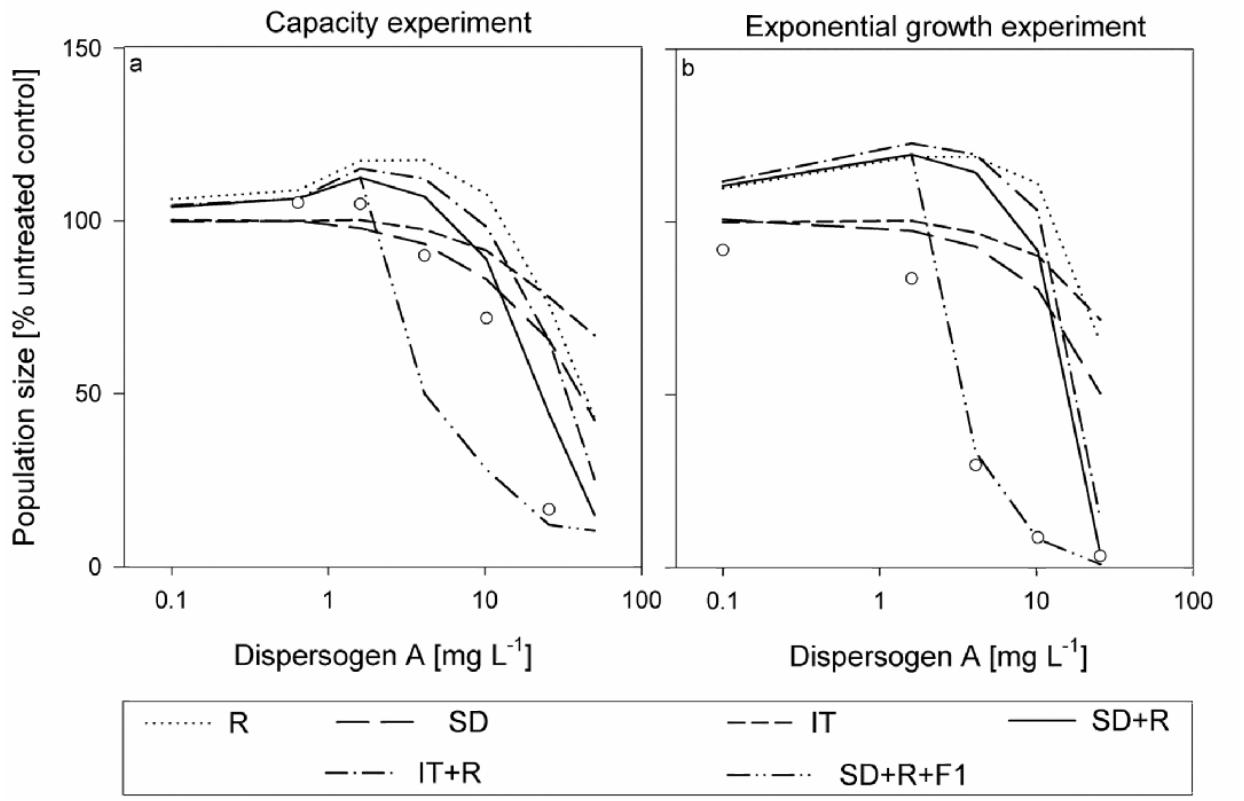

Fig. 3.3. Mean population size as a percent of the control for the different effect models in relation to Dispersogen A in the capacity experiment (a) and in the exponential growth experiment (b). Open dots show measured data; R: Reprotox; SD: Stochastic death; IT: Individual tolerance; F1: additional effects on the F1 generation; "+" submodels combined.

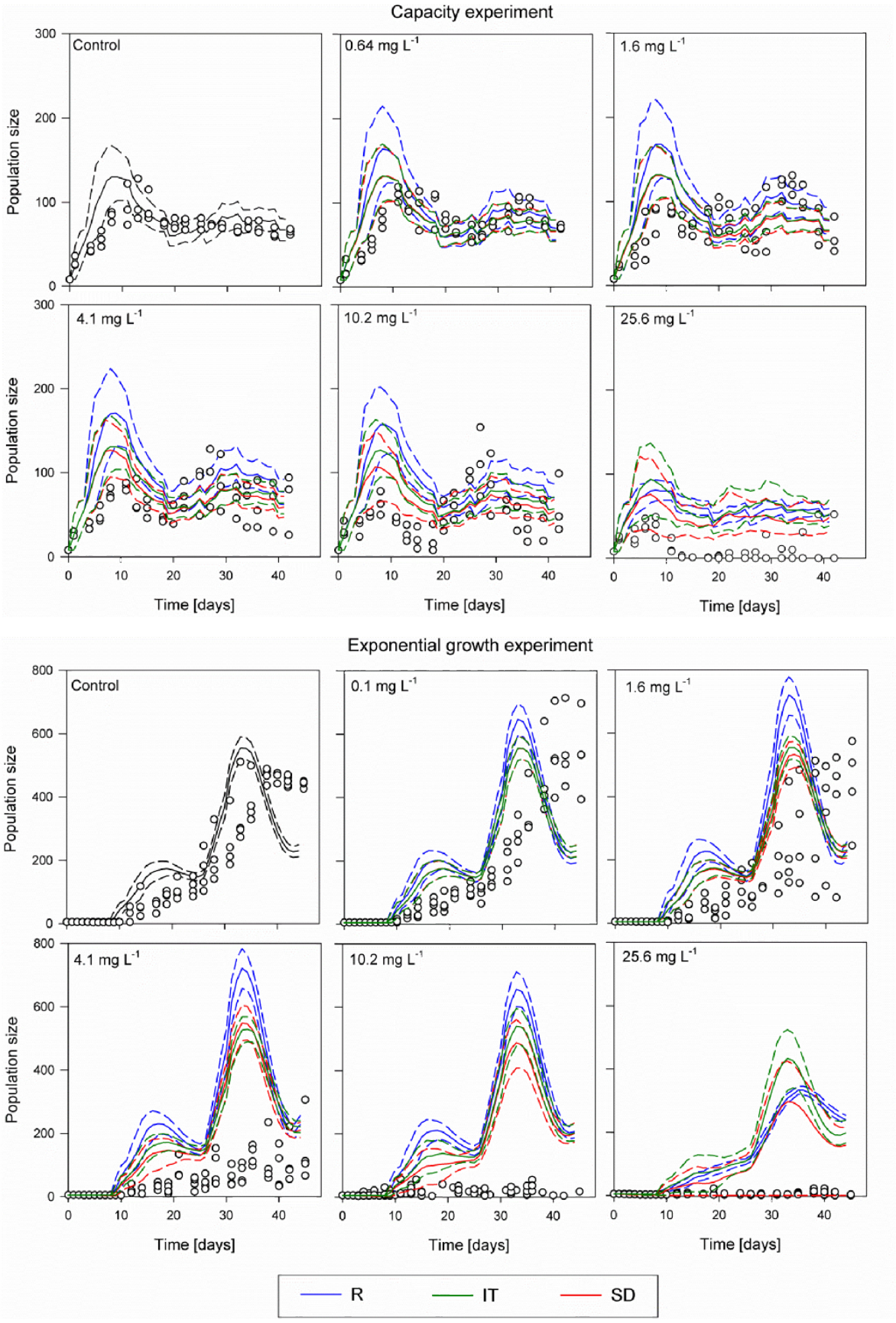
Capacity experiment
Control
0.64 mg L-1
1.6 mg L-1
4.1 mg L-1
10.2 mg L-1
25.6 mg L-1
Population size
Time [days]
Exponential growth experiment
Control
0.1 mg L-1
1.6 mg L-1
4.1 mg L-1
10.2 mg L-1
25.6 mg L-1
Population size
Time [days]
R
IT
SD

Fig. 3.4. Predictions of population dynamics with the two effect models for survival, stochastic death (SD) and individual tolerance (IT), and the Reprotox model (R) in the carrying capacity experiment and in the exponential growth experiment. Dots show the measured data. Solid lines show the average of 1000 Monte-Carlo simulations, dashed lines the 95th percentiles.

The sums of squared errors, SSE (Fig. 3.5 a and b), indicate no differences between the two survival models and the Reprotox model in predicting population dynamics for the capacity experiment, whereas for the exponential growth experiment, effects on survival were a better predictor than effects on reproduction.

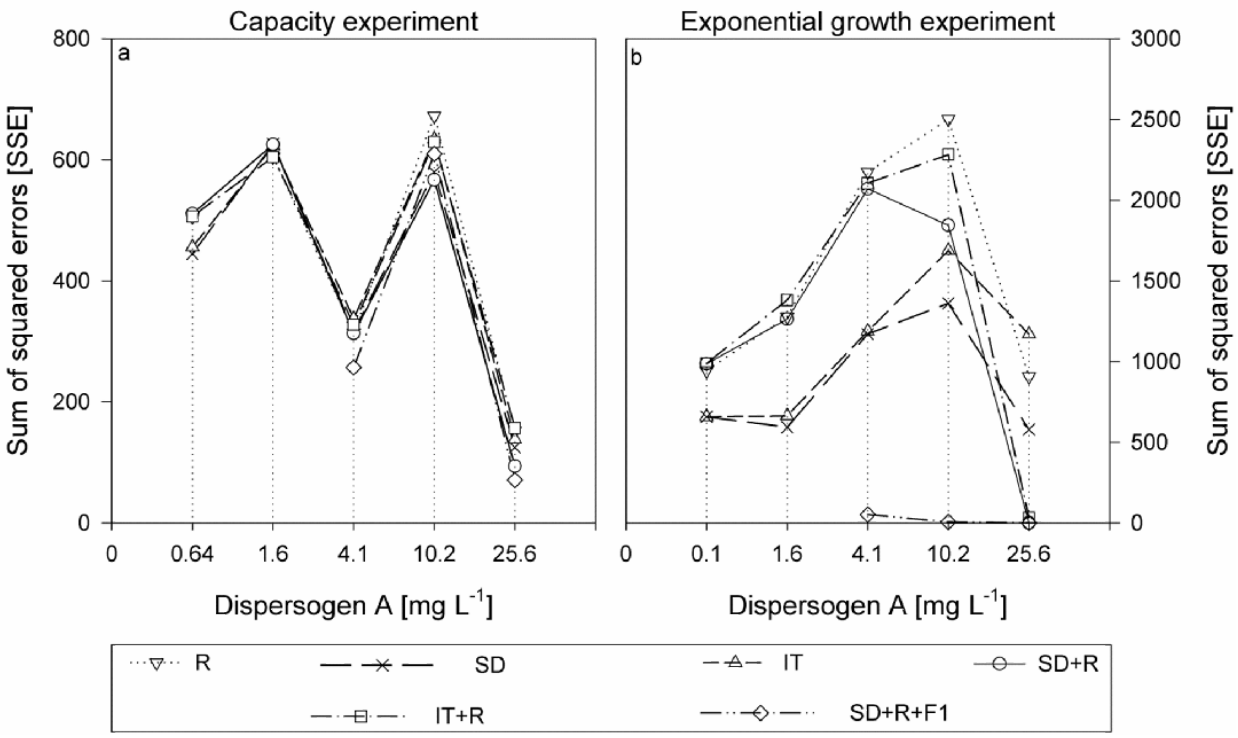

Fig. 3.5. Sum of squared errors for the different effect models in the capacity experiment (a) and the exponential growth experiment (b) in relation to Dispersogen A concentrations. Acronyms of the effect(s) models are as in Fig. 3.3.

Furthermore, single simulation results using the single effect models SD, IT, or Reprotox (Fig. 3.6) reflected the fluctuations observed in the population dynamics of the capacity experiment with the Reprotox model, but not with the survival models.

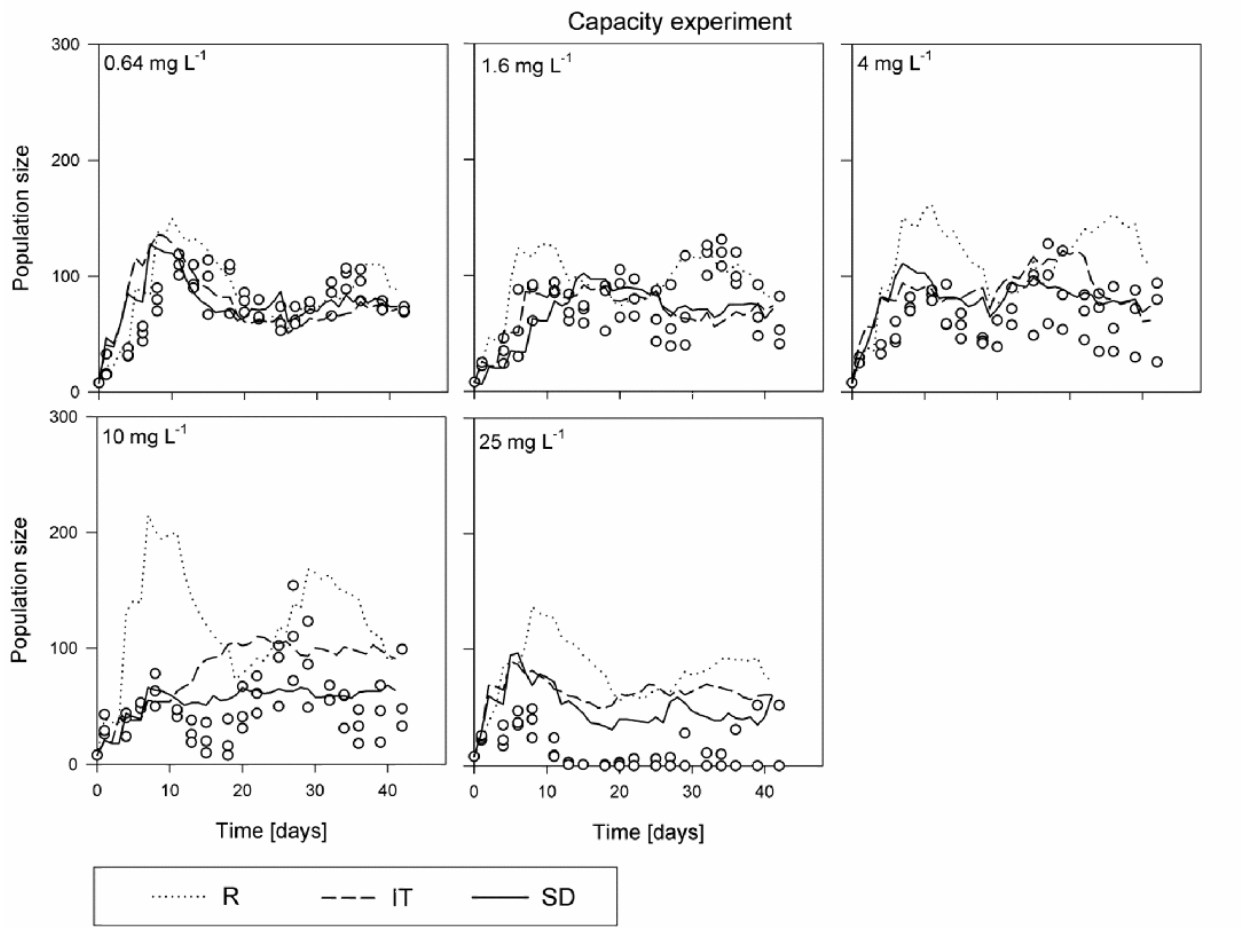

Fig. 3.6. Single simulation results using the two effect models for survival, stochastic death (SD) and individual tolerance (IT), and the Reprotox model (R) in the capacity experiment. Dots show the measured data. Solid lines show the simulated pattern.

3.3.3. Assuming combined effects on reproduction and survival

In both population experiments, IT+R (IT combined with Reprotox) and SD+R (SD combined with Reprotox) effect models described similar population behaviour with an increase at the two lowest exposure levels, followed by a decrease from 4.1 mg L^{-1} onwards (Fig. 3.3 a and b). Nevertheless, the higher the concentration, the more accurate the predictions with SD+R became compared to IT+R. Additionally, SSE calculated for the SD+R model was smaller than that of the IT+R model in both datasets (except at 1.6 mg L^{-1} in the capacity experiment).

Therefore, in the case of Dispersogen A, SD+R appeared to be the most accurate individual-level effect model for predicting population size.

Fig. 3.7 shows population dynamics predicted by the SD+R model for both experiments (from 4.1 mg L^{-1}), and Fig. 3.8 shows the predictions for the dynamics of size classes in the capacity experiment. In the capacity experiment, the SD+R model matched the measured population size over the entire experimental time quite well. Likewise, the measured dynamics of the neonates and the juveniles were accurately captured by this model. However, the adult fraction of the population was over-estimated at low concentrations.

In the exponential growth experiment, the model fitted better to the measured population size at the first and the second phases until day 35, when population size was over-predicted at all exposure levels.

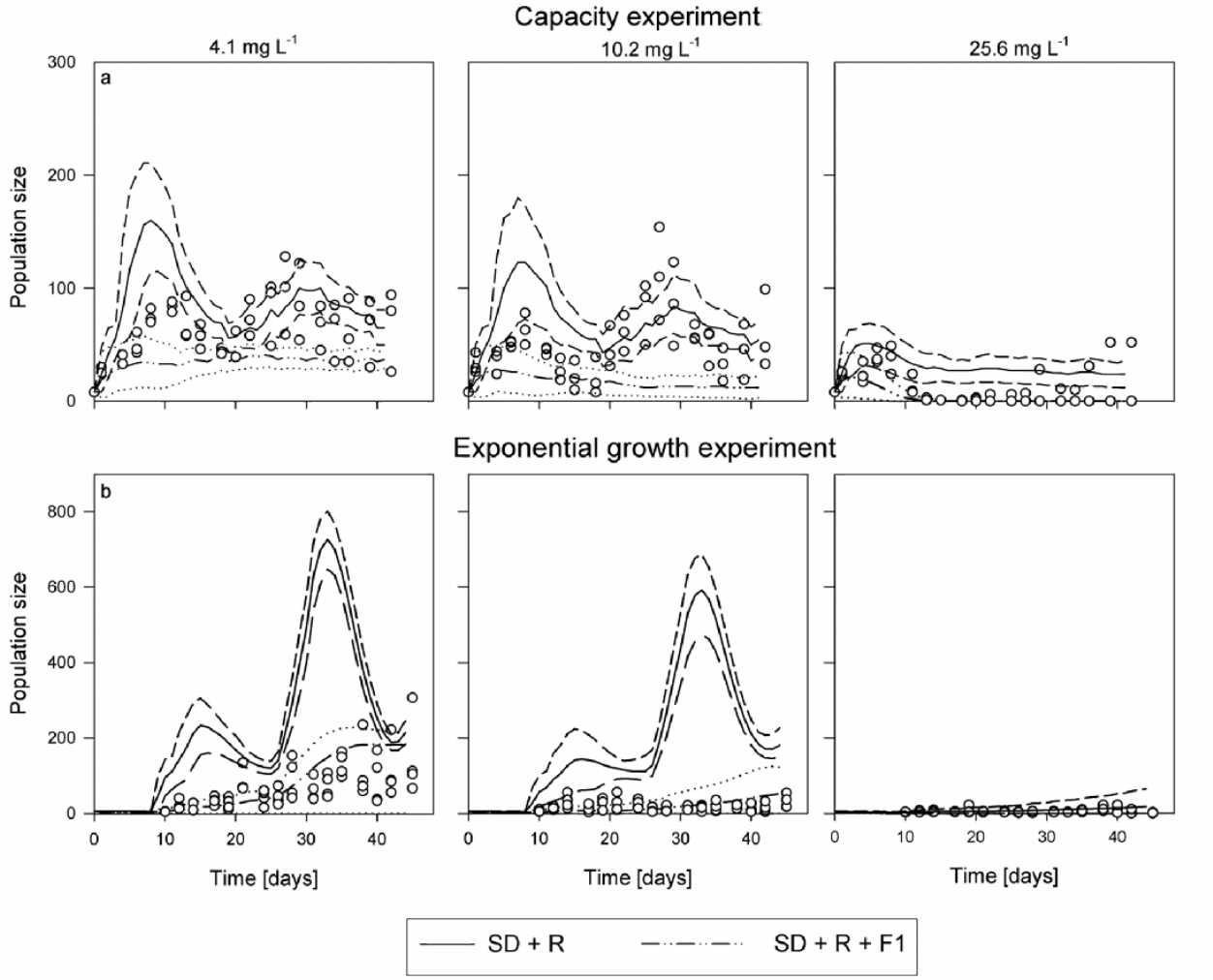

Fig. 3.7. Comparing the predictions of SD+R effect model obtained with and without F1 generation effects in the capacity experiment (a) and in the exponential growth experiment (b). Open dots show the measured data. Solid and pecked lines show the average of 1000 Monte Carlo simulations in the SD+R and the SD+R+F1 models, respectively. Dashed and dotted lines show the 95th percentiles in the SD+R and SD+R+F1 models, respectively. Acronyms of the effect(s) models are as in Fig. 3.3.

