The very basic core of a man's living spirit is his passion for adventure. The joy of life comes from our encounters with new experiences, and hence there is no greater joy than to have an endlessly changing horizon, for each day to have a new and different sun.

— Christopher McCandless

Promoters

Prof. dr. Colin Janssen
Environmental Toxicology Unit (GhEnToxLab)
Department of Applied Ecology and Environmental Ecology
Ghent University

Prof. dr. ir. Frederik De Laender
Laboratory of Environmental Ecosystem Ecology
Research Unit in Environmental and Evolutionary Biology
Department of Biology
University of Namur

Dean

Prof dr. ir. Marc van Meirvenne

Rector

Prof. dr. Anne De Paepe

Karel VIAENE

Improving ecological realism in the risk assessment of chemicals: Development of an integrated model

Thesis submitted in fulfilment of the requirements for the degree of
Doctor (PhD) in Applied Biological Sciences

Proefschrift voorgedragen tot het bekomen van de graad
Doctor in de Bio-ingenieurwetenschappen

Dutch translation of the title:

Verhoging van de ecologische realiteit in de risicoschatting van chemische stoffen: Ontwikkeling van een geïntegreerd model

Refer to this work as:

Viaene K., 2016, Improving ecological