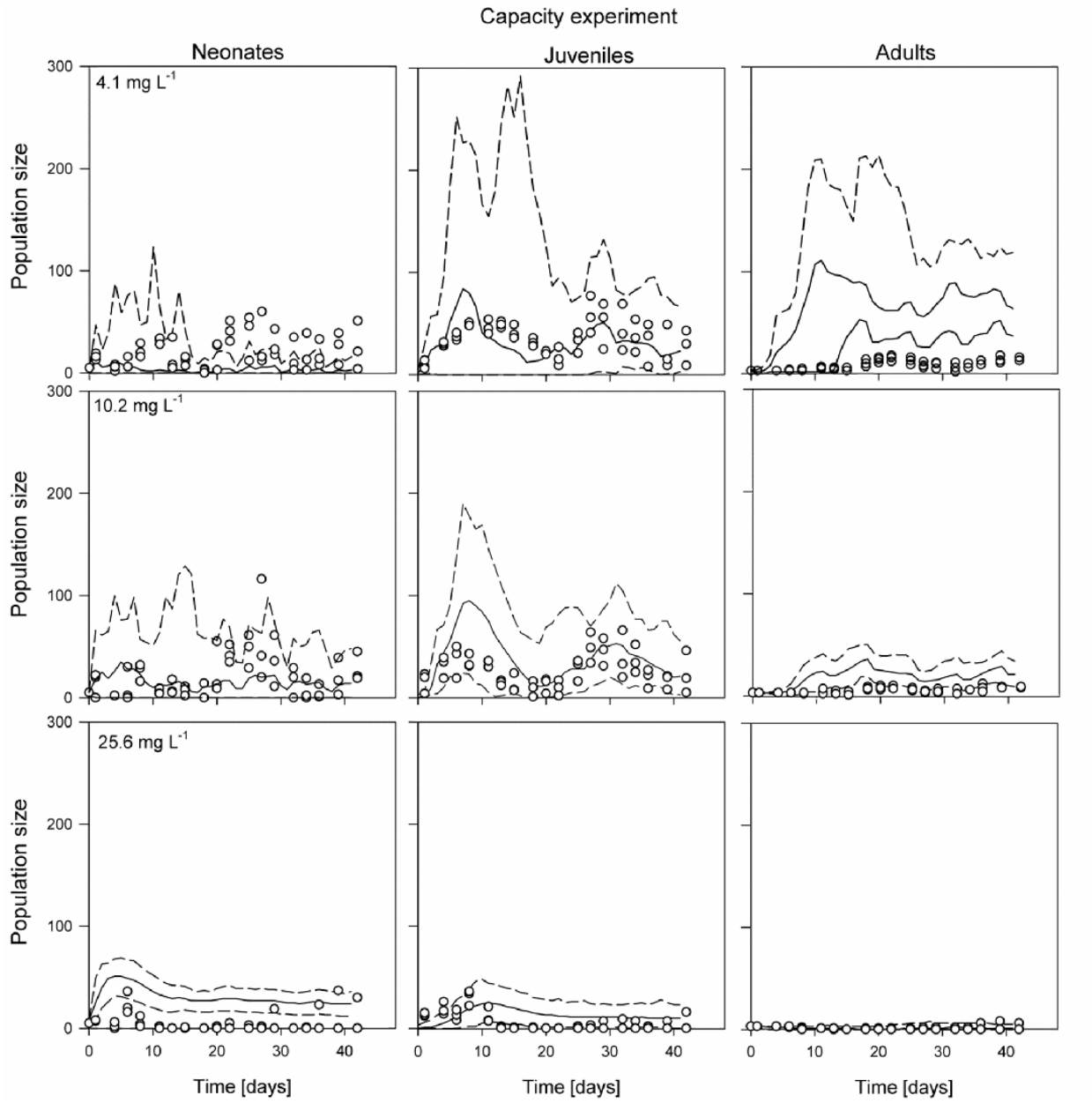

Fig. 3.8. Predictions of the dynamics of size classes in capacity experiment population obtained with the SD+R model. Open dots show the measured data. Solid lines show the average of 1000 Monte Carlo simulations using the SD+R effect model, dashed lines the minimum and maximum.

3.3.4. F1 generation effects

After accounting for the combined effects on survival and reproduction, there was still a reduction in the population size at the highest concentrations which was explained by none of

the effect models (Fig. 3.3 a, b). Fig. 3.3 shows the results of the variation in the mean population size compared to the control, including the F1 effect model where survival was modeled using the SD assumption (SD+R+F1). The simulated population dynamics including the F1 generation model were compared to the SD+R model in Fig. 3.7 (a and b) in both experiments.

In the capacity experiment, at 4.1 and 10.2 mg L^{-1}, model simulations including F1 effects overestimated the effects on population dynamics and mean population size (Fig. 3.3 a and 3.7a). However, at 25.6 mg L^{-1}, they were more accurate than those of SD+R and the reduction in the population size compared to the control was fully captured (Fig. 3.3 a). Additionally, model predictions including F1 effects yielded the smallest SSE compared to the other models at that concentration (Fig. 3.5 a).

In the exponential population growth experiment, F1 reduced the deviations between SD+R simulations and the measured data also at the lowest concentration considered 4.1 mg L^{-1} (Fig. 3b and 7b), along with smaller SSE values (Fig. 3.5 b). Values of 94, 98 and 99 % of the measured reductions in the population size as a percent of the control were obtained with the SD+R+F1 model at 4.1, 10.2 and 25.6 mg L^{-1}, respectively. The advantage of SD+R+F1 over SD+R was more evident at 4.1 and 10.2 mg L^{-1} whereas it provided a similar fit to the data at 25.6 mg L^{-1} (Fig. 3.3 b) but with smaller SSE than SD+R model (Fig. 3.5 b).

3.3.5. Ranking model scenarios based on prediction quality

Statistical measures for the prediction quality of the different model approaches are summarized in Table 3.2. In the capacity experiment, SD+R and SD+R+F1 effect models had the highest r^2 values (0.52 and 0.48, respectively) followed by the SD model (0.42). Adequacy

values of SD+R+F1 and SD+R models were also the highest (0.41) whereas the most reliable models were SD+ R and SD (0.31).

In the exponential growth population experiment, SD+R+F1 had the highest r^2, adequacy and reliability values compared to the other effect models (0.61; 0.48 and 0.29, respectively), followed by SD+R and SD which showed similar adequacy values (0.33) but different reliability (0.18 and 0.16, respectively) and r^2 (0.27 and 0.3, respectively) values. The low reliability values recorded for all submodels mean that the uncertainty levels yielded by the model predictions were high compared to the capacity experiment, and also that the SD+R+F1 effect model was the most reliable.

Table 3.2. Predicted measured and area comparison statistics between the measured data and the different models' simulations in the capacity experiment and in the exponential growth experiment. Acronyms of the effect(s) models are as in Fig. 3.3

	Capacity experiment				
	Slope	intercept	r^2	Adequacy[1]	Reliability[1]
Simulated control	0.99	10.48	0.76	0.55	0.26
R	0.5	11.2	0.33	0.38	0.22
SD	0.76	1.03	0.42	0.38	0.31
IT	0.62	7.4	0.29	0.33	0.29
SD+R	0.62	8.4	0.52	0.41	0.31
IT+R	0.56	8.9	0.4	0.35	0.27
SD+R+F1	0.57	25.48	0.48	0.41	0.24
	Exponential growth experiment				

	Slope	intercept	r^2	Adequacy	Reliability
Simulated control	0.9	35.2	0.73	0.32	0.37
R	0.33	9.51	0.21	0.2	0.18
SD	0.49	-0.39	0.3	0.33	0.16
IT	0.42	2.8	0.26	0.16	0.14
SD+R	0.34	10.9	0.27	0.33	0.18
IT+R	0.36	10.7	0.25	0.32	0.2
SD+R+F1	0.64	42	0.61	0.48	0.29

[1] Output of the method developed by Scholten and Van der Tol (1998); see text.

3.3.6. Extinction probability

Extinction probability (Fig. 3.9) for Dispersogen A clearly depended on the individual-level effect scenario. With the Reprotox model, the concentration that was lethal to 50 % of the population, the $LC_{50(pop)}$, was 29.6 mg L^{-1}. Although there was a large difference between extinctions obtained with the SD approach ($LC_{50(pop)}$ = 55.4 mg L^{-1}) and with IT approach ($LC_{50(pop)}$ = 244.9 mg L^{-1}), this difference was significantly reduced when survival models were combined with the Reprotox model. Finally, there was still a difference between the $LC_{50(pop)}$, obtained with the combined reproductive and lethal effect simulation (SD+R) and with the inclusion of F1 generation effects, which were found to be 15.6 mg L^{-1}.

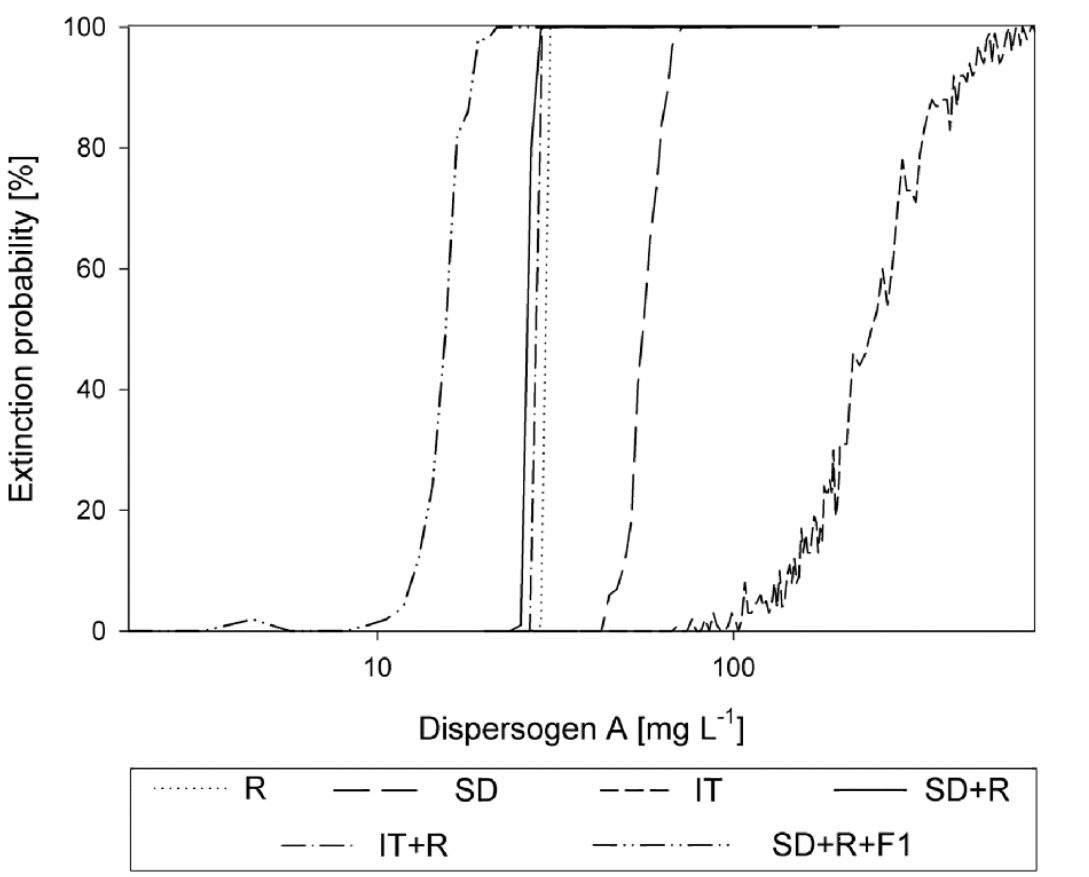

Fig. 3.9. Extinction probability of the population obtained with the different effect models; Acronyms of the effect(s) models are as in Fig. 3.3.

3.4. Discussion

3.4.1. Relevance of the test compound for ecology and ERA

We perceive Dispersogen A as an important case study for two reasons. From an ecological point of view, Dispersogen A mimics the mechanism of action of natural infochemicals (Klaschka, 2008) that induce a shift in the biomass allocation to reproduction in *Daphnia* (Coors et al. 2004; Hammers-Wirtz, 2002; Hammers-Wirtz and Ratte, 2000). Tradeoffs between size and number of neonates are a common strategy in *Daphnia* when faced with changes in environmental conditions such as crowding (Cleuvers et al. 1997), food scarcity (Ebert, 1993), or predation (Coors et al. 2004). The presence of a toxicant with a similar

mechanism of action might alter the adaptive responses of the daphnids (Hammers-Wirtz, 2002). Moreover, if adaptive strategies induce the production of fitter generations, the presence of Dispersogen A induces the contrary effects (Hammers-Wirtz and Ratte, 2000).

From a regulatory point of view, the multiple and complex effects of Dispersogen A on individual daphnids led to the observed underestimation of the effects on laboratory populations, which justifies the need to proceed with further investigations to ensure the conservatism of current risk assessment for Dispersogen A to field populations. Standard tests do not identify the mechanism underlying the observed effects on individuals; even from the adopted reproduction tests (OECD, 2008) or immobilization tests (OECD, 2004), no inferences on mechanisms can be made. Population growth experiments have been recommended as a surrogate for reproduction tests (Hammers-Wirtz and Ratte, 2000) because they provide additional information on other population-level endpoints (population growth rate, population size), and are therefore more reliable (Agatz et al. 2012). However, they still do not allow for inferences on the mechanisms underlying the effects on populations. We conclude that it is important for establishing an accurate risk assessment of chemicals to understand, as a first step, the mode of action of the toxicant in question.

Chemicals with multiple toxicity effects are therefore particularly challenging for risk assessment. Here, we discuss the modelling approach used in the present study to understand the multiple modes of action of Dispersogen A and their effects on populations, and we highlight its advantages and implications for the risk assessment.

3.4.2. Modelling approach

3.4.2.1. Reproductive effect models

A biphasic relationship existed between Dispersogen A concentration and the reproductive output of individual daphnids: low doses trigger a stimulatory reproductive response followed by a declining phase at high concentrations. The interest in including sub toxic concentrations in dose-response relationships has been expressed in studies on *Daphnia* for diverse substances like heavy metals (Bodar et al. 1988) and insecticides such as chlorpyrifos and triazophos (Li and Tan, 2011), whereas interpreting and reporting test results for chemicals' effects in ERA still traditionally rely on the use of a monotonous sigmoidal function, ignoring stimulatory effects (Conolly and Lutz, 2004). However, dismissing the hormetic aspect of the dose response curve is not a correct methodology, particularly for Dispersogen A, because stimulatory effects were observed not only at low exposure concentrations, but up to relatively high concentrations (10.2 mg L^{-1}). In this study, with an adequate parameterization of the Brain and Cousens model (Eq. 1), we could describe the entire dataset, allowing predictions to be made at any concentration point in the curve and allowing for an efficient modelling of reproductive effects at the population level. To integrate these measured sublethal effects, using an IBM is optimal for extrapolating effects from the individual to the population level (Preuss et al. 2010).

3.4.2.2. Survival models

Whereas toxicity effects of 3,4-dichloraniline on populations could be efficiently predicted with IDamP (Preuss et al. 2010) by implementing simple dose-response relationships for survival and for reproduction, a dynamic effect model was necessary to simulate the survival of daphnids exposed to Dispersogen A. Toxicodynamics were expressed through the increased

sensitivity of daphnids over time, with an EC_{50} in acute toxicity tests (48 h) of 167 mg L^{-1} which decreased to 16.5 mg L^{-1} in chronic tests. The increased sensitivity of daphnids over time calls for the use of a TK/TD model in the present study (Ashauer et al. 2011; Ashauer and Escher, 2010).

According to the single effect models tested within our IBM, reproductive toxicity effects predicted an increase in population size at low exposure concentrations (in the capacity experiment). The subsequent decrease at high concentrations was due to effects both on reproduction and on survival. Yet, the reduction in the population size (Fig. 3.3 a and b) obtained with the Reprotox model had steeper slopes than with the survival models in both the capacity and the exponential growth experiments. This suggests that the effects caused by Dispersogen A on populations were mainly due to effects on reproduction. Nevertheless, it was necessary to account for the combined effect scenario incorporating survival and reproductive toxicity because it yielded more accurate predictions of the real measured population size than any of the single effect scenarios tested.

At the highest Dispersogen A concentrations, even when lethal and sublethal effects were simultaneously integrated, the population dynamics was still not fully captured. Effects on reproduction and survival appeared to be the determinants of population size in the capacity experiment, but in the exponential growth experiment, large deviations were observed at 4.1 mg L^{-1} and higher concentrations. Additionally, with the adopted assumption for survival (SD) which over-estimated the effects of the chemical at the individual level (at 25.6 mg L^{-1}, Fig. 3.2 a), effects were still underestimated at the population level. This suggests that further mode(s) of action triggered the observed reduction in population size. The results obtained by incorporating the F1 generation effects, even using a simple approach, greatly improved the

agreement between the model predictions and the measured data. This is clearly observed in the exponential growth experiment in which all the statistical indicators of goodness of fit improved greatly compared to those of SD+R scenario. In the carrying capacity experiment, the adequacy of the model remained constant but its reliability decreased. This means that the model captured the same area of the measured data but with higher uncertainty levels. This was to be expected since we introduced another effect model (F1 model) to capture the effects at the highest concentration (25.6 mg L^{-1}). Therefore, the increase in variability is due to considering the F1 effects at 4.1 and 10.2 mg L^{-1} (also observed in Fig. 3.5 a) which did not improve the fit of the model at these concentrations. Furthermore, the sum of squared errors was greatly reduced in both experiments with the SD+R+F1 model. These findings clearly demonstrate that the F1 generation effects are needed to fully explain population-level effects.

Population extinction probabilities calculated using the combined scenarios differed only slightly from the population extinction due to reproductive toxicity effect alone, indicating a stronger implication of reproductive effects in predicting population-level effects. Nevertheless, the extinction probability of the population obtained by integrating the F1 model increased to a greater extent than with the survival models, suggesting, in accordance with the results mentioned above, that F1 generation effects might control the effects on population more than survival effects.

Following these results, we were able to rank the individual-level effects of Dispersogen A according to their importance and their role in determining population-level effects: the hormetic effects on individuals' reproductive output accompanied by the reduction of the neonates' fitness were the strongest predictors of population size, followed by the F1 generation

effects and finally, the effects on individual survival that appeared to have the lowest impact on populations.

3.4.3. Advantages and implications for risk assessment

Using an IBM combined with a TK/TD model, we were able to capture the multiple organism-level effects of Dispersogen A and detect the potential mechanisms controlling *Daphnia* populations by testing several effect scenarios. This study explicitly showed that separately considering the impact of single toxicity effects on individual survival and reproduction might underestimate the effects on populations. Even a combination of these two effects still did not capture all the observed effects on populations. Not accounting for the multiple effects explains why the risk assessment was not protective for daphnid populations in the laboratory, and disputes the robustness of risk assessment procedure in the case of Dispersogen A for field populations. No other tool allows such an investigation and therefore this study highlights the potential of mechanistic effect modelling to supplement current risk assessment approaches and to improve their robustness. To achieve these insights, it was critical that we used a combination of population-level data with mechanistic effect models to inversely determine the modes of action of the toxicant at the individual level. This cross-level use of data is a key element of pattern-oriented modelling (Grimm et al. 2005; Grimm and Railsback, 2012); it reflects the fact that within higher-order biological organizations, the performance and behaviour of individuals is affected by the size and structure of the population, but at the same time, population size and structure emerge from the individuals' performance and behaviour. Pattern-oriented modelling aims to capture and use these mutual effects to find the most appropriate representation of structures and processes.

3.5. Conclusion and recommendations

According to the findings of this study, we suggest that the measured individual-level endpoints in ERA should be revised and re-adjusted for the case of Dispersogen A. Additionally, in cases where the chemical in question induces hormetic effects at the individual level, we strongly recommend that further population tests or F1 generation tests be conducted and their results be taken into account in the risk assessment. The use of validated population models in combination with laboratory population experiments is a powerful tool for investigating toxicity effects in various experimental settings, and also for simultaneously incorporating the effects of multiple stressors and exposures, which reflects reality. We believe that mechanistic effect modelling has considerable potential for improving the accuracy of ERAs of chemicals in the future and would greatly assist in achieving efficient and trustworthy decision making.

CHAPTER 4

The importance of interactive maternal traits and environmental factors in determining offspring size in *Daphnia magna*

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4.1. Introduction

Owing to its essential role in food-web dynamics of freshwater ecosystems and to its importance as a test organism in ecotoxicology, the cladoceran *Daphnia magna* (Straus) is frequently used in modelling studies to answer diverse questions on the biology and ecology of this organism (Kooijman, 2000; Vanoverbeke, 2008; Preuss et al. 2009), or to elucidate complex mechanisms behind its responses to natural (Gergs et al. 2013) or anthropogenic (Preuss et al. 2010) stressors. Depending on the addressed question, ecological models can, in general, be based on empirical description of processes (Preuss et al. 2009) or on energetic concepts (Kooijman, 2000; Vanoverbeke, 2008). Yet, an important limitation of these available models is that none of them accounts for the natural variability in the offspring size (OS) in *Daphnia magna*. This trait, in fact, determines vital individual processes like growth (Hammers-Wirtz and Ratte, 2000), size at first reproduction (Barata and Baird, 1998) or size-selective predation (Lampert, 1993; Gergs and Ratte, 2009), which in turn affect higher-order processes such as population growth rate, maturation (Sakwinska, 2004; Rinke, 2006), population survival rates (Sakwinska, 2004; Dudycha and Lynch, 2005) or resistance to starvation (Tessier and Consolatti, 1991).

To efficiently capture the variation in the OS in *Daphnia*, it is essential to simultaneously account for the relevant variables that induce a change in this life history trait. Changes in OS in *Daphnia* are frequently a consequence of maternally-mediated responses to environmental effects. As explained by Mousseau and Fox (1998), the experienced environmental changes are transmitted from mothers to their offspring, whose development is thereby altered. For example, mother daphnids reduce the size of their progeny in the presence of large-selective predators such as fish (Stibor, 1992), and do the opposite in the presence of

small-size selective predators such as *Chaoborus* (Stibor and Lüning, 1994; Coors et al. 2004). OS may also vary in response to differing maternal food levels (Tessier and Consolatti, 1991; Glazier, 1992; Ebert, 1993; Enserink et al. 1993; Lampert, 1993; Guinnee et al. 2004, 2006) or to varying population densities (Gliwicz and Guisande, 1992; Goser and Ratte, 1994; Burns, 1995; Cleuvers et al. 1997; LaMontagne and McCauley, 2001).

In this study, we investigated the interactive effects of multiple maternal traits and environmental rearing conditions on the neonate body size in *Daphnia magna.* We then parameterized a multivariate model describing OS variation with these variables. Finally, we validated the resulting model using an independent dataset.

4.2. Material and Methods

4.2.1. Experimental dataset

Data from previous experimental studies made at the Institute of Environmental Research, RWTH Aachen University (Cleuvers, 1995; Popovic, 1996; Goser, 1997; Coors, 1999; Agatz, unpublished; Table 4.1) were used for parametrizing the model. Daphnids (≤ 24 h old) used in these tests originated from third broods of acclimated mothers. Mothers had been individually reared in cultures kept under constant temperature and light conditions (20 ± 1 °C, 16-h light: 8-h dark photoperiod in a climate-controlled chamber) for several generations (almost 30 years) in the laboratory. These cultures were fed three times a week with log-phase *Desmodesmus subspicatus*. The algae were harvested from batch cultures grown in medium as described by Kuhl and Lorenzen (1964), centrifuged and re-suspended in 80 ml Elendt M4 medium. Mothers belonged to two different clones (Table 4.1): clone 5 was reared under laboratory conditions, and clone Tonne originated from field sampling near Aachen (Coors, 1999). Experiments a, b and c were conducted under flow-through conditions at constant food

levels and a flow rate of 12 ml min^{-1} (according to Goser and Ratte, 1994). Experiments d and e were run under semi-static scenario, where daphnids were fed daily and the culture medium changed every Monday, Wednesday and Friday.

Table 4.1. List of the different data sources used in the study and description of the experimental conditions using *D. magna*

Experiment (literature)	**Clone**	**Density** (ml per daphnid)	**Food concentration** (mg C per daphnid per day)	**Scenarios**
a (Cleuvers, 1995)	5	12.5-50	0.1	FT
b (Popovic, 1996)	5	12.5-50	0.1	FT
c (Goser, 1997)	5	1.25 - 80	0.1 ; 1	FT
d (Coors, 1999)	Tonne	15-80	0.05; 0.075; 0.1; 0.2	SS
e (Agatz, unpublished)	5	80	0.2	SS

FT and SS stand for flow-through and semi-static scenarios, respectively.

4.2.2. Measured variables

We tested the following maternal traits as potential variables affecting the OS: the maternal body length and age, the brood size, the brood number and the amount of ingested carbon per mother. Food concentration and density-dependence effects on OS were tested as potential environmental variables influencing the OS.

4.2.2.1. Determining the maternal body length, age, brood number and brood size

Within each released brood, the maternal body length and age, as well the brood size and number were recorded, and the average OS per brood was calculated. The individual

measurements of neonates and mothers' lengths were made within 12 hours of the time of brood release. Body-lengths were measured (from top of eye to base of posterior spine) using a software designed at the institute (Preuss, unpublished). The mother and all her released offspring were placed in a Petri dish using a pipette and the surplus of water was removed to prevent the movement of the animals. The Petri dish was scanned (Canoscan 8800F) at X1200 dpi resolution. Once the scan finished, the mother was transferred into the medium and the neonates were discarded.

4.2.2.2. Calculating the ingested carbon per mother daphnid

Because the experiments were run under different feeding scenarios (semi-static and flow-through), we used the ingested carbon for each individual mother as an explanatory variable for OS instead of the food concentration administered. To calculate the ingested carbon, we used the IDamP model **(Chapter 2)** which was set to the environmental conditions of each experiment described in Table 4.1. A dynamic simulation is especially necessary for semi-static conditions, where food availability might be triggered by the ingestion rate of the daphnid and the volume of the beaker. IDamP model dynamically simulates the ingestion of daphnids based on the equation of McMahon and Rigler (1963), including the maximum filtration rate, a half-saturation constant (K_s) and the incipient limiting level (ILL). This function describes the filtration rate of daphnids to be constant below the ILL at the maximum filtration rate and to decrease with increasing food concentration above the ILL (Lampert, 1987). This behaviour leads to a constant ingestion rate at higher food concentrations (Preuss et al. 2009). The maximum filtration rate for the daphnid is calculated as a function of body length. In this equation, feeding is expressed by means of daily ingested carbon per individual (IC, Eq. 1),

which depends on the filtration rate (F, in ml h^{-1}) and on the food concentration (C, in mg C ml^{-1}).

$$IC = F \times C \times 24 \quad (1)$$

The filtration rate (Eq. 2, Preuss et al. 2009) is calculated as a function of maternal body length (L, in mm) and the concentration of algae (c, in cells ml^{-1}).

$$F = \frac{p \times L^s \times K_s}{K_s + c - ILL} \quad (2)$$

with p = 0.5 ml mm^s h^{-1}: Factor for filtration rate

s = 2.45: scaling factor for filtration rate

K_s = 30644 cells ml^{-1}: half saturation constant

ILL = 8506 cells ml^{-1}: incipient limiting level

4.2.3. Data analysis

Statistical analyses were made using SPSS software (IBM SPSS statistics version 20) and the graphs were plotted in Sigma Plot (Systat Software, Inc. Sigma Plot SPW 11.0). We examined the relationships between OS and all the measured variables mentioned in the previous sections.

4.2.3.1. Variation of OS with the environmental variables: density-dependence effects

Experiments a, b, c and d (Table 4.1) contained data from both control and density conditions, where the daphnids were reared in groups in the same beaker and the available volume per daphnid varied from 1.25 to 80 ml (Table 4.1). This dataset was used separately to build up the regression model relating OS variation to density-dependence effects. Determining this relationship was done stepwise: to discard food level effects, we considered data relative to

only one food concentration (0.1 mg C x daphnid^{-1} x day^{-1}). For each experiment, we used the data from 'control' daphnids to make linear regressions relating OS to maternal body size (relative to 0.1 mg C x daphnid^{-1} x day^{-1}). The obtained equations were used to derive the expected sizes of offspring released from mothers under density conditions. The average relative OS (measured/expected OS) was then plotted against the density values (expressed as available volume per daphnid).

4.2.3.2. Variation of OS with the maternal traits

In order to check for the existence of genetic (clonal) differences within our dataset, we ran an analysis of Variance (ANOVA, $P < 0.05$) where the homogeneity of variances (Levene's test) and the normality of the distribution (Shapiro-Wilk test) were verified. OS variation with the maternal body size was studied for the different food concentrations and regression analyses were made to assess the significance of the different relationships. Effects of brood size and brood number on OS were tested using simple linear regressions. Similarly to density dependence effects, the relationship of OS to the ingested carbon was determined by deriving the mean expected OS from the regression equation relative to data for 0.2 mg C (Fig. 4.1 a). Then, the relative OS, which is a function of maternal body length, was plotted against the daily amount of ingested carbon.

4.2.3.3. Multiple regression analysis

Stepwise multiple linear regression analysis (MLR) was conducted in order to generate equations linking OS to effects of maternal traits and environmental factors (maternal body size, maternal age, brood size, ingested carbon and brood number). This approach accounts for the effect of each variable after controlling for the effects of other variables on OS. In these analyses, the independence of the errors, homoscedasticity and normality of errors were

verified. The significance of maternal body size, age, brood size, brood number and the ingested carbon were tested. The accuracy of the obtained models was judged by the value of the coefficient of determination (r^2) and the significance of each predictive variable (t-test). Once the significant variables were identified, we tested the goodness of fit to the data by i) plotting the measured against the predicted data and determining the r^2, and ii) determining the mean (Eq. 3) and the maximum deviation of the measured data to the model.

$$Deviation\ (\%) = \frac{Measured\ OS - Predicted\ OS}{Measured\ OS} \times 100 \qquad (3)$$

4.2.4. Model validation

The ability of the developed model to predict OS variation was tested against an independent dataset (Glazier, 1992) obtained from *D. magna* reproduction tests using two genetically distinct clones (L-F and P-S1). The culture medium (120 ml of ASTM hard water) as well as food source (*Chlorella vulgaris*) differed from the dataset used for developing the model. The experiments were carried out in 120 ml ASTM media with individual daphnids. Daphnids were daily fed 0.3 mg C L^{-1} (0.036 mg C x $daphnid^{-1}$ x day^{-1}) and 1.5 mg C L^{-1} (0.18 mg C x $daphnid^{-1}$ x day^{-1}). The carbon content of *C. vulgaris* was transformed to its equivalent for *D. subspicatus*, assuming that *D. subspicatus* has an average carbon content of 1.95×10^{-8} mg C $cell^{-1}$ (Sokull-Kluettgen, 1998; unpublished results from the Institute of Environmental Research, RWTH Aachen University). The data provided information on the egg dry mass. The neonate's dry weight (DW) was derived from egg dry mass using the relationships given in Glazier (1992: Table 3). Accordingly, newborns were 22.5 % heavier than eggs for clone L-F and 47.05 % for clone P-S1. Finally, a regression model (Kooijman, 2000) was fitted (Eq. 4) to convert the dry weight (DW) of neonates into body length (mm):

$$DW = 11.89 \times Length^3 \quad (4)$$

4.3. Results

In our dataset, OS was not significantly influenced by clonal differences (ANOVA, $P > 0.05$).

4.3.1. OS dependence on maternal traits

4.3.1.1. Effects of maternal body size and age

OS showed significant positive linear relationships with maternal body size and age at all food concentrations (Fig. 4.1 a and b). The slopes of the different regression equations linking OS to these two variables were in general steeper at low (0.05 and 0.075 mg C) than higher food concentrations (0.1 to 1 mg C). Large females (body size ≥ 3.5 mm) reared at high food concentrations produced smaller offspring than the low-fed ones of the same size (Fig. 4.1 a). However, for the smaller mothers (body size ≤ 3 mm), OS had a non-linear response with food: it increased with decreasing food concentration from 1 to 0.1 mg C and then decreased when food decreased down to 0.05 mg C. In comparison, females of the same age produced smaller offspring with increasing food concentration (Fig. 4.1 b).

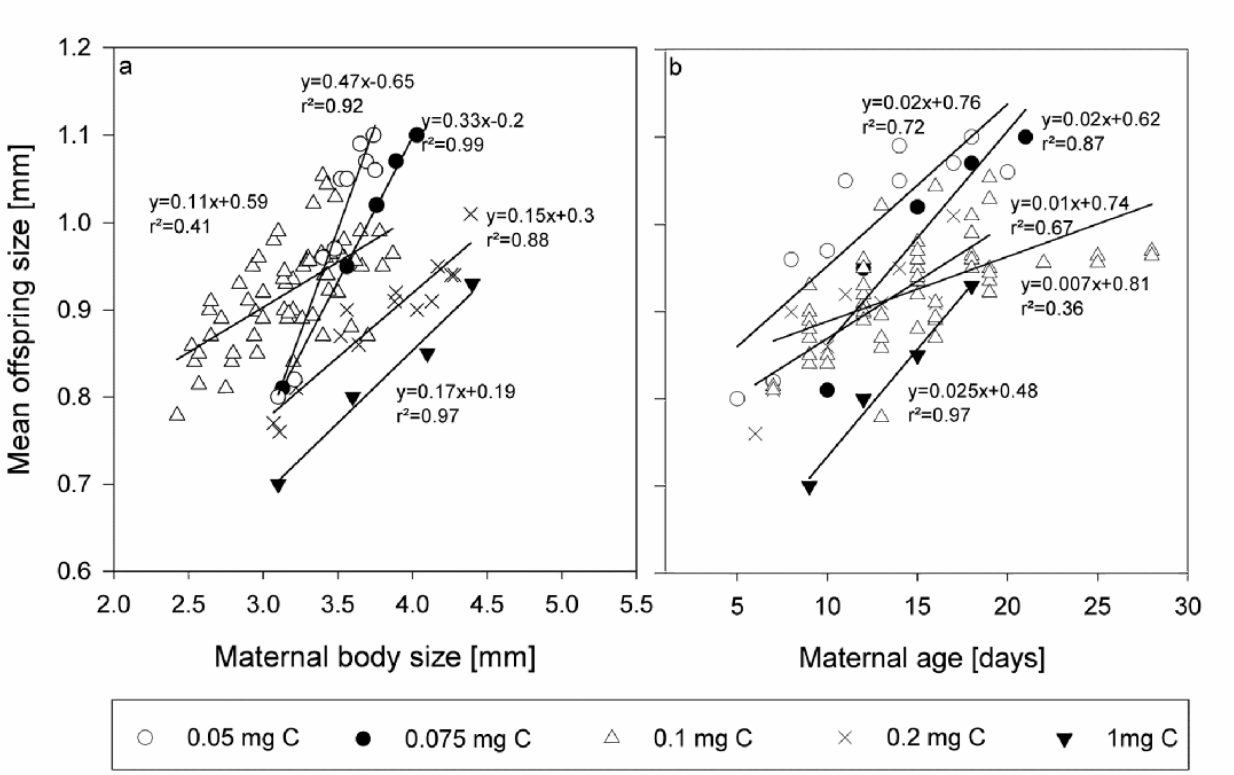

Fig. 4.1. Dependence of the mean OS on the maternal body size (a) and age (b) in *D. magna* in relation to food level (n = 118).

4.3.1.2. Effects of the daily amount of ingested carbon

Independently of the maternal body size, there was a significant negative relationship between OS and the ingested carbon ($r^2 = 0.52$, n =118; Fig. 4.2): the largest neonates were born to mothers with the lowest ingested carbon and the smallest neonates to mothers with the highest ingested carbon.

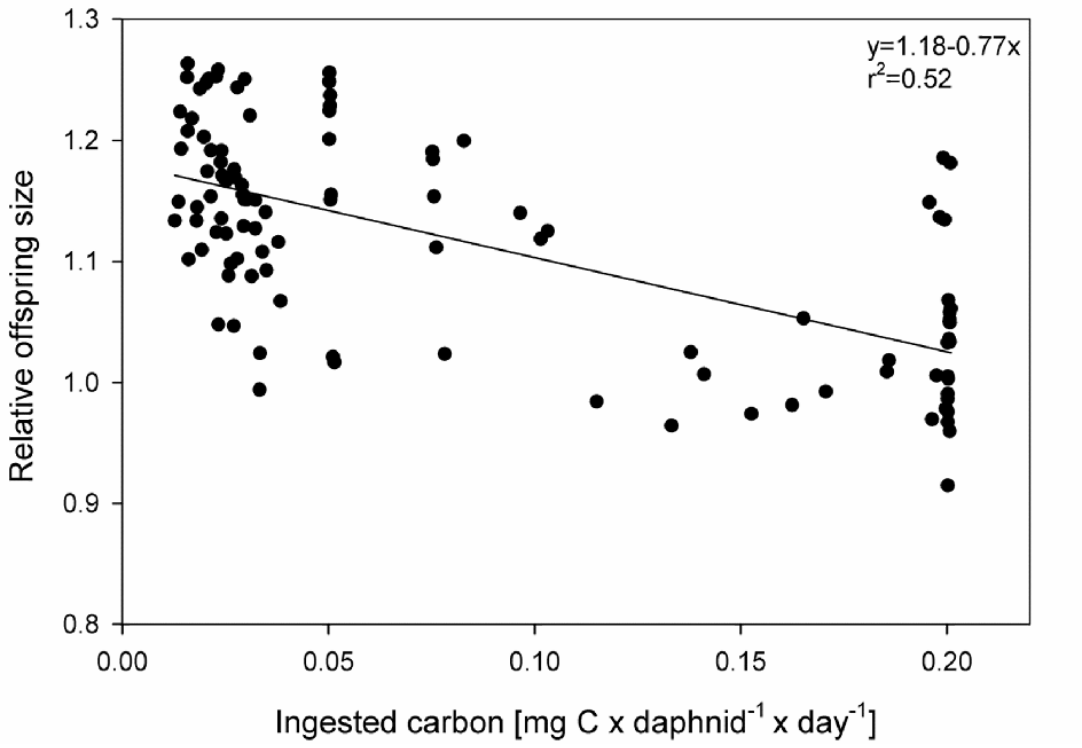

Fig. 4.2. Dependence of the relative OS on the daily amount of ingested carbon in *D. magna*. The relative OS is the ratio between measured values and model predictions based on the maternal body size.

4.3.1.3. Effects of the brood size

There was no data available on the brood size for daphnids reared at 1 mg C. Based on the remaining dataset, OS tended to decrease with larger broods, but the relationship was not significant ($r^2 = 0.051$, n = 114; Fig. 4.3).

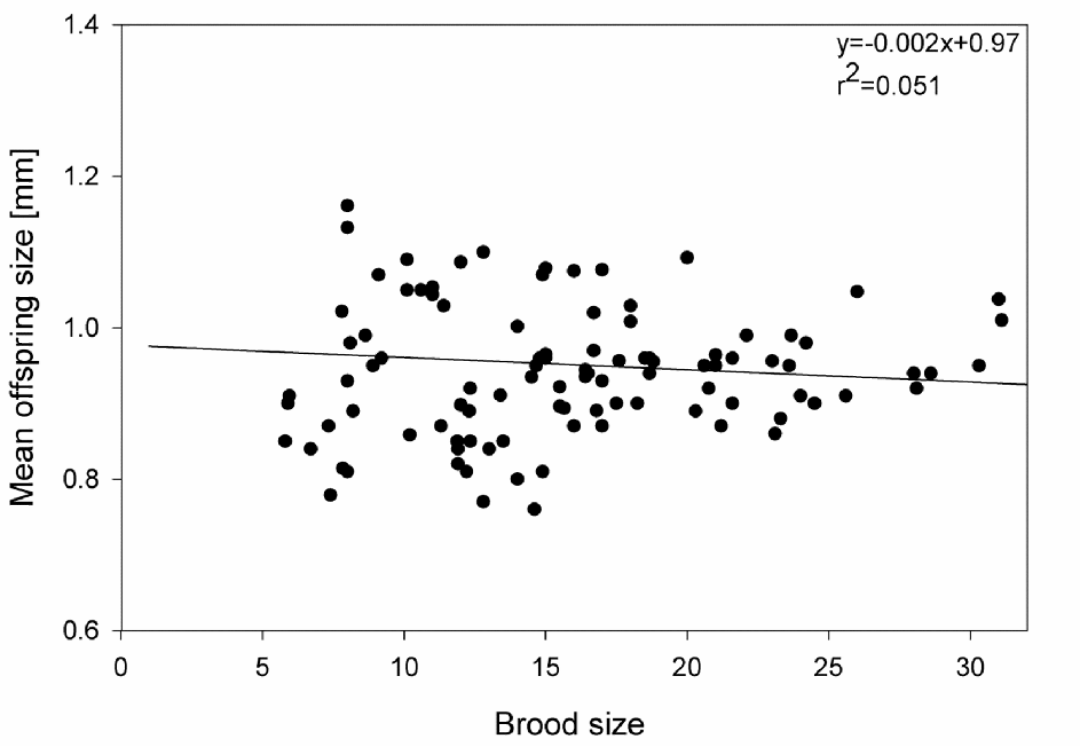

Fig. 4.3. Dependence of the mean OS on the brood size (n = 114) in *D. magna* (excluding the dataset for 1 mg C in reference c, Table 4.1).

4.3.2. OS dependence on the maternal environment

The variation in the relative OS (based on the regression of OS on maternal body length and the food concentration) followed a two-parameter exponential-decay function (Eq. 5, Fig. 4.4).

$$Relative\ offspring\ size\ = 1 + a\ \times\ e^{-b \times density} \qquad r^2 = 0.94\,; n\ = 169 \quad (5)$$

with a = 0.1266; b = 0.0659; and density expressed in ml per daphnid.

OS varied significantly with density (P < 0.05). At minimal density values ranging between 80 and 50 ml per daphnid, there was no variation in the OS. However, the OS significantly increased with increasing densities. Density effects were most pronounced when

the volume decreased below 20 ml per daphnid. Under these conditions, the predicted OS was significantly smaller than the observed OS.

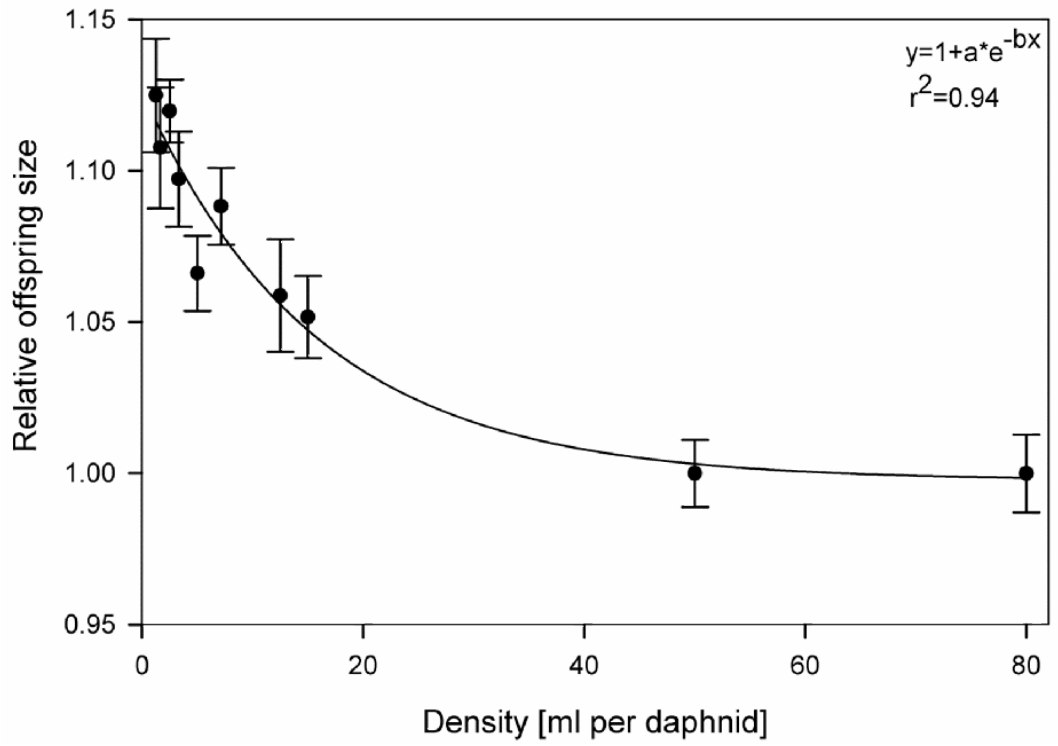

Fig. 4.4. Dependence of the relative OS on density in *D. magna* (n = 169). The relative OS is the ratio between measured values and model predictions based on maternal body size. Food effect was excluded by considering data deriving from only one food level (0.1 mg C x daphnid^{-1} x day^{-1}). Error bars indicate 95 % confidence intervals.

4.3.3. The multivariate model for OS

The parameters obtained by stepwise MLR and their significance are summarized in Table 4.2. Effects of maternal body size, brood size and the ingested carbon were significant (P < 0.0001). Maternal age had a P value of 0.039 whereas brood number was not a significant variable (P = 0.185) and was removed from the model. OS showed a positive dependency on maternal body size and age, and negative dependencies on the ingested carbon and brood size. The model was able to describe 71 % of the variability within the dataset.

Table 4.2. Multiple linear regressions of OS on maternal body size, maternal age, brood number, brood size and the ingested carbon. All dataset (except 1 mg C in experiment c) were used in this analysis

	Factors	**Coefficient**	**Standard Error**	**t-test**	***P***
Step one	Constant	0.630	0.031	20.560	< 0.0001
	Maternal body size	0.090	0.008	10.776	< 0.0001
Final step	Constant	0.495	0.045	11.052	< 0.0001
	Maternal body size	0.156	0.018	8.599	< 0.0001
	Brood size	-0.005	0.001	-6.758	< 0.0001
	Ingested carbon	-0.536	0.112	-4.793	< 0.0001
	Age	0.002	0.001	2.175	0.039

Because maternal body size and age are strongly related variables, and age was the last variable introduced last in the MLR model, we ran another MLR excluding this variable. The results (Table 4.3) show that all variables included in the analysis contributed significantly to determining the OS. Moreover, by removing the maternal age, the collinearity between the explanatory variables was significantly reduced without affecting the model's goodness of fit (r^2 = 0.7). Therefore, the final model describing OS variation with the maternal body size (ML), ingested carbon (IC) and brood size (BS) as explanatory variables is shown in Eq. 6.

$$OS = 0.436 + 0.184 \times ML - 0.595 \times IC - 0.00567 \times BS \qquad r^2 = 0.7; n = 114 \quad (6)$$

Table 4.3. Multiple linear regressions of OS on maternal body size, brood size and the ingested carbon. All dataset (except 1 mg C in experiment c) were used in this analysis

	Factors	**Coefficient**	**Standard Error**	**t-test**	***P***
Step one	Constant	0. 634	0.031	20.544	< 0.0001
	Maternal body size	0.089	0.008	10.634	< 0.0001
Final step	Constant	0.436	0.037	11.799	< 0.0001
	Maternal body size	0.184	0.0134	13.722	< 0.0001
	Brood size	-0.00567	0.00079	-7.185	< 0.0001
	Ingested carbon	-0.595	0.11	-5.387	< 0.0001

The model fitted well to the measured data (Fig. 4.5 a) with a mean deviation of 4.01 ± 3.2 % and a maximum deviation of 16.92 %. OS dependence on density was considered by multiplying the MLR by the non-linear density effect function. The model describing OS variation to density in addition to maternal body size, brood size and ingested carbon was obtained by multiplying the MLR equation (6) by the density-effect equation (5). The resulting equation is:

$$OS = [0.436 + 0.184 \times ML - 0.595 \times IC - 0.00567 \times BS] \times \left(1 + 0.1266 \times e^{-0.0659 \times density}\right)$$

$$r^2 = 0.65; n = 169 \quad (7)$$

The model described OS variation in a good agreement with the measured data (r^2 = 0.65, Fig. 4.5 b) with a mean and a maximum deviation of 4.07 (± 3.16 %) and 16.92 %,

respectively.

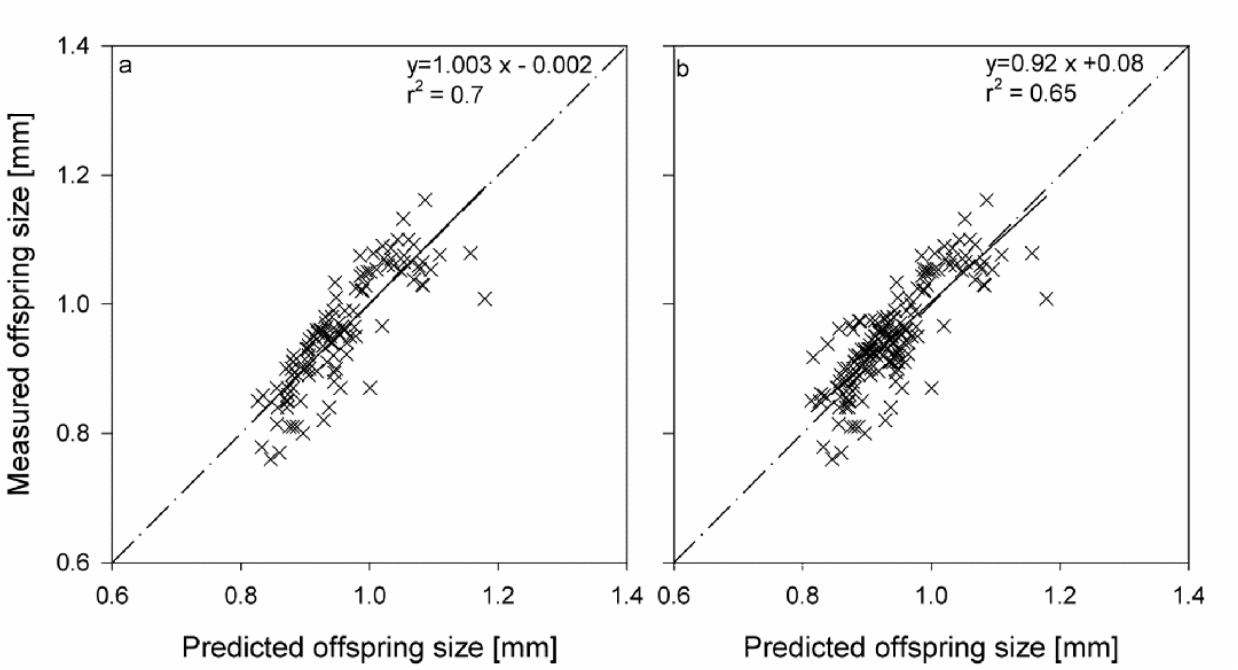

Fig. 4.5. Predicted-measured statistics for the OS (excluding the dataset for 1 mg C in reference c, Table 4.1). Predicted values were obtained by means of multiple linear regressions without (a, n = 114) and with (b, n = 169) density effects. Full lines show the regressions and dashed lines the optimal 1:1 prediction.

4.3.4. Model validation

There were no validation data available for density dependence effects. Thus, the results in Fig. 4.6 show the validation of the MLR model obtained under density-free conditions (Eq. 6), predicting the variation of OS with maternal body size, brood size and ingested carbon. The model appears to be largely validated because the measured OS varied with the predicted OS with a slope of 1.03 and an intercept of 0.007, which should ideally equal 1 and 0, respectively. The model was able to describe 36 % of the variability in the data.

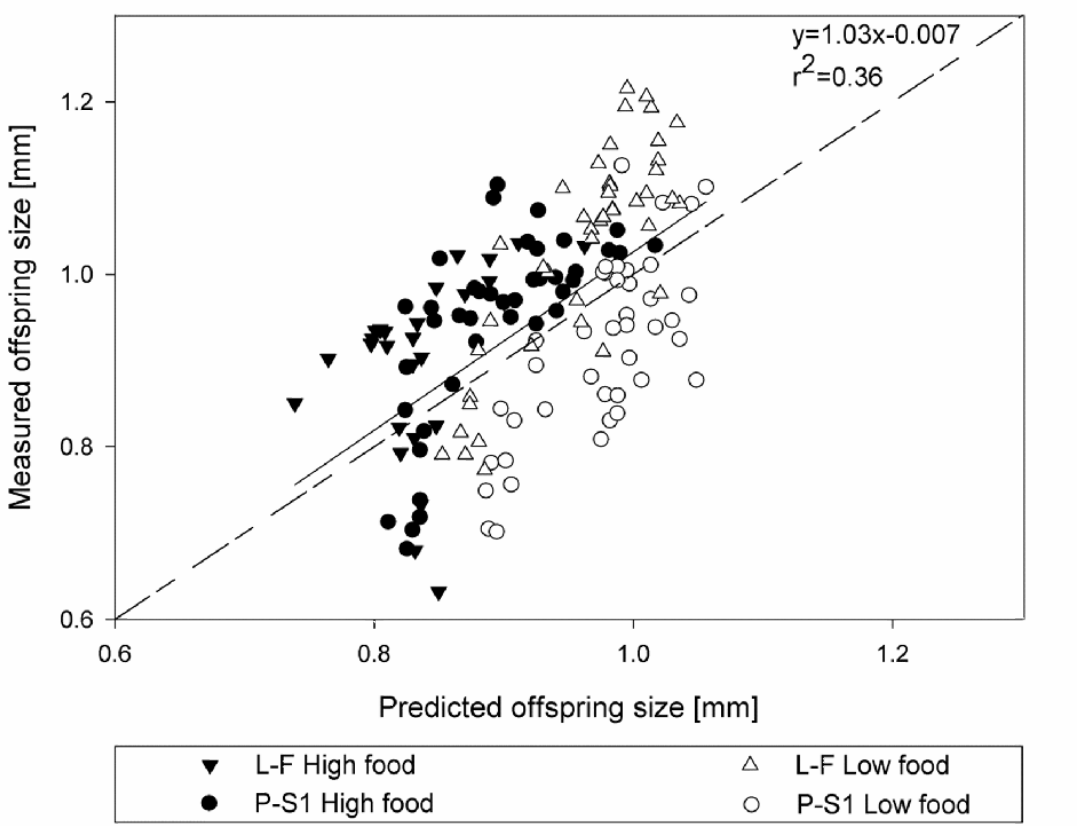

Fig. 4.6. Test of the model on independent data using two distinct clones: P-S1 and L-F, and two different food levels. Data (n = 83) originated from Glazier (1992). The full line indicates the model predictions and the dashed line the optimal 1: 1 prediction.

4.4. Discussion

In this work, we set up an empirical model describing the variation in the size of offspring born from mothers reared under different feeding and density conditions. The model was tested against an independent dataset using two genetically different clones (which also differed from the clones used in the parameterization of the model), a different food source and culture medium, as well as different food levels. It is important to mention that all the allometric relationships of brood mass, brood size and egg mass were significantly different between the two clones (see Glazier, 1992 for details). Besides, if we used life-cycle data from flow-through and semi-static tests to develop the model, validation data was obtained at semi-static

conditions. Despite these divergences, the multivariate model accurately described the variation in OS (Fig. 4.6).

The amount of ingested carbon was the most determining factor of OS variation, contrary to brood size which accounted for the least effects. Even though the two variables were correlated, maternal body size was a better predictor than maternal age. Additionally, the brood number did not significantly influence the OS and therefore, the exclusion of these two variables from the model did not affect its efficiency to describe the variation in OS. Our results support the findings of Ebert (1993), showing that age and brood number did not affect OS in two *D. magna* populations from different artificial ponds, but the food level, maternal body size and brood size strongly affected OS. However, opposite results were observed for *D. galeata* (Sakwinska, 2004) where the juvenile growth increment differed between young, intermediate and older mothers, leading to a dependence of OS on maternal age. The observed variability in the factors determining OS in *Daphnia* could be attributed to inter-species differences.

4.4.1. OS dependence on maternal body size

Positive linear relationships between the size of the mother and that of her offspring were obtained under high, intermediate and low food levels. Other studies have shown similar relationships where the effort per offspring (in terms of size or mass of eggs or neonates) increased with maternal body size for different clones of *D. magna*, under high and low food conditions (Glazier, 1992; Ebert, 1993; McKee and Ebert, 1996). Positive effects of maternal body size on OS were observed in other *Daphnia* species like *D. galeata* (Sakwinska, 2004) or *D. hyalina* (Burns, 1995) and in other organisms (Bernardo, 1996) as well.

4.4.2. OS dependence on the ingested carbon

Mothers clearly showed a dynamic shift in the way they provision their offspring when food decreases, changing from an emphasis on egg number in food-rich environments to egg size in food-poor environments. These results support the findings of Glazier's (1992) humped-shape model, predicting a positive relationship between OS and the ingested carbon at low food levels and a negative correlation at high food levels. The negative relationship observed in the present study is also found in other *Daphnia* species like *D. pulex* (Taylor, 1985), *D. pulicaria* and *D. hyalina* (Guisande and Gliwicz, 1992). By contrast, positive covariation between OS and food level was found in two different studies with *D. pulex* (Lynch, 1989; LaMontagne and McCauley, 2001). In these studies, either very low food concentrations were used (equivalent to 0.01-0.06 mg C per *Daphnia* per day, LaMontagne and McCauley, 2001) or the daphnids were not daily fed as done in the present study or in Glazier (1992)'s study which was used to validate our model. These two experiments thus fit into the left hand side of the humped-shape model.

4.4.3. OS dependence on maternal body size and food level

In large females (body size $\geq$ 3.5 mm), OS showed a negative relationship with food concentration. In small females (body size $\leq$ 3 mm), the increase in OS with decreasing food concentrations (down to 0.1 mg C x daphnid^{-1} x day^{-1}) and its decrease at the lowest food concentrations (down to 0.05 mg C x daphnid^{-1} x day^{-1}) are consistent with the humped- shaped model of Glazier (1992, Fig. 7), where both positive and negative relationships between OS and food demand are hypothesized. At very low food levels, egg mass becomes smaller in *Daphnia* because very small females are structurally and energetically incapable of producing large eggs (Glazier, 1992). This hypothesis may also explain the steeper slopes of the regressions relating

OS to maternal body length at the lowest food levels: under these conditions, small females were constrained to produce small offspring because of energy constraints and the spatial limitations in the brood pouch. However, large females may have had enough energy reserves to show the adaptive response of producing relatively large offspring, as was observed in other *Daphnia* studies (Glazier, 1992; Ebert, 1993; Lampert, 1993; Boersma, 1995, 1997). At higher food levels, the slope was less steep because small females were not as energy limited, and large females were favoured to produce many small offspring. In this way, small daphnids continued to produce smaller offspring than the larger ones because of spatial limitations of the brood pouch. However the difference was not as great because energy limitation is less important, resulting in the observed shallower slopes. The results obtained in this study support optimal offspring investment theory, which predicts that larger offspring should be produced under low compared to high food conditions (Goulden et al. 1987), as long as the mothers are not too small (thus preventing them from making larger offspring, as predicted by Glazier's (1992) OS response model).

Our analysis of the OS with both maternal body length and food concentration showed that, even at a laboratory scale where it is purposely attempted to reduce experimental variability, mother daphnids have different reproductive strategies according to their interactions with the environment, which had important repercussions on OS.

4.4.4. OS dependence on brood size

OS was larger in small broods compared to large ones. Most studies observed the same pattern in *D. magna* (Ebert, 1993; Boersma, 1997) and other cladoceran species (Taylor, 1985; Gliwicz and Guisande, 1992). However; the brood size accounted for only a small proportion of variability compared to the other factors; i.e. the ingested carbon and the maternal body size.

The importance of this variable in determining the OS might be indirectly related to its interaction with other variables, such as the food level (Ebert, 1993).

4.4.5. OS dependence on density

It is well known that *Daphnia* changes its reproductive strategy under varying density conditions. At high densities, daphnids grow more slowly and produce fewer offspring (Guisande, 1993; Goser and Ratte, 1994). Density effects were also shown to propagate to the F1 generation whereby daphnids living singly but descending from ancestors living in groups produced significantly larger offspring (F2) than daphnids descending from singly living ancestors (Cleuvers et al. 1997). Our results show that the daphnids responded to increasing density conditions by an increase in OS at the expense of brood size which decreased. This was observed by disentangling the food level effects. Our results are similar to those obtained by other authors (Cox et al. 1992; Naylor et al. 1992) who observed that at low densities, *D. magna* produces more and smaller neonates than at higher stocking densities where there were fewer and larger neonates produced. Cleuvers et al. (1997) explained that the daphnids shift their reproductive strategy from producing a higher quantity to a higher quality of neonates (heavier offspring) when the available culture volume is minimal. The increase in OS with increasing density conditions was also observed for other daphnid species like *D. pulex* (Ban et al. 2009), other Daphnidae species like *Simocephalus vetulus* (Perrin, 1989), as well as other aquatic invertebrates like copepods (Cooney and Gehrs, 1980). However, contrasting results were recorded for other *Daphnia* species: Burns (1995) showed that for *D. hyalina* and *D. galeata*, mothers kept in density conditions (≥ 150 individuals per liter) produced smaller offspring and smaller broods.

4.4.6. Adaptive value

In addition to the strong relationships between OS and maternal life-history traits, mothers were able to change their reproductive strategy in accordance to changes in the environmental conditions, i.e. available food and density, and this shift was manifested by a change in OS. The observed patterns of OS variation with maternal traits and environmental factors suggest an adaptive shift from quantity to quality of offspring as food availability per individual decreases. During spring and early summer, *Daphnia* populations grow rapidly (Hülsmann, 2003, Wagner et al. 2004), resulting in a depletion of available resources. At the end of the spring algal bloom, newborn daphnids have few available resources, exerting high physiological stress on individuals, which results in an elevated non-consumptive mortality (Hülsmann, 2003). These processes lead to a declining population size of *Daphnia*, which in some cases, particularly in eutrophic waters, can directly proceed to the initiation of a midsummer decline of daphnids (Hülsmann and Weiler, 2000; Hülsmann, 2003). In this context, producing fewer larger (fitter) offspring at low food levels and high population densities, but many small offspring at high food levels and low population densities may be adaptive responses for increasing population survival and growth, respectively.

4.5. Conclusion

Our study shows that multiple maternal and environmental variables significantly affect OS in *D. magna*. As a result, future models addressing ecological or biological questions regarding *Daphnia* populations should include the natural variability of OS in relation to relevant maternal and environmental variables. This would ensure a more realistic prediction of individual behaviour, thereby leading to a more accurate characterization of *Daphnia* population dynamics under natural or stressed conditions.

CHAPTER 5

Linking individual exposure to effects on populations: the role of biological interactions in determining the sensitivity of populations to chemicals

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5.1. Introduction

The release of chemicals into the environment can result in adverse effects on the living organisms and on the functioning of ecosystems. To prevent adverse effects from occurring, chemicals in the EU are authorized to be placed in the market only after an extensive assessment of their impact on organisms belonging to different trophic levels (Galic, 2012). However, a lot of criticism surrounds the current ecological risk assessment (ERA) methodology for many reasons. On one hand, while protection goals are often targeting the population level (EFSA, 2010; Hommen et al. 2010), ERA of chemicals is based on results of laboratory tests conducted on individual organisms. The toxicant concentration assumed to have no effects on populations is then derived simply by applying uncertainty factors to the calculated toxicity endpoints (as also stated in **Chapter 3**). On the other hand, these laboratory tests are conducted under favourable conditions, ignoring the complex interactions that occur in natural systems between the tested species and several environmental variables like the food level (Orcutt and Porter, 1984; Nandini and Sarma, 2003), predation (Boeing et al. 2005) or inter- and intra-specific competition (Goser and Ratte, 1994; Foit et al. 2012). Yet, these variables control vital individual-level physiological processes, and they might also interact with chemicals and alter their effects on the living organisms (Coors and De Meester, 2008; Rubach et al. 2011). Ignoring multiple stress exposure on individuals might therefore lead to a wrong interpretation of the real risk of chemicals on populations (Forbes and Calow, 2012; Knillmann et al. 2012 b).

Nevertheless, it is a challenging task to adequately assess the effects of multiple stress exposure on populations because of the lack of efficient tools to grapple with the required complexity. In fact, it is almost impossible to experimentally assess the joint effects of chemical

and non-chemical environmental stressors on organisms due to the testing efforts required and to the difficulty of simulating realistic scenarios at a laboratory scale (Grimm et al. 2009). Neither can this be done based on knowledge of the separate effects of each stressor because interactions between chemical and environmental stressors don't always lead to additive effects (Folt et al. 1999; Heugens et al. 2006). Furthermore, if the use of mesocosms was proven efficient to integrate more environmental realism into the ERA schemes (Brock et al. 2006) they remain costly and time consuming (Forbes, 2010), which makes decisions often based on a limited dataset. Therefore, approaches that allow extrapolations to other scenarios or biological levels are needed (Folt et al. 1999).

In this context, the 'virtual ecologist' approach was developed as a method to circumvent data limitations. It relies on the use of powerful models that allow testing complex, realistic scenarios (Zurell et al. 2009). In particular, individual-based models have the ability not only to link laboratory measured individual-level effects to the population level (Grimm et al. 2009) but also they easily integrate various environmental variables, as well as the life-cycle processes of the organism in question, allowing for a mechanistic understanding of the ecological impacts on populations (Vignati et al. 2007; Preuss et al. 2009, 2010). These features make them powerful virtual laboratories for testing hypotheses about population properties and constitute a major step towards increasing our understanding of environmental and chemical interactions (Berger et al. 2008).

In this study, we use the IDamP model as a virtual laboratory to investigate the changes, caused by the presence of environmental stressors, in the population sensitivity to chemicals with different modes of action on individuals. Chemicals targeted key physiological endpoints of *Daphnia* life cycle, including the individual reproductive output, survival, feeding rate or the

somatic growth rate. As environmental variables, we focused on the food level and on the ecological interactions with competition or predation, for their importance in controlling the dynamics of populations (Knillmann et al. 2012 b; Beketov and Liess, 2006; Liess and Foit, 2010).

5.2. Material and Methods

5.2.1. The individual based model IDamP

The IDamP model for *D. magna* was used to simulate the effects of different hypothetical chemicals and species interactions on the population level.

5.2.2. Hypothetical chemical stress

In IDamP, hypothetical chemical toxicity targeted one of the following endpoints: the reproductive output (reduction in the clutch size), the survival, the feeding rate, or the somatic growth rate of individuals (see **Chapter 2**). Examples of chemicals with effects on the reproduction of *Daphnia* include the insecticide carbaryl (Coors and de Meester, 2008) in addition to the chemical compounds 3,4-dichloroaniline (Preuss et al. 2010), nonylphenol (Preuss et al. 2008) or dispersogen A (Hammers-Wirtz and Ratte, 2000). *Daphnia* feeding rate was found to be affected by toxicants like the insecticide imidacloprid (Agatz et al. 2013) while chemicals like carbaryl (Coors and de Meester, 2008) or dispersogen A (Hammers-Wirtz and Ratte, 2000) affected the growth rate of individual daphnids. All these chemicals induced mortality at high exposure levels (Hammers-Wirtz and Ratte, 2000; Coors and de Meester, 2008; Preuss et al. 2010; Agatz et al. 2012; Agatz et al. 2013). An overview of the implementation of these different endpoint processes in IDamP is provided in **Chapter 2**. More detailed information can be found in Preuss et al. (2009).

IDamP was designed so that toxicity levels on individuals can be assigned as a percent inhibition from the control. Thereby, specific inhibition levels of 25 %, 50 %, 75 %, and 95 % were chosen for each chemical's mode of action. This choice of a wide range of inhibition levels was to ensure that it covers all possible toxicity levels chemicals might have in reality.

5.2.3. Hypothetical environmental stress: species interactions

The effects of a competitor, represented by a different *Daphnia* species (inter-specific competition) were implemented in IDamP in a dynamic manner. The individuals of the competitive population undergo the same life cycle processes as those of the original one. They feed on algae, grow, reproduce and die according to the same modelling framework. Both populations compete for one food source and for space (crowding). In this approach, two main assumptions were applied regarding the competitive population. Firstly, we assumed that it has a slightly lower filtration rate (by 10 %) than the original one. A competing population with equal or higher feeding rates than the population of interest will result in extinction of that population and therefore would make this kind of analysis impossible. This difference in the feeding rates (10 %) between the original and the competitive population was assumed to be constant and independent of the population abundance. Secondly, in the simulations, the competitor was assumed to be not sensitive to chemical stress. Overall, this approach can be seen as a worst-case competitor for a population under chemical stress exposure, because in reality chemicals might affect the competitive population as well. In addition, different food sources might be available which would allow coexistence of both species.

Predation was accounted for by implementing the feeding behaviour of *Chaoborus crystallinus* larvae on *Daphnia*. In IDamP, each *Chaoborus* feeds daily on 15 daphnids of a maximum size of 1.4 mm (Swift, 1992). Therefore, predatory effects are a function of

population abundance. The density was set to one *Chaoborus* per one-liter beaker and was assumed to be constant during the whole simulation time. This assumption emerges from the fact that the life cycle of *Chaoborus* is slower than that of *Daphnia*. Subsequently, as the *Daphnia* population grows, the influence of predation on the mortality rate is reduced, resulting in inverse density-dependence effects on the population. Therefore, predatory effects are a function of population abundance. As for competition, in the simulations, *Chaoborus* was assumed not sensitive to the chemical.

5.2.4. Tested scenarios

Population simulations were run assuming four different effect scenarios: In the first scenario, the effects on populations caused by exposure to chemical stress solely were tested. Thereby, simulations were run for each toxicity endpoint and inhibition level (as stated previously). In the second scenario, the population dynamics were simulated including species interaction processes (competition, predation or both competition and predation) under toxicant-free environment. Finally, in the third and fourth scenarios, the simultaneous effects of both chemical stress and species interactions were tested, whereby either competition or predation was introduced in the simulated environment.

5.2.5. Simulation conditions

Monte Carlo simulations were run over 365 days for all scenarios, including the untreated control population. All simulations started at day zero with 5 neonates and 3 adults, whose initial lengths (mean and standard deviation) were set to 0.9 ± 0.2 mm and 4.1 ± 0.2 mm, respectively (paragraph 2.5 in Preuss et al. 2009). Exposure to chemical and/or non-chemical stress was assumed to be constant and was applied from day zero till the last day of the simulation. All simulations were run at one low (0.05 mg C per population per day) and one

high (0.3 mg C per population per day) food level, except for the second scenario where simulations were run at food levels ranging between very low (0.05 mg C per population per day) and very high (1 mg C per population per day) concentrations. This was done to check whether the population response to environmental stressors was sensitive to changes in the food level.

5.2.6. Calculated population endpoints

In all simulations, the mean population size over time and the 95 % percentiles were recorded. For the first and second scenarios, the average sizes of the populations exposed to chemical stress and to species interactions were calculated as a percent of the untreated control population (relative population size). The resulting values will be used to calculate the endpoints for the other scenarios; thus, they are referred to as P_{chem} and P_{env}, respectively.

In the third and fourth scenarios, our main endpoint was the change caused by species interactions (due to predation or competition), in the population response to chemical stress exposure. This endpoint was calculated in the following way. The average size of the exposed population to both chemical and environmental stresses was estimated as a percent of the size of the exposed population to the corresponding environmental stressor only (P_{env}). The resulting value is referred to as $P_{chem+env}$. Thereby, the change in toxicity effects on the population size due to the environmental stressor was calculated by subtracting $P_{chem+env}$ from P_{chem}.

If this difference is positive, the environmental stressor increased the chemical toxicity on populations. In this case, the interaction between both chemical and environmental stresses led to additive, synergistic or potentiating effects on populations (Coors and De Meester, 2008). Additive effects occur when the joint effects of chemical and environmental stressors equal the sum of each single stressor's effect solely. When the effect of both stressors on populations

exceeds the sum of each effect taken solely, we have synergistic effects (Coors and De Meester, 2008). Finally, potentiating effects occur when a factor of stress with no impact on populations becomes harmful in the presence of another stressor.

However, if the calculated difference is negative, toxicity was reduced by the environmental stressor and we have in this case antagonistic effects.

5.3. Results

Population dynamics were simulated for different stressor scenarios at several food concentrations. Within the following paragraphs the impact of single stressors and afterwards combination of stressors on population dynamics for these food concentrations will be described. The untreated control population showed similar dynamics at the two extreme food levels. It grew until reaching a maximum size of 91 and 128 individuals on average at low (0.05 mg C × population^{-1} × day^{-1}) and high (0.3 mg C × population^{-1} × day^{-1}) food levels, respectively. Then, when food became limiting the population decreased until reaching a plateau with (~) 24 individuals at 0.05 mg C × population^{-1} × day^{-1} and (~) 86 individuals at 0.3 mg C × population^{-1} × day^{-1}. Overall, the mean population size was 24.35 ± 9.88 at low food level and 86 ± 8.04 at high food level.

5.3.1. Impact of single chemical toxicity stress on the population size

The average population size was least sensitive to toxicity effects on the somatic growth rate (Fig. 5.1 a, b). Even very strong effects on this endpoint decreased the population size by 3 % and 5 % only, at 0.05 and 0.3 mg C × population^{-1} × day^{-1}, respectively. Similarly, toxicity effects on individual feeding rates reduced the population size at only high toxicity levels for both food concentrations. However, when survival and reproduction were the targeted endpoints, the population was reduced at as low as 25 % or 50 % toxicity levels, respectively.

Independently of the inhibition level, high food concentration increased the sensitivity of populations to toxicity effects on survival compared to low food. In contrast, effects on reproduction manifested stronger at low food where the impact on populations started already at lower reproductive toxicity effects (50 % inhibition level) and the percent reduction was higher. Population size was thus mostly sensitive to toxicity effects on reproduction at low food level and on survival at high food level.

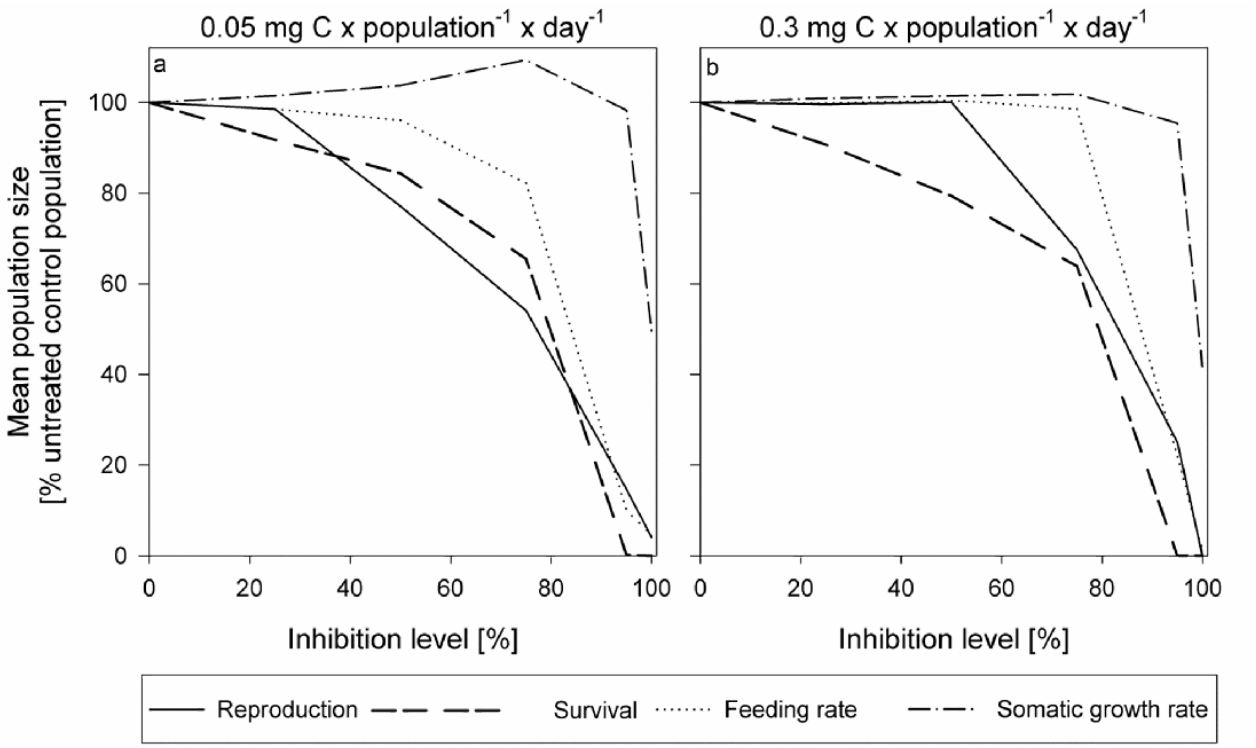

Fig. 5.1. Mean variation in the predicted population size (% of the mean untreated control population) with different toxicity inhibition levels of survival, reproduction, somatic growth rate and feeding rate at low (0.05 mg C × population^{-1} × day^{-1}, a) and high (0.3 mg C × population $^{-1}$× day^{-1}, b) food concentrations.

5.3.2. Impact of species interactions on the population size

The population size was reduced to a greater extent by *Chaoborus* predation than by competition (Fig. 5.2). No food dependency was observed for competition effects whereas

predation effects were reduced with increasing food levels. The lowest percent reduction in the population size due to predation (28 % reduction) was observed at 1 mg C × population^{-1} × d^{-1} and the highest (74 % reduction) at 0.05 mg C × population^{-1} × day^{-1}. In comparison, competition reduced the population size by 0.46 % to 12 % only. Synergistic effects of predation and competition, independent of the food level, were observed on the population size and the exposed population didn't exceed 20 % of the untreated control population.

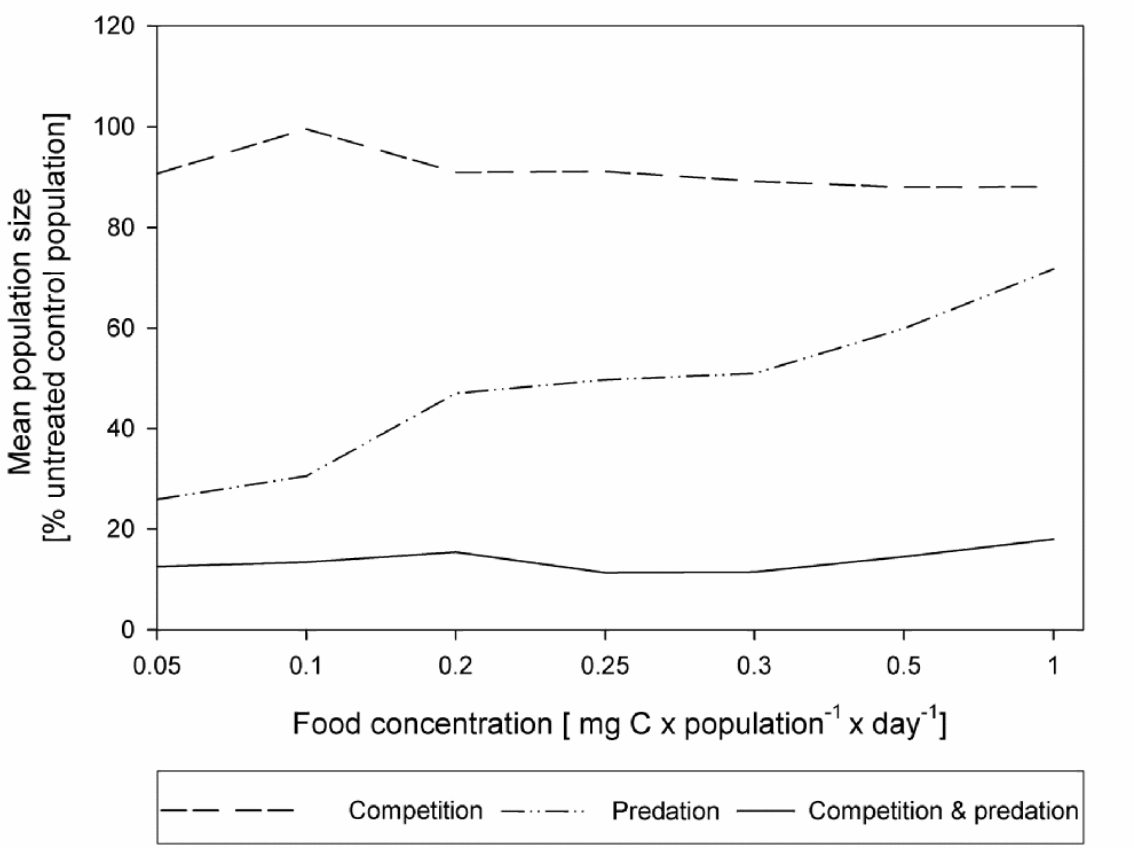

Fig. 5.2. Mean variation in the population size (as a percent to the mean untreated control population size) with predation and competition alone or in combination, in relation to food concentration.

5.3.3. Impact of species interactions on the population response to chemical stress

5.3.3.1. Competition effects

In general, most of the competition-induced changes in toxicity effects fell in the range of 50-75 % toxicity inhibition levels (Fig. 5.3 a, b). Except for the inhibition of the feeding rate, all toxicity effects were enhanced by competition at both food levels. However, some toxicity endpoints led to a higher reduction in the population size at low food level (survival) and others at high food level (reproduction and the somatic growth rate). The increase in the population sensitivity to chemical stress manifested though different interactive mechanisms: Synergistic effects were observed between competition and toxicity effects on survival at as much as 50 % at both food levels: whereas competition alone reduced the population size by 9 to 12 % and an inhibition of survival up to 50 % reduced the population by a maximum of 20 % (Fig. 5.1), a combination of these two stressors led to a reduction of 70 % in the population size (Fig. 5.3 a, b). However, potentiating effects were observed at 0.3 mg C × population^{-1} × day^{-1} where 25-50 % reproductive toxicity had no impact (Fig. 5.1 b), but reduced the population size by 61 % in the presence of competition (Fig. 5.3 b). Additionally, an inhibition of the somatic growth rate had no effect on the population size (Fig. 5.1 a, b), but with competition, it reduced the population size by up to 80 %.

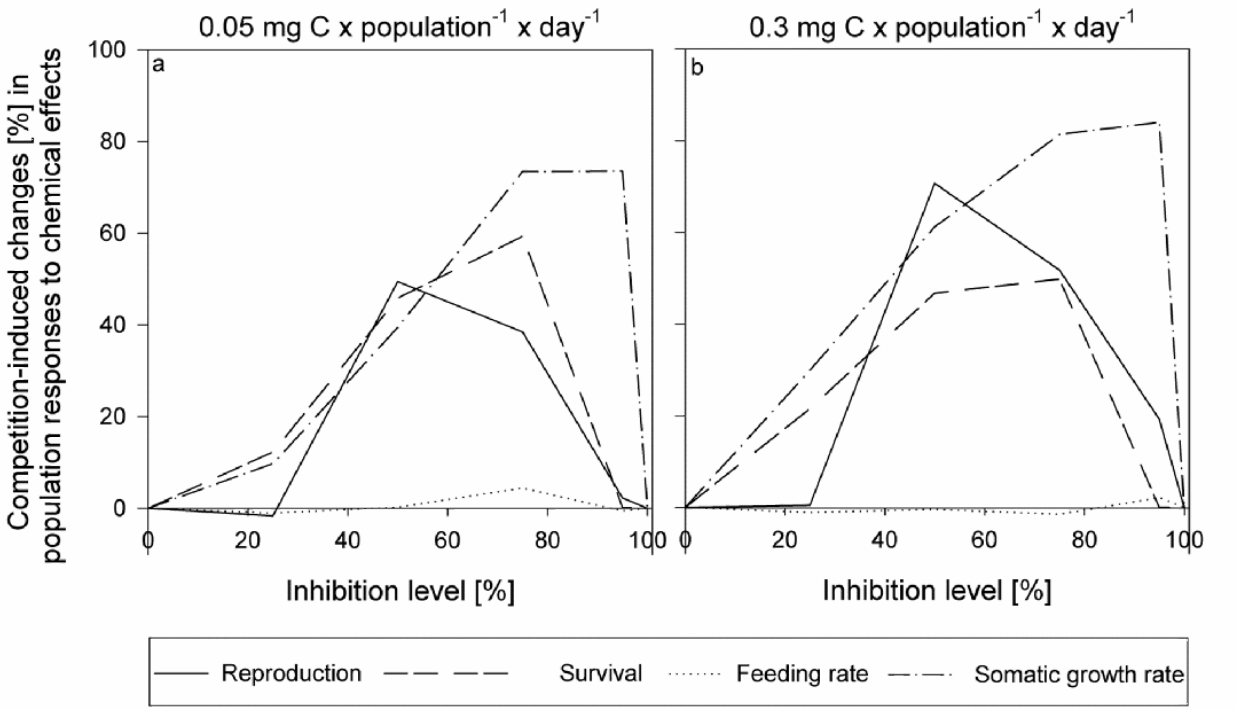

Fig. 5.3. Competition-induced changes in the abundance of populations exposed to different toxicity inhibition levels at low (0.05 mg C × population $^{-1}$ × day^{-1}, a) and high (0.3 mg C × population $^{-1}$ × day^{-1}, b) food concentrations. Positive and negative values indicate respectively, an increased and a reduced sensitivity of the population to the chemical's effect.

5.3.3.2. Predatory effects

The effects of predation by *Chaoborus* on the population response to chemical stress differed with the food level and with the targeted toxicity endpoint (Fig. 5.4 a, b). Similar to competition, predation did not seem to affect population response to toxicity effects on the feeding rate. Toxicity effects on the population due to an inhibition of the somatic growth rate interacted synergistically with predation and the effects were stronger at high food level. In addition, at low food level, predation increased the population sensitivity to toxicity effects on survival (synergistic effects, Fig. 5.4 a). At high food level, however, if the population was reduced by 56 % with predation solely, its sensitivity to toxicity effects on survival was not affected by predation (Fig. 5.4 b).

Nevertheless, and in contrast to competition, not all the other toxicity effects were enhanced by predation: antagonistic effects could be observed when reproduction was inhibited at 0.05 mg C × population^{-1} × day^{-1} (Fig. 5.4 a). At that food level, predation acting solely reduced the population size by 74 % (Fig. 5.2) whereas an inhibition of reproduction reduced the population size by a maximum of 90 % (Fig. 5.1). But in combination, only a maximum of 50 % reduction in the population size was recorded. This was not observed at high food level where predation stimulated the reproductive toxicity effects and the population size was reduced further by 40 % (Fig. 5.4 b).

To explain these contrasting patterns, we examined the size structure of the population exposed to predatory effects under low and high food concentrations (Fig. 5.5). We chose an inhibition level of 75 % on reproduction because it is the highest concentration causing the observed antagonistic effects at low food concentrations. At low food conditions, the juveniles represented the population fraction that was predominantly reduced by predation (down to 9 % of the total population) whereas the neonate fraction remained constant. In comparison, at high food conditions, the juvenile fraction was reduced by only 23 % with predation while the neonates' fraction increased by 36 %.

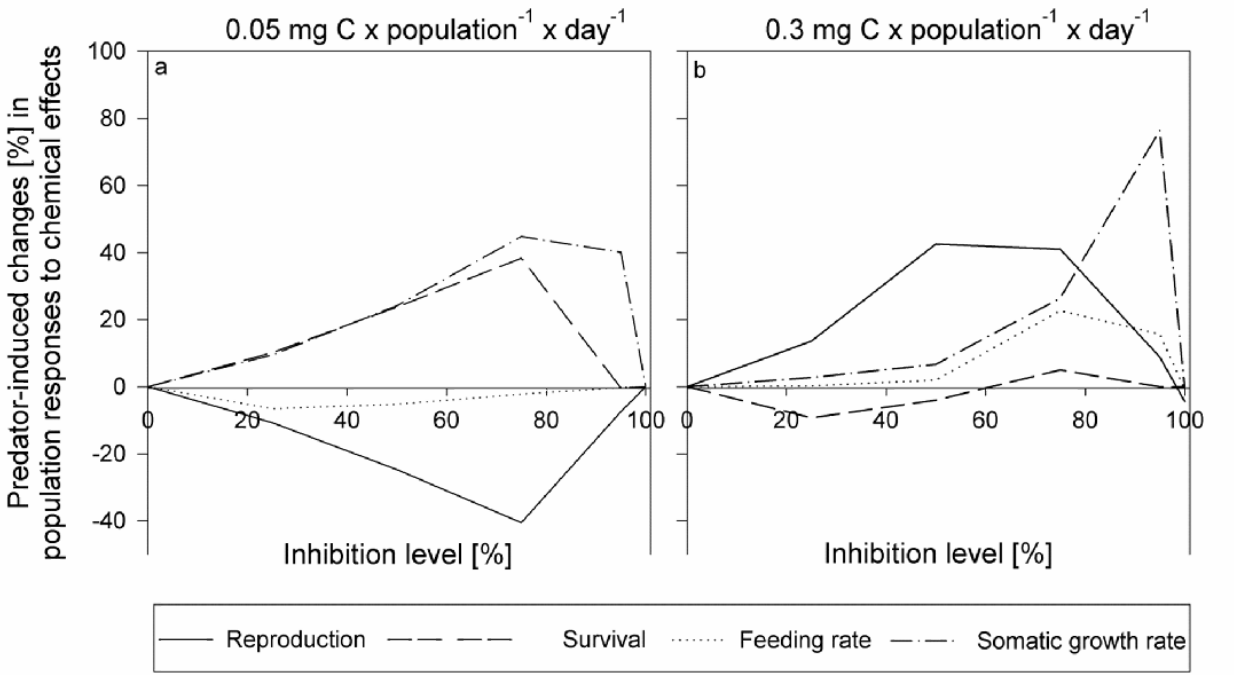

Fig. 5.4. Predator-induced changes in the abundance of populations exposed to different toxicity inhibition levels at low (0.05 mg C × population $^{-1}$ × day^{-1}, a) and high (0.3 mg C × population $^{-1}$ × day^{-1}, b) food concentrations. Positive and negative values indicate respectively, an increased and a reduced sensitivity of the population to the chemical's effect.

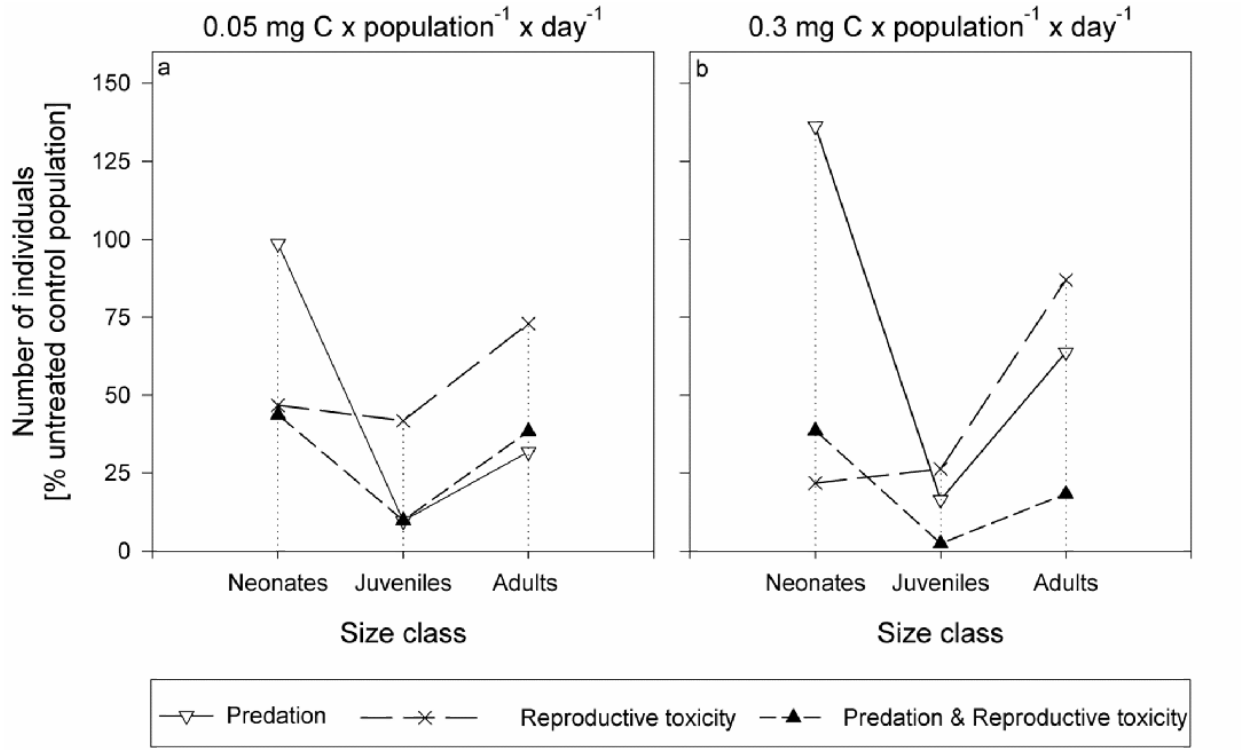

Fig. 5.5. Variation in the abundances of the neonate, juvenile and adult fractions in the exposed population to predation, reproductive toxicity, or to both predation and reproductive toxicity. Abundances of the different size fractions were expressed as a percent of the abundance of their respective fractions in the control population. An inhibition level of 75 % was chosen to represent reproductive toxicity effects on the population level.

5.4. Discussion

5.4.1. Relevance of the simulated scenarios

Under natural conditions, the daphnids are exposed to a multitude of chemical and environmental stressors which influence their life-history strategies and alter the population dynamics and size structure. In order to link multiple stress exposure of individuals to effects on populations, we used an individual-based model and tested different scenarios of chemicals with different modes of action on *Daphnia* acting with environmental variables important to *Daphnia*.

5.4.2. Relevance of the endpoint response, the relative population size

In the present study, we compare the magnitudes of change in daphnid population abundances, caused by different stress scenarios, ranging from single chemical toxicity to multiple stress exposure. To this end, the choice of a suitable population endpoint is crucial especially for multiple stress exposure as it should accurately address the potential of the environmental stressor (in our case, food and species interactions) in altering the population sensitivity to the toxic effect. In this context, the absolute population size is a commonly used endpoint to compare the different scenarios in terms of the risk of extinction they represent on populations, but this would not be relevant for the aim of the present study. In comparison, our use of the relative population size (mean population size as a percent of the control) allowed such investigation and clearly demonstrated the contribution of environmental stressors (or interaction of stressors) in altering population sensitivities to chemical stress exposure.

5.4.3. Population responses to chemical stress exposure

Populations were mostly sensitive to toxicity effects on survival and reproduction whereas much higher inhibition levels of the two other tested endpoints (somatic growth rate and filtration rate) were necessary to cause similar reductions in the population. A reduction in the abundance of populations is the result of a decrease either in the reproductive potential of individuals, or their survival. Furthermore, reproduction is related to the processes of feeding rate, growth rate and juvenile development rate (as described in **Chapter 2**). Thus, an inhibition of the reproductive potential occurs also indirectly, when the feeding rate or the somatic growth rate are inhibited. Thereby, a toxicant acting on the feeding rate will affect all the individual processes of *Daphnia* (all processes depend on the feeding rate in IDamP, **chapter 2**) as also demonstrated experimentally (Agatz et al. 2013). In comparison, a reduced growth rate does

indirectly affect the feeding rate (because it depends on the length of *Daphnia*). Nevertheless only slight effects on populations are observed if the feeding rate or somatic growth rate are inhibited. These causalities can only be understood by taking into account the dynamic interactions implemented in the model and which take place in reality. In equilibrium, the population ingests all the algae available per day, if higher food levels are available, the population abundance will increase until the whole food amount is consumed. If the feeding rate is inhibited, the animals will feed less, which would result in reduced reproduction and growth. Yet, the remaining algae in the system will be consumed later. Subsequently, the ingested amount of algae by the population will stay constant unless the feeding rate becomes extremely low that it does not allow the consumption of the full amount, and only then that the population starts to decline. Therefore effects on the feeding rate impacted the population size only at 75 % (at 0.05 mg C per population per day) or at 95 % (at 0.3 mg C per population per day) inhibition levels.

The same mechanism holds true for the inhibition of growth, since animals are smaller, the feeding rate and reproduction are reduced which leads to a higher amount of food in the beaker that is available for the daphnids. Thus, reproduction is promoted and subsequently, even a higher population abundance than in the control could be reached (as observed at low food level, Fig. 5.1 a). In comparison, an inhibition of reproduction due to embryo mortality (as simulated here and also the case for 3,4-dichloroaniline (Preuss et al. 2010) or direct mortality lead to a drainage of energy (here algal food) from the system which results in more pronounced effects at the population level.

In the present study, food concentration determined the vulnerability of daphnid populations to chemical stress, in accordance with the common finding in the literature of food-

dependent toxicity for *Daphnia* (Foit et al. 2012; Heugens et al. 2006; Klüttgen and Ratte, 1994) and other species as well (Cecchine and Snell, 1999). This dependency was manifested by an increase in the population sensitivity at low food compared to high food concentration. We could even identify scenarios with non-visible chemical effects on populations at high food but that yielded significant reductions of the population size at low food concentration (e.g. up to 50 % reproductive toxicity or 75 % inhibition of the feeding rate). These results have direct implications on the currently adopted ERA methodology: According to OECD guidelines (OECD 211, 2008), chemicals' effects on individual reproduction, survival and sometimes growth of individuals are assessed in laboratory tests at food concentrations which should be enough to ensure the reproduction of the species. These concentrations are often higher than those occurring in natural conditions (even those characterizing eutrophic waters) where organisms are more likely to cope with food limitation rather than unlimited food conditions (Klüttgen and Ratte, 1994). The higher sensitivity observed at low food conditions in our study would mean that non-protective predictions of toxicity effects on field populations can be made when food is a limiting factor. This fact was mostly reflected when chemical toxicity targeted the reproductive output, the key physiological endpoint used for decision making in ERA Extrapolating toxic effects from laboratory tests to the population level should therefore account for the variability in the food resources as this occurs in natural field situations.

5.4.4. Population responses to multiple stress exposure

The environmental stressors i.e. predation and competition considered in the present study represent two extreme case scenarios for species interactions: While predation removes a certain fraction of neonates and juveniles from the original population, competition limits the accessibility of the individuals of this population to food resources (contest competition), and

thus hampers their maturity. In this way, both interactions act directly on the population growth rate, which is a very important determinant of population resilience.

Species interactions with competition or predation greatly influenced the sensitivity of populations to chemical stress exposure (Figs. 5.1, 5.3 and 5.4). The change in the responses (increased or reduced toxicity) as well as its magnitude depended on the toxicity endpoint and on the environmental variable (a predator or a competitor was present). Moreover, population sensitivity did not emerge from the joint effects of chemical and the environmental variable only, but from their interactions with the food concentration as well. In the following, we discuss these statements with examples from our results obtained with competition and afterwards with predation.

Competition alone did not significantly affect the abundance of the daphnid populations (Fig. 5.2), but significantly increased the population vulnerability to toxicants (Fig. 5.3 a, b). This is particularly important for scenarios where chemicals with non-visible impact on populations (e.g. up to 50 % reproductive toxicity at high food level, or inhibition of the somatic growth rate at both food levels, Fig. 5.1) became toxic with competition (more than half of the population was reduced, Fig. 5.3). This pattern was designated by Artigas et al. (2012) as collateral stress where an insignificant stress factor fosters other stressors to affect organisms.

Moreover, in our study, if the predicted sensitivity of *Daphnia* populations to chemicals was always increased with competition, the magnitude of effect was controlled by the food conditions. For instance, competition had higher impact on population vulnerability to reproductive toxicity at high compared to low food levels. At high food, the competing population takes advantage of the availability of resources and increases its abundance. The original population is subsequently exposed to increasing crowding and adapts its life cycle to

inter-specific and intra-specific competition as well. To compensate for the density-dependence effects, the daphnids naturally reduce the number of their progeny at the expense of producing fitter (larger) individuals (Goser and Ratte, 1994). This important feature of density-dependence adaptive behaviour is included in the IDamP model (see **Chapter 2** and also Preuss et al. 2009). If reproduction is further inhibited by chemical stress, this would induce greater reductions in the population at high food compared to low food where density-dependence effects are minor. Competition-induced increase in toxicity effects on daphnids was observed in experimental studies using *Daphnia* (Foit et al. 2012) and other organisms as well like trichoptera (Beketov and Liess, 2005) or mayflies (Foit et al. 2012). However, the advantage of our study over the previous ones is that by using a modelling approach compared with laboratory experiments, we could not only identify the extent of change in chemicals' effects on populations, but also we determined a toxicity inhibition-level threshold below which there would be no need to account for competition as an additional stress factor. This was exemplified in the population responses to low chemical inhibition levels of reproduction (below 25 %) which remained unchanged with competition regardless of the food level.

Comparing the results observed with single chemical stress and those with species interactions indicates that the same environmental variable might induce contrasting effects on populations, depending on the other variables that constitute the full environmental scenario. This is illustrated in the higher sensitivity of populations to chemicals under low food level, but a high food level fostered the sensitivity of the population to chemicals in the presence of a competitor. These contrasting observations raise the importance of considering not only the interactions between chemical and environmental variables only, but the interactions among the

environmental variables themselves might lead to a change in the impact that one factor exerts on populations.

In contrast to competition, populations exposed to predation were not always more sensitive to chemical stress than the unexposed ones. Food concentration was the main factor determining population sensitivity. This was reflected in our results in two situations: Firstly, a particularly intriguing result was that predation reduced reproductive toxicity effects on populations at low food conditions (at as much as 40 %, Fig. 5.4 a), resulting in antagonistic effects. Yet, at high food conditions, predation exerted the opposite effect on the chemical whereby its impact on populations was promoted (by 40 %, Fig. 5.4 b). These contrasting patterns can be explained by the alteration in the size structure of the population (Fig. 5.5). The observed increase in the neonate fraction at high food level results from the inverse density dependence created by predatory effects: as the predator feeds on neonates and juveniles, the density of the population is reduced, leading in return to more food available for the remaining populations. Mother daphnids use the higher amount of food to produce more neonates, resulting in a larger neonate fraction. This important feature of adaptive traits of *Daphnia* to changes in environmental factors is included in the IDamP model. This increase in the neonates fraction might suffice to reach that of the control population (the case at 0.05 mg C per population per day; Fig. 5.5) or even exceed it (the case for 0.3 mg C per population per day), meaning that the reduced neonate fraction due to predation was compensated for, but to a less extent at 0.05 mg C per population per day because food is a limiting factor. Changes in the size structure of the population due to predation are known in ecology as the Allee effect (De Roos et al. 2003) which designates a positive feedback induced by the predator on the prey population for the benefit of its own consumption.

Furthermore, the population exposed to reproductive toxicity (at an inhibition level of 75 %) experienced mainly a reduction in the small-sized daphnids (neonates and juveniles) but with a higher impact on the juvenile fraction at low food concentration. Thus, the reduced reproductive toxicity at low food conditions could be attributed to the predation pressure which suppressed the juveniles, the most sensitive fraction of the population to toxicity effects on reproduction. This was not observed at high food conditions because of the larger fraction of neonates and the smaller impact of predation on the juveniles. This finding is concomitant with the results of a study using *Notonecta maculata* as a predator on daphnid populations exposed to pulses of Nonylphenol (Gergs et al. 2013), where a reduced toxicity was attributed to the size-specific mode of action of the toxicant that was inhibited by the predator acting on the same fraction of the population.

Secondly, survival toxicity effects on populations were enhanced by predation at low food but not at high food level. This is a consequence of the density-dependence effects on the size structure of the population. In fact, it is well known that the higher the food supply is, the more likely the populations will be exposed to crowding effects. This feature is included in IDamP as well-fed *Daphnia* produce a higher amount of neonates **(Chapter 2)**. One of the consequences of the crowding effects in IDamP is the reduced neonate and juvenile survivals **(Chapter 2)**. This means in our case, that the fraction of neonates and juveniles compared to the total population size will be smaller at high (0.3 mg C per population per day) compared to low (0.05 mg C per population per day) food level where the crowding effects are minor. Given that predation acts on the neonate and juvenile fractions, this explains the higher sensitivity of populations exposed to chemical effects on survival at low compared to high food levels. This observation illustrates how population resilience emerges from the interactions between

ecological factors (food concentration, predation), life cycle properties of the species (adaptation to density-dependence conditions) and chemical stress exposure.

Comparing our results with literature, it was argued that the responses of organisms to toxicants may be considerably stronger with predation pressure (Beketov and Liess, 2006). We showed that this observation might hold true for daphnids at a favorable food supply, but not when food is a limiting factor. Even that high toxicity effects at the population level were cancelled out by predation at low food supply, and the model revealed that the interactive effects among the environmental variables themselves were the reason behind the observed divergences in the population responses to chemical stress with predation.

5.4.5. Advantages of theoretical modelling and implications for risk assessment

Although there is a general awareness of the necessity to include multiple stress exposure in risk assessment schemes (Vignati et al. 2007; Artigas et al. 2012; Duquesne and Liess, 2003), little efforts have been deployed so far in this direction. ERA faces an increasing pressure to test more chemicals at a lower cost (Forbes and Calow, 2012). Experimental studies, in addition to being costly and time consuming, provide limited information to specific test conditions e.g. toxicant concentrations, food levels, toxicity endpoints (Duquesne and Liess, 2003; Heugens et al. 2006; Vignati et al. 2007; Knillmann et al. 2012 b). In comparison, our use of an individual-based model allowed predictions to be made at any toxicity inhibition level along with various combinations of environmental stressors. We could demonstrate that population responses to the same chemical's mode of action strongly depended on the environmental conditions. The importance of this statement is reflected for instance at low toxicity inhibition levels on reproduction (up to 25 % inhibition), where the model did not predict a change in the effects on populations with competition, but predicted a different

sensitivity with predation. As chemical application causes side effects to populations even at low concentrations (Forbes, 2010), accounting for the co-occurrence of multiple stresses and their effects on *Daphnia* populations for the low chemical doses might be a step forward towards reducing the uncertainty when extrapolating toxicity effects to the population level. Since predictions of toxicity effects are still relying on laboratory test results, we think that our results, obtained with a model that is applicable for laboratory populations, may already serve as a model-guide to narrow up the wide range of possible toxicity design experiments (Vignati et al. 2007; Artigas et al. 2012) by selecting relevant combinations of abiotic factors with the mode of action of the toxicant in question.

5.4.6. Limitations of the approach for field situations

Owing to the high specificity of the IDamP model, results of the present study have some limitations regarding extrapolation to field situations. If, by employing such approach, we could successfully solve the problem of extrapolating multiple stress effects on individuals to the population level, a topic that raises high debate in ERA, this study remains limited when it comes to extrapolation of effects to *Daphnia* populations in real field situations. There, other environmental variables, in addition to more complex, dynamic mechanisms for species interactions might enter into play, leading to more diverse population responses than those depicted in the present study. It is therefore necessary to define the ecological scenario of the species of interest in detail, including the necessary biological interactions, to reach the defined protection goals (EFSA, 2013).

5.5. Conclusion

According to the findings of the present study, we conclude that species interaction processes should be accounted for in the ERA of chemicals. Validated ecological models, by

their potential to simultaneously incorporate physiological processes of individual organisms and several relevant environmental variables and processes, would significantly contribute to our understanding of the interactions between the species, the chemical stressor(s) and the environmental variables, and their consequences for populations. We believe that ecological models will help improve ERA efficiency in multiple stress exposure scenarios, which is the case in real field situations.

CHAPTER 6

Predicting population recovery from chemical stress: the influence of the environmental scenario on recovery patterns

6.1. Introduction

Understanding recovery processes is one of the main objectives set up by the European directives towards improving environmental risk assessment (ERA) of chemicals (SANCO, 2013; EFSA, 2013). In aquatic systems, recovery of organisms from chemical disturbance does not result from the exposure pattern and the mode of action of the toxicant only (Sherratt et al. 2010; Barnthouse, 2004), but also depends on important ecological factors like the life cycle of the species (Van den Brink et al. 1996; Dalkvist et al. 2009; Nienstedt et al. 2012), inter-specific competition as well as predation (Beketov and Liess, 2005; Liess and Foit, 2010; Reynaldi et al. 2011; Knillman et al. 2012). In addition, environmental variables like temperature (Van Wijngaarden et al. 2005; Solomon et al. 2008), food resources (Roessink et al. 2005; Traas et al. 2004) or the connectivity of the contaminated site to its surrounding environment (Niemi et al. 1990; Van den Brink et al. 1996; Galic, 2012) control the speed of recovery of populations exposed to chemical stress.

Furthermore, it is increasingly being recognized that the confounding effects of these different variables are very important as they might either hamper or foster the recovery of populations from chemical stress exposure (Barnthouse, 2004; Spanhoff and Arle, 2007; Stampfli et al. 2011; SANCO, 2013). In this context, the usefulness of mesocosm studies in providing accurate predictions of recovery times has been acknowledged by the European authorities (EFSA, 2013). However these experimental designs remain limited when it comes to extrapolation between the different exposure scenarios or extrapolation to field situations (Barnthouse, 2004; Solomon et al. 2008; Hommen et al. 2010).

Up to now, there is no scientifically sound basis that accounts for these multiple ecological effects on recovery (Stampfli et al. 2011; SANCO, 2013) in ERA. The need for a

comprehensive framework that encompasses the full complexity as in natural conditions has been raised by environmental scientists (Barnthouse et al. 2004; Brock et al. 2006; Hommen et al. 2010) and the use of ecological models has been suggested as a useful tool to circumvent the limitations of experimental approaches (Hommen et al. 2010; SANCO, 2013; Solomon et al. 2008). The advantages of ecological models are manifold: They enable the extrapolation of effects between different exposure profiles (Hommen et al. 2010; Forbes et al. 2010) and integrate the necessary ecological knowledge, leading to a more realistic chemicals' effect assessment (Van Straalen, 2003). Individual-based models in particular have the additional ability to predict effects on populations which emerge directly from the properties of each individual within that population (Preuss et al. 2009, 2010; **Chapters 3 and 5**). This constitutes an important feature for estimating population dynamics under chemical exposure especially under the impact of environmental interactions (Preuss et al. 2009, 2010; Gergs et al. 2013; **Chapter 5**).

In the present study, we use the IDamP-IBM for *Daphnia magna* as a virtual laboratory to determine how the recovery of populations from exposure to hypothetical lethal effects is influenced by the environmental scenario. Chemicals targeted the survival of individuals at different effect strengths. As environmental variables, we focused on food level, temperature and on the ecological interactions with competition or predation.

6.2. Material and Methods

We used the IDamP model for *D. magna* to simulate different chemical lethal effects in combination with abiotic and biotic variables, on population recovery.

6.2.1. Hypothetical chemical stress

For this study we used lethal toxicity levels on individuals as probability to die in percent. In IDamP, each individual daphnid dies if the probability to die exceeds a random number assigned to each individual daphnid at birth (Preuss et al. 2009). As an example, if the probability to die is 50 %, a daphnid with a random number of 49 will die immediately, but another daphnid with a random number of 51 will survive. Thereby, we analyzed specific mortality levels of 40 %, 50 %, 70 %, 80 % and 90 %. For lower effect levels, no significant effects on population level could be detected for most environmental scenarios and will therefore not allow estimating recovery times (as also seen in Fig. 6.1).

6.2.2. Biotic stressors

The effects of inter-specific competition as well as predation by *Chaoborus crystallinus* were implemented in IDamP following the same approach used in **Chapter 5** (paragraph 5.2.3.).

6.2.3. Tested scenarios

Population simulations were run assuming three different effect scenarios: In the first scenario, the effects on populations caused by exposure to chemical stress solely were tested. Thereby, simulations were run for each mortality level (stated previously). In the second and third scenarios, the simultaneous effects of both chemical and environmental stresses were tested, whereby either competition or predation was introduced as a non-chemical, environmental stressor.

6.2.4. Simulation conditions

One hundred simulations were run over 600 days for all scenarios, including the untreated control population. All simulations started at day zero with 5 neonates and 3 adults (as in section 5.2.5). In the simulations, we assumed a one-day exposure to the chemical (at day 60, because populations have already reached their carrying capacity by that date). In contrast, exposure to biotic stressors (for combined scenarios) was continuous during the entire simulation period. All simulations were run at food concentrations of 0.05; 0.1; 0.3; 0.5; 1 and 2 mg C per population per day. To check for the effect of temperature, we compared population recovery from chemical exposure at 10 °C and 20 °C. In addition, we ran simulations using exposure to the toxicant at temperatures ranging between 7 °C and 30 °C, to detect potential changes in population responses with temperature.

6.2.5. Calculated population endpoints

We define population recovery in this study as the number of days needed to return to an abundance that is not significantly different from the abundance of the control population under the same simulated environmental conditions. Thereby, for simulations including species interactions, the control population is the population exposed to either competition or predation.

In all simulations, the mean population size over time and confidence intervals were recorded. Since most statistical tests (e.g. the t-test or ANOVA) depend on the number of replicates, which is arbitrary for a model analysis, we used the percentiles as a measure to define a significant difference (Environment Canada, 2007). If the percentiles of the control and treated population abundances do not overlap, this means that there is a significant difference between these two populations. In the opposite case, no significant difference is assumed.

Simulations including chemical stress exposure were used to also compare the predictions of recovery times obtained with the commonly used logistic growth model for predicting recovery (Barnthouse, 2004; Solomon et al. 2008) and those obtained with our individual-based model. Thereby, the population growth at each time unit was calculated following:

$$dN_t = N_{t-1} \times r \times N \times \left(\frac{K - N}{K}\right) \quad (1)$$

with N being the population size at time t; r and K being respectively the growth rate and the carrying capacity of the population. r was obtained by fitting the logistic growth model to modelled population dynamics for the simulated environment. Different K values ranging between 10 and 1000, were tested. For all simulations, the population was always initialized to 8 daphnids and the dynamics were simulated for 365 days.

The reduction in the population size due to the disturbance event which occurred at day 60 was calculated (Eq. 2).

$$N_{t=60} = N_{t=59} \times \left(\frac{100 - \%\, effect\ strength}{100}\right) + dN_{t=60} \quad (2)$$

Subsequently, time to recovery (TTR) was estimated as the time that the population takes to return to the defined carrying capacity value K. For this analysis, recovery was estimated at 20 °C for chemical mortality levels ranging between 10 and 99 %.

For scenarios including biological interactions (predation or competition), our calculated population endpoint was the change caused by the presence of this environmental stressor, in the population recovery from chemical lethal effects. To represent this change, we calculated the deviation of TTR from the joint biological interactions and chemical stressors, to that obtained with single chemical stress exposure (Eq. 3).

$$Deviation\ (\%) = \frac{TTR_{joint\ stress} - TTR_{single\ chemical\ stress}}{TTR_{\ single\ chemical\ stress}} \times 100 \quad (3)$$

If the deviation is positive, this means that the interaction with competition or predation delayed recovery. A negative difference means that the population recovered faster under the impact of competition or predation.

6.3. Results

6.3.1. Predicting recovery using the logistic growth model and the IDamP model

The logistic growth model predicted the same TTR of populations with different capacities (10, 100 and 1000, Fig. 6.1). In contrast, IDamP predicted different recovery times in relation to different food concentrations. Furthermore, with the logistic growth model, recovery tended to increase linearly with increasing lethality levels whereas our model predicted a three stage response curve: Chemicals with effect strengths below a threshold of 30 % mortality had no effects on the population at any food concentration. At lethal effects ranging between 40 and 70 %, TTR followed a plateau and afterwards increased linearly for lethal effects above 70 %.

Finally, if the logistic growth model was conservative at very low toxicity levels (≤ 30 %), recovery times were always under-estimated compared to those obtained with the IDamP model from 40 % mortality. At toxicity levels higher than 90 % (95 % and 99 %), the logistic model predicted recovery within 12 days maximum while with IDamP, recovery even did not occur.

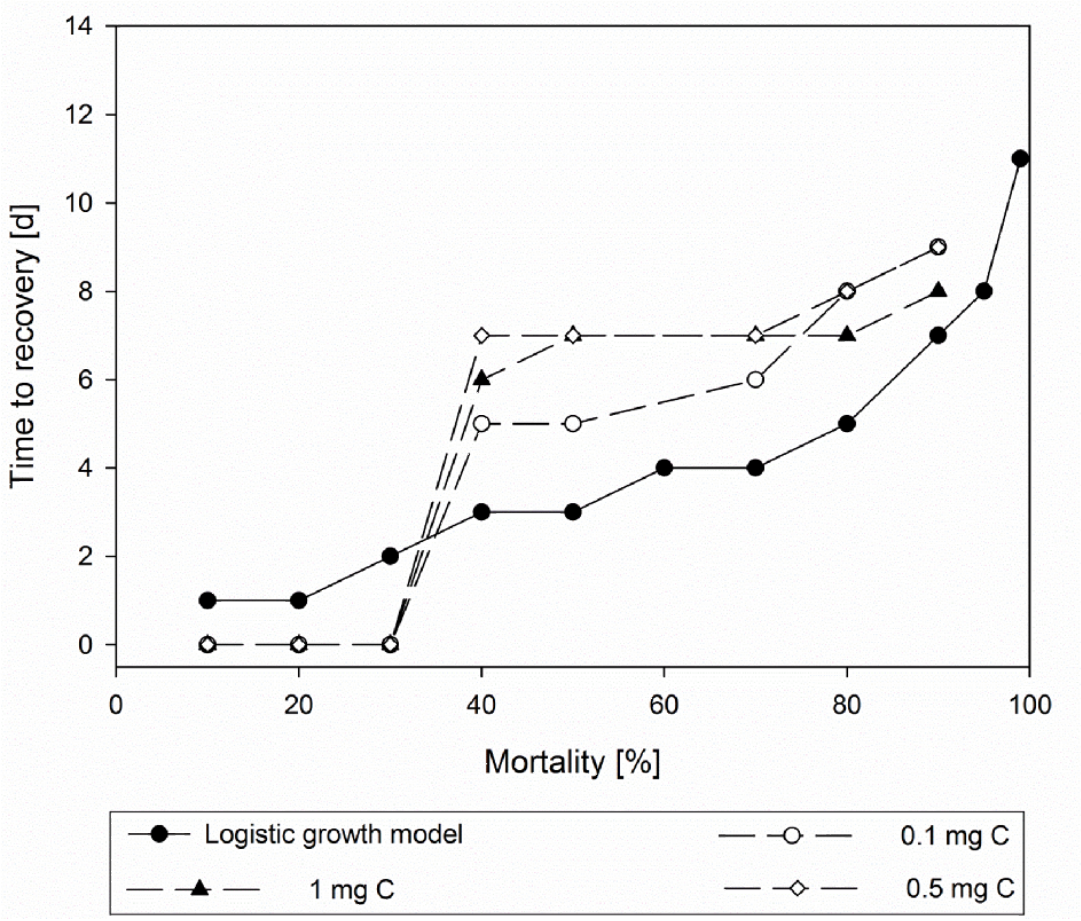

Fig. 6.1. Predictions of TTR (in days) of populations from exposure to chemicals with different lethal effects using the IDamP model (dashed lines) and the logistic growth model (full line). TTR was estimated for carrying capacities of 10, 100 and 1000 with the logistic growth model, and for food concentrations of 0.1; 0.5 and 1 mg C with the IDamP model. All simulations were run at 20 °C.

6.3.2. Variation of TTR with food level at different temperature conditions

TTR of populations from lethal effects at different food levels are shown in Fig. 6.2 at 10 °C (a) and 20 °C (b). Recovery was usually slower at low compared to high temperatures, except at the highest food concentration where populations took longer to recover from high effect strengths (70 % and 90 %) at 20° C. At both temperatures, the general trends in TTR differed with toxicity levels: at low to medium effects (40-70 % mortality), TTR decreased at low food levels, so the populations recovered faster compared to high food levels. However, at

the highest effect strengths (80-90 %), TTR increased with decreasing food at 10 °C, whereas at 20 °C, no tendency could be observed.

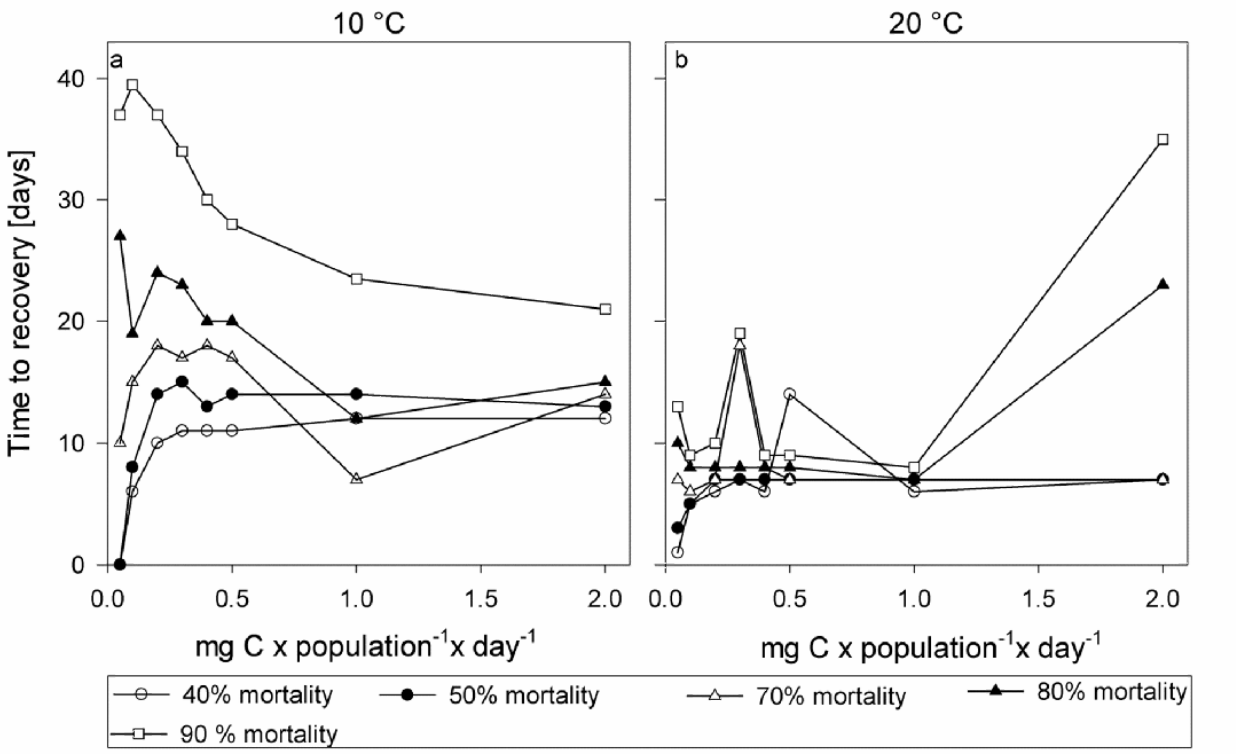

Fig. 6.2. Variation in TTR of populations from exposure to chemicals with different effect strengths (from 40 % to 90 % lethality) in relation to food concentration at 10 °C (a) and 20 °C (b).

6.3.3. Variation of TTR with temperature at different food conditions

Patterns of variations of recovery times at low and high food concentrations were quite similar (Fig. 6.3 a, b). However, TTR showed different patterns of variation with temperature and lethality levels: At high lethal effects (90 % mortality), TTR increased exponentially with decreasing temperatures below 15 °C but tended to follow a linear pattern at temperatures higher than 15 °C. In comparison, recovery from low mortality (50 %) was less influenced by variations in temperature conditions.

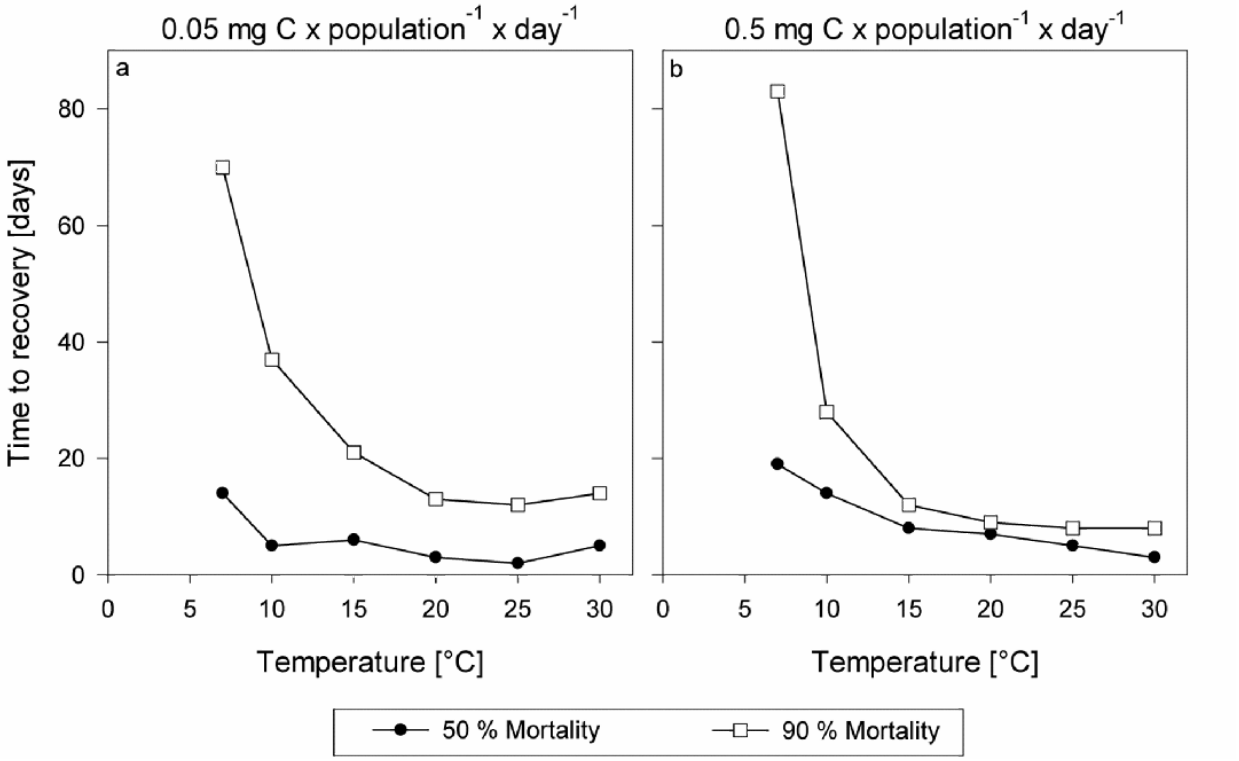

Fig. 6.3. Variation in TTR of populations from exposure to chemicals with low (50 % mortality) and high (90 % mortality) effect strengths in relation to temperature at low (0.05 mg C per population per day, a) and high (0.5 mg C per population per day, b) food concentrations.

6.3.4. Variation of TTR with biotic factors

6.3.4.1. With competition

Competition effects on TTR differed with temperature, food level and the lethal effect (Fig. 6.4 a, b). Populations usually recovered faster from low lethality levels (40 and 50 % mortality) in the presence of a competitor except at 0.1 mg C per population per day where recovery was delayed by competition. At 70 % mortality, competition induced a slower recovery with a maximal effect occurring at 1 mg C per population per day. This delay was 4 times greater at 20 °C than at 10 °C. By contrast, at 90 % mortality, competition had a stronger effect at low temperature and populations did not recover between 0.5 mg C and 1.5 mg C per

population per day. It is only at the highest food concentration (2 mg C per population per day) that recovery occurred (at day 105). In comparison, at 20 °C, recovery from 90 % effect was delayed by more than 10 weeks (800 %) and when food concentration exceeded 0.5 mg C per population per day, competition had no longer effects on TTR.

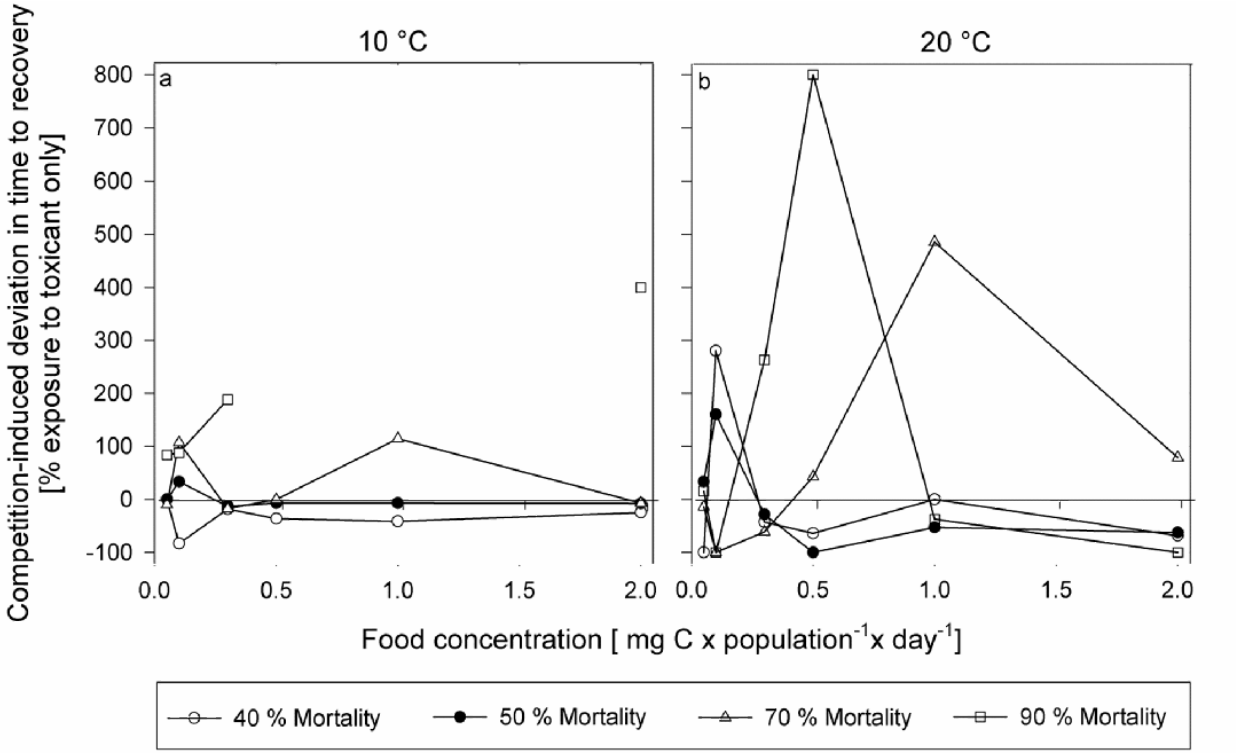

Fig. 6.4. Competition-induced deviations (in % exposure to toxicant only) in TTR of populations from chemical exposure in relation to food concentrations at 10 °C (a) and 20 °C (b).

6.3.4.2. With predation

At low temperatures (10 °C, Fig. 6.5 a) predation had the strongest impact on population recovery at 0.3 mg C per population per day whereas at high temperatures (20 °C, Fig. 6.5 b), the strongest effects were often observed at 1 mg C per population per day. TTR decreased afterwards with increasing food concentrations, and at the highest one (2 mg C per population per day) there was no effect of predation on population recovery. Similarly than with

competition, predation fostered the recovery of populations from low to medium effects (40-50 % mortality), but the observed cases remain fewer than those with competition. Additionally, recovery from strong effects was delayed by predation, but to a less extent than by competition.

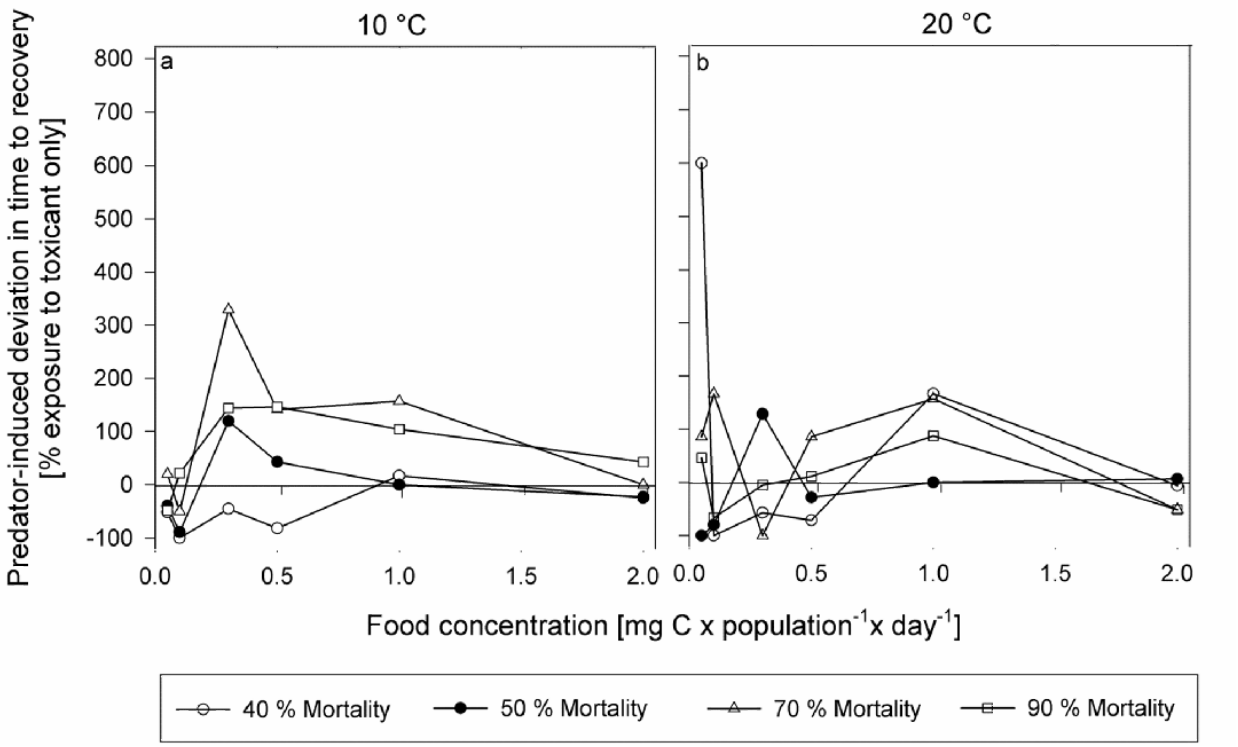

Fig. 6.5. Predator-induced deviations (in % exposure to toxicant only) in TTR of populations from chemical exposure in relation to food level at 10 °C (a) and 20 °C (b).

6.4. Discussion

In the present study, we used an individual based model as a virtual laboratory to analyse the recovery of *D. magna* populations at different stress scenarios including pulse exposure to a hypothetical chemical acting alone or in combination with different environmental factors (temperature and food conditions) and biological interactions (predation or competition). We showed that temperature and food level have a strong influence on population recovery from chemical stress exposure. Additionally, interactions of the chemical with inter-specific competition or predation often hampered the speed of recovery and even drove the populations

did not recoverin some cases. Nonetheless, the opposite response was also observed in few scenarios where lethal effects on population recovery were compensated for by competition or predation and consequently induced, in close relation with the biotic and abiotic factors, a faster recovery. In the following, we discuss the role of the environmental scenario in determining population recovery from chemical stress exposure.

6.4.1. Comparing recovery times using the logistic growth model and the individual-based model

The logistic population growth model is commonly used to easily estimate the recovery potential of populations from toxicity events (Barnthouse, 2004; Solomon et al. 2008). Comparing our model predictions with those of the logistic growth model showed that, at the exception of the lowest mortality levels (< 40 % effect), the logistic growth model always underestimated recovery time of populations (Fig. 6.1). Furthermore, and independently of the food concentration, our model predicted similar recovery times from low toxicity levels ranging between 0 and 30 % (for which there was no effect on populations) as well as medium toxicity intervals (between 40 % and 70 % mortality). These findings are supported by the experimental study of Liess and Foit (2010), demonstrating that population abundance of *D. magna* took the same time to recover from different toxicity inhibition levels in two experimental set ups at two different carrying capacities (30-55 % effect mortality in experiment A, and 60-73 % effect mortality in experiment B; Fig. 3). In comparison, the logistic growth model predicted a gradual increase in TTR with increasing toxicity levels.

In addition, recovery times predicted with IDamP depended on the capacity of the population, in agreement with the same experimental studies (Fig. 3 A, B in Liess and Foit

2010), but the predictions of the logistic growth model always led to unvariable recovery times at the different carrying capacities (Fig. 6.1).

From these results, we can infer that the simple logistic growth model is not totally wrong and could be used to provide general estimates on the expected population recovery rates (Barnthouse, 2004). Yet, this model results very likely in under-predicting the TTR of populations, even when chemical effects only are considered. In comparison, powerful ecological models that include detailed information on the species are more reliable and allow realistic and conservative estimations of population recovery.

6.4.2. Effects of food and temperature on population recovery from chemical stress exposure

Results of the simulations using single chemical stress exposure show that populations do not react equally to the same stressor but that their sensitivity strongly depends on the environmental scenario. Our results disclose several case scenarios with important implications in ERA. First, the impact of the environmental scenario on recovery from chemical exposure was different between strong and weak mortality levels. For instance, at 10 °C (the average temperature characterizing central European waters is 12 °C, according to the European Environmental Agency, 2012), low food conditions delayed recovery from high mortality levels (≥ 80 % effect strength) but induced the opposite effect at low to medium mortality levels (40-70 %) whereby recovery was fostered. These results indicate that an extrapolation of recovery times between the different toxicity levels is not straightforward, even when the same environmental context applies.

The potential of food supply in fostering recovery from strong lethal effects observed for the *Daphnia* population in the present study is supported experimentally for *Daphnia*

populations exposed to Fenvalerate (Pieters and Liess, 2006), but also for other organisms like populations of different *Chaoborus* species. In fact, *Chaoborus obscuripes* recovered faster from strong lethality levels of the insecticide lambda-cyhalotrin in the eutrophic mesocosm compared to the mesotrophic one (Roessink et al. 2005). The same was observed for *Chaoborus crystallinus* exposed to alpha-cypermethrin (Strauss et al. 2007).

Yet, our study showed that this buffering capacity of high food concentrations for the lethal effects is exhausted by elevated temperatures (2 mg C per population per day, 20 °C; Fig. 6.2) and food becomes an additional stress factor for populations. This finding contradicts the general statement that a good nutritional status always serves in favor of population resilience (SANCO, 2013; Pieters et al. 2005) and the compensating effect of food seems to depend on other environmental factors (temperature in our case) as well as the effect strength of the toxicant. These observations stipulate that the interactions among environmental variables determine the fate of exposed populations rather than the variables themselves.

Second, it has been found in mesocosm studies that elevated temperatures (20-28 °C) delayed the recovery of cladoceran populations from strong (1 to 10 µg L^{-1}) as well as weak (0.01 to 0.1 µg L^{-1}) chemical mortalities caused by an insecticide compared to "cool temperatures" (16- 18 °C, Van Wijngaarden et al. 2005). In comparison with our study, we found no significant difference in the recovery of daphnid populations from weak effects between these same intervals for warm and cool temperatures (Fig. 6.3 a, b), but detected a delay in recovery from the strong mortality effect at the cool compared to the warm temperature interval (90 %; Fig. 6.3 a). Colder temperatures (below 15 °C) even caused stronger delays (Fig. 6.3 a, b) and this delay was more pronounced at high (90 % mortality) than lower (50 % mortality) chemical effect strengths.

6.4.3. Effects of predation and competition

Biological interactions induced opposite effects on recovery at low and at high mortality levels (Figs 6.4 and 6.5). In fact, and in contrast to high toxicity effects (≥ 70 % mortality) which were increased by species interactions, in most of the scenarios with exposure to low-medium toxicity (40 -50 % mortality), both interaction types did not represent additional stress and even sometimes induced a faster recovery (Figs. 6.4 and 6.5) compared to populations exposed to chemical stress only. These findings are in contradiction with the generally accepted argument that species interactions (with competition or predation) constitute additional stress factors to the exposed populations to chemicals (Beketov and Liess, 2006; Liess and Foit, 2010; Knillmann et al. 2012 b).

The faster recovery from low mortality levels can be explained as follows: Predation reduces the neonate and juvenile fractions, leading to less competition for food and more released neonates. This feature of producing larger broods at higher food supply is a driving factor of population dynamics in IDamP **(Chapter 2)**. The predation rate is assumed constant in the present study (15 individuals per day), creating at low lethality levels, an inverse density-dependence effect which results in increasing the size of the population. This increase might even favor the recovery of populations from low/ medium lethality levels (40-50 % mortality). Inverse density dependent effect of predation is nevertheless not able to compensate for strong mortality effects, as the population, including the adult fraction, is severely depleted by the chemical and the number of neonates added to the population remains very low. With competition, the filtration rate and subsequently the growth rate of individual daphnids are reduced. A slow growth induces a faster maturity process leading to the production of fewer neonates (in IDamP, the number of neonates is proportional to the body size of the mother,

Chapter 2). This subsequent reduction in the population size results in more food available that adults will use to produce more neonates. Consequently, competitive effects turn out to have no adverse effects of the population size at sufficient food supply (Fig. 6.4 a, b). As seen with predation, the production of a higher number of neonates might favor population recovery from low chemical effects (40 and 50 % mortality; Fig. 6.4), but not from strong effects. In this latter case, the population size is severely affected and the food depletion caused by resource competition further reduces the population size in profit of the competitive population which increases its abundance. We remind here that competition effects were implemented following a worst-case approach; the competitive population being not affected by the toxicant.

These results support the argument of Gergs et al. (2013) stipulating that population resilience is the result of buffer mechanisms emerging from interactions of the species with the environmental factors. These buffer mechanisms do occur in nature but only to a certain capacity, which is defined by a too strong or a prolonged effect (Gergs et al. 2013). In the present study, the buffering capacity of predation and competition effects was limited to a toxicity threshold of 50 % effect strength, beyond which species interactions are not able to alleviate toxicity effects anymore, but it was also controlled by temperature and food conditions.

Another important observation which can be depicted from our results obtained at strong (≥ 70%) mortality levels, is that the delay in recovery caused by the presence of predation or competition effects was in turn, cancelled out by very high food concentrations (2 mg C per population per day; Figs. 6.4 and 6.5). In this context, Gergs et al. (2013) showed that buffer mechanisms for natural stressors' effects can even be triggered by the toxicant itself, as found for predatory effects by *Notonecta maculata* which were compensated for by a change in the

internal organization (population structure) of *D. magna* populations exposed to pulse exposures of Nonylphenol, a toxicant acting in a size dependent manner (Preuss et al. 2008).

From these different findings, we can infer that no specific role can be attributed to any abiotic or biotic variable in isolation. Only the complex interactive mechanisms between the different factors constituting the full environmental scenario can determine their mutual roles in controlling the resilience of populations to chemical stress exposure. Unless these combinations of factors and effects are simultaneously taken into account in the framework of ERA of chemicals, we cannot achieve a complete understanding of the mechanisms behind the recovery of populations from exposure to chemicals.

6.4.4. Application of model predictions to environmental risk assessment

The important question for an effective use of the recovery concept in ERA is whether recovery measured in mesocosm or population experiments represents a realistic worst case for recovery under field conditions. Mesocosm studies (Hanazato and Yasuno, 1990; Roessink et al. 2005; Van Wiindergarden et al. 2005) as well as population experiments (Pieters and Liess, 2006; Foit et al. 2012; Knillmann et al. 2012 b) usually investigate recovery from medium to high effect strengths. These experiments are normally conducted under mesotrophic or euthropic conditions to achieve a high number of individuals. Commonly, exposure experiments are conducted in spring, and recovery is observed in summer until autumn (EFSA, 2013). Thus, we can expect average temperatures of about 10 to 20 °C in central Europe during the period of the experiment. As for biological interactions, in the case of *Daphnia*, we definitely have competitors, and in some studies, predators are present in the mesocosms (Beketov and Liess, 2006; Reynaldi et al. 2011). Under field conditions, particularly in an agricultural landscape, we expect low to medium toxicity effect strengths, eutrophic conditions in the water body and

chemical application in spring or summer. Competition and predation will also occur under field conditions. To summarize, in ERA, we extrapolate TTR from strong effects at medium food conditions with biotic interactions as measured in mesocosms to small effects at high food conditions with biotic interactions in field conditions. From our analysis, it can therefore be concluded that recovery measured in mesocosms seems to be conservative for field situations for daphnid populations because at strong effects, biotic interactions are more pronounced and at higher food supply, TTR increases for strong effects but decreases for medium to low effects.

Nonetheless, it remains unclear whether this conservative aspect for daphnid populations holds true for other aquatic organisms especially those with longer generation time like univoltine or semivoltine insects (Stark et al. 2004 b; Solomon et al. 2008; SANCO, 2013), for whom a slower recovery is to be expected. Furthermore, the question of the presence of competitors or predators is highly species-dependent and might be different for other species, e.g. *Asellus aquaticus* (Galic, 2012).

6.4.5. Limitations of mesocosm experiments and contributions of ecological models in improving the assessment of population recovery in the field

In the technical guidance directives for ERA, the conditions of the mesocosm experiments for recovery studies "… should be sufficiently representative of natural ecosystems in terms of species composition, species interactions (predation, competition) and natural stressors…" (EFSA, 2013). This statement is vague and does not reflect the variability in the possible environmental scenarios, which would result in failure to address their different outcomes on population recovery. In fact, if we consider the case of daphnid populations, potential predators in field situations such as *Chaoborus* (Swift, 1992), *Notonecta* (Gergs et al. 2013) or fish (Beklioglu and Moss, 1996) will exhibit different predatory behaviours, and this

influences differently the response of the prey population to chemical exposure. Additionally, the same environmental factor will control differently the predatory potential of these different species. For instance, predation by fish has a stronger impact on daphnid populations in test systems with higher nutrient concentrations (Scheffer et al. 2000). By contrast, high nutrient levels reduce the predatory impact by *Chaoborus* on daphnid populations as observed in the present study as well as experimentally (Hanazato, 1991). Similar to predation, the strength of inter-specific competition on the population of interest in the field is triggered by the type of competition (contest versus scramble competition), leading to differences in population resilience to chemical stress. Lastly, in field situations, some natural conditions favor the expansion of a certain population at the expense of another one, whose resilience is thereby altered. For instance, Van Wijngaarden et al. (2005) found that increases in copepods and rotifers coincided with reductions of cladoceran communities. Another study (Hanazato and Yasuno, 1990) showed that the relatively rapid recovery of a *Chaoborus* species from exposure to Carbaryl interrupted that of cladocera in pond experiments (Hanazato and Yasuno, 1990).

Accounting for such variety in possible sets of field scenarios and for the interactions between their different components goes far beyond the capacities of mesocosm experiments. Since mesocosms are still the method used to assess recovery of populations from chemical stress exposure, we propose the use of validated mechanistic effect models in supplement to these experiments to allow testing different environmental scenarios and extending results to further chemical effect strengths and species interactions (Traas et al. 2004; Bednarska et al. 2013). This would definitely result in a reduced uncertainty in recovery estimates under field conditions. Using such a powerful tool, the identification of the most potent combinations of

natural and chemical stressors (in accordance to Holmstrup et al. 2010) is also feasible and with no additional cost.

6.5. Conclusion

The highly heterogeneous responses of recovery times of populations from chemical stress exposure with the environmental scenario support the necessity of specifying the full ecological scenario for mesocosm experimental setups in the technical guidance directives for ERA. Ecological modelling can help to define this kind of scenario and will assist in extrapolating effects from mesocosm or other test systems to this scenario.

CHAPTER 7

Synthesis and outlook

The ultimate goal of ecological risk assessment (ERA) in the context of authorization or registration of chemicals is to protect (non-target) populations in natural systems from adverse effects due to exposure to the chemical. Extrapolation of chemical effects from laboratory single species tests to the population level is a prominent challenge in ERA, which is now being circumvented by the use of safety or assessment factors. For a more scientifically sound approach for extrapolation, the use of mechanistic effect models is being increasingly advocated (Hommen et al. 2010; Preuss et al. 2010; Thorbek et al. 2010; Galic, 2012), as they allow accounting for the toxicant properties as well as for the relevant processes that intervene in the natural regulation of population dynamics (as explained in **Chapter 1**).

In my thesis, I contributed to demonstrating the power of this tool by addressing research questions that are currently perceived as hampering the realism of ERA. To this end, I applied individual-based modelling not only as a tool that captures toxicity effects on individuals and determine their consequences on different population endpoints (**Chapters 3, 5 and 6**) but also conversely, to trace back toxicity effects on the population response endpoint to the individual level and subsequently identify the toxicant modes of action that led to the observed population-level effects **(Chapter 3**). Such a use of multiple patterns at different hierarchical levels is a defining feature of pattern-oriented modelling (Grimm et al. 2005).

The major outcome of this thesis is that only a simultaneous consideration of multiple effects emerging from species-specific properties, environmental properties and the mode of action of the chemical allows determining the real impact of toxicant stress on populations. In this chapter, I come back to the main important findings of my research and highlight their importance for an effective prediction of toxicity effects on populations.

7.1. Hormesis

Hormetic effects were particularly important to investigate in the case of Dispersogen A (**Chapter 3**) because the stimulatory effects on the targeted process (reproductive output) did not manifest only at low toxicant concentrations such is the case for most substances, but within a concentration interval ranging between 0.001 and 10 mg L^{-1}. Our analysis of population extinction risk (Fig. 3.9) using single effect models revealed that these promoted effects had the strongest impact on populations, and even preceded toxicant-induced mortality. An important conclusion of this finding is that population-level effects do not always depend on the most sensitive individual-level endpoint (as also stipulated by Jager et al. 2004), which strikingly confirm the necessity of understanding the mode of action of chemicals on individual traits and efficiently extrapolate these effects to populations. Such a task cannot be achieved by any tool other than mechanistic effect models.

7.2. Time course of toxicity

Toxicity effects are more accurately addressed when we consider the concentration of the chemical inside the organism rather than in the medium. This is because the internal concentration, not the external one, is causing the effect. The internal concentration depends on the chemical properties as well as on individual characteristics (e.g. the body size; Preuss, 2007;

Baas et al. 2010; Kulkarni et al. 2013) and thus, is not constant over time, which in turn leads to different toxicity effects on the organism. This was expressed in our study by the increased toxicity of Dispersogen A on daphnids with time **(Chapter 3)**, which was captured by using the TK/TD model. The advantage of these kinds of models in providing more scientifically sound predictions of toxicity effects on survival is increasingly being recognized (EFSA, 2013; SANCO, 2013). The ability of IBMs to allowing individuals in the population to change their life history traits (growth rate, time to reach maturity, fecundity) in time (Forbes et al. 2008) and to simultaneously account for the dynamics of toxicity and its consequences on individual endpoints by incorporating complex TK/TD models, is greatly advantageous for an effective and realistic chemicals' risk assessment on populations.

7.3. Elucidating assumptions behind toxic effects

The application of mechanistic effect models allowed explaining the assumptions behind the toxicant effect. For instance, in **Chapter 3**, we demonstrated that for the case of Dispersogen A, effects on survival were not induced by a toxicity threshold concentration beyond which individuals would incur an instant death (individual tolerance); instead, death was rather a chance process (stochastic death). These two approaches lead to different predictions for survival over time at constant as well as pulse exposure scenarios (Jager et al. 2011). Powerful tools such as mechanistic effect models, which allow identifying the assumptions behind the dynamic processes underlying a toxic response are needed to efficiently extrapolate effects beyond test conditions (Ashauer and Escher, 2010).

7.4. Maternal effects and individual fitness

The role of newborn fitness in determining important population responses has been widely acknowledged. Resistance to toxic stress is also a matter of the individual's fitness acquired at birth, which is in turn highly correlated to the maternal investment of energy into reproduction. Mothers decide on the amount of energy they allocate to their progeny in close relation with environmental conditions. The individual's fitness (size or weight) is accounted for in different manners in the existing biological theories. For example, in the DEB (dynamic energy budget) theory, there is a fixed maturity threshold for birth, and maternal effects on egg weight are incorporated by positively correlating the amount of energy reserves allocated by the mother to its eggs, and the ingested food. This relationship does not seem to hold for all organisms. Daphnids for example exhibit a different mechanism of energy allocation to offspring: under decreasing food levels, they produce fitter neonates at the expense of the clutch size which is reduced (as shown in the different experiments used in Chapter 4). Other organisms display different mechanisms of energy allocation, such as pond snails whose number of eggs stays rather constant but a change in the age or size at maturity is observed (Zimmer, 2013). The high variability in egg size or weight in many animals, even in the same mother and within the same clutch, makes the establishment of a mechanistic explanation of such a feature difficult (Jager et al. 2013). Therefore, a descriptive approach as done in **Chapter 4** is suitable for describing such an aspect.

The consideration of the neonate fitness is essential to adequately estimate important population attributes (abundance and size structure). Unless this natural variation is adequately accounted for, we cannot capture the real toxicant effects on individuals or their consequences

on the population level, particularly for chemical compounds which directly act on this individual trait, like Dispersogen A (**Chapter 3**).

7.5. Multiple stress interactions: effects on population resilience

Results of **Chapters 5** and **6** demonstrate the ability of the IBM to simultaneously incorporate different environmental and toxicological stressors and evaluate their consequences on population dynamics and size structure as well as population recovery. The different environmental factors influenced populations in different manners so that exposures to the same chemical induced different population responses. Important environmental factors also proved to be very important in triggering population responses to chemical effects, like density dependence or the Allee effect.

The critical conclusion of the implication of environmental stressors is that our assessment of effects of chemicals from laboratory studies is not conservative in most cases, over protective in fewer cases, but almost never accurate, unless these interactions are taken into account.

7.6. Assessing recovery based on environmental properties

Our analysis of recovery times (**Chapter 6**) may not reflect totally the reality of recovery in populations in field situations. There, and in addition to different competition and predatory mechanisms (as explained in **Chapter 6**), the connectivity of the exposed system to the surrounding water bodies is of primary importance (Galic, 2012) because it usually represents a source of external recovery due recolonization from undisturbed systems. Nonetheless, the isolated nature of the simulated experiment is not that important for *Daphnia* compared to other organisms with slower generation times throughout the year, for which

external recovery is important (ex: *Asellus aquaticus)*. For such species, a spatially explicit individual based model is needed (Galic, 2012).

7.7. Conclusion

Following the scientific progress in the fields of ecology and ecotoxicology, more complex but also more reliable and scientifically sound methodologies are anticipated to be used in the future to complement the standard toxicity tests for a more ecologically relevant decision making. Ecological models constitute the most prominent tools and different types of models are being developed to make better use of existing toxicity and ecological data and provide answers to different challenges in ERA.

Nonetheless, the main advantage of the current ERA is that it employs simple tools whose outcomes can be easily understood by all stakeholders. In comparison, ecological models can be at a very high level of complexity that it becomes hard for the involved parties to understand and trust the delivered outputs (Grimm and Railsback, 2005). For this reason, establishing a culture for good modelling practice through the use of TRACE documentation constitutes a prominent step towards the acceptance of ecological models as decision making tools (Schmolke et al. 2010; Grimm et al. submitted for the latest updated version). This standardized documentation process provides details and evidence on model quality and credibility pertaining to all the stages of model development, analysis and application.

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LIST OF PUBLICATIONS

Peer-reviewed journal articles

Gabsi F, Schäffer A, Preuss TG. 2014. Predicting the sensitivity of populations from individual exposure to chemicals: the role of ecological interactions. *Environmental Toxicology and Chemistry*.http://onlinelibrary.wiley.com/doi/10.1002/etc.2409/abstract

Gabsi F, Hammers-Wirtz M, Grimm V, Schäffer A, Preuss TG. 2014. Coupling different mechanistic effect models for capturing individual- and population-level effects of chemicals: lessons from a case where standard risk assessment failed. *Ecological Modelling*. 280:18-29

Gabsi F, Glazier DS, Hammers-Wirtz M, Ratte HT, Preuss TG. 2014. How do interactive maternal traits and environmental factors determine offspring size in *Daphnia magna*? *International Journal of Limnology*. 50: 9-18

Grimm V, Focks A, Frank B, Gabsi F, Kulakowska K, Johnston A, Liu C, et al. 2014. Towards better modelling and decision support: documenting model development, testing, and analysis using TRACE. *Ecological Modelling*. 280: 129–139

Agatz A, Hammers-Wirtz M, Gabsi F, Ratte HT, Brown KD, Preuss TG. 2012. Promoting effects on reproduction increase population vulnerability of *Daphnia magna*. *Environmental Toxicology and Chemistry* 31(7): 1604–1610

Conference contributions

Gabsi F, Preuss TG. 2014. Recovery of populations from chemical stress exposure is a matter of the environmental scenario. Platform presentation at SETAC Europe 24th Annual Meeting (Basel, Switzerland)

Gabsi F, Hammers-Wirtz M, Grimm V, Schäffer A, Preuss TG. 2013. Using a modeling approach to capture individual and population level effects of a chemical where standard risk assessment failed to be protective for populations. Platform presentation at the Symposium for the European Freshwater Sciences (Münster, Germany)

Gabsi F, Hammers-Wirtz M, Grimm V, Schäffer A, Preuss TG. 2013. Coupling different mechanistic effect models for capturing individual and population level effects of chemicals: lessons from a case where standard risk assessment failed. Platform

presentations at the CREAM Open Conference "Mechanistic Effect Models for Ecological Risk Assessment of Chemicals" (Leipzig, Germany)

Gabsi F, Schäffer A, Preuss TG. 2013. Using an individual-based model as a virtual laboratory to assess population responses to multiple-stress exposure in *Daphnia magna.* Poster presentation at the CREAM Open Conference "Mechanistic Effect Models for Ecological Risk Assessment of Chemicals" (Leipzig, Germany)

Gabsi F, Schäffer A, Preuss TG. 2013. A modelling approach to characterize sub-lethal responses of *Daphnia magna* populations to chemicals in the presence of environmental stressors. Platform presentation at the 23rd SETAC annual meeting (Glasgow, United Kingdom)

Gabsi F, Hammers-Wirtz M, Schäffer A, Preuss TG. 2012. Do we need modelling for a conservative risk assessment of chemicals? An investigation on *Daphnia magna* populations. Poster presentation at the 6th SETAC World Congress/ SETAC Europe 22nd Annual Meeting (Berlin, Germany)

Gabsi F, Preuss TG. 2011. Modelling individual and population dynamics of *Daphnia magna* under toxicant exposure. Platform presentation at the International Symposium Cladocera (Verbania, Italy)

Gabsi F, Preuss TG. 2011. Modelling the effects of toxicants on population recovery and extinction – Example of *Daphnia magna* for toxicants with different mechanisms of action. Poster presentation at the Marie Curie Researchers Symposium (Warsaw, Poland)

Gabsi F, Hammers-Wirtz M, Preuss TG. 2011. The importance of maternal environment in determining offspring size in *Daphnia magna* population models. Poster presentation at SETAC Europe 21st Annual Meeting (Milan, Italy)

CURRICULUM VITAE

Personal

Name: Faten Gabsi

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Education

2010-2014	PhD student at the Institute for Environmental Research, RWTH Aachen University, Germany
2008-2010	Researcher at the University of Sfax (Tunisia) in collaboration with the University of Caen (France)
2006-2008	Master of Science in Biodiversity and Aquatic resources at the University of Sfax, Tunisia
2002-2005	Biological engineering studies at National School of Engineers of Sfax (Tunisia)
2000-2002	Preparatory classes for entrance to the Engineering schools (IPEIS- Sfax, Tunisia), Biology/Geology section
Jun 2000	Baccalauréat in Experimental Sciences. Lycée Majida Boulila (Sfax, Tunisia)

Membership

Since 2010	Society of Environmental Toxicology and Chemistry (SETAC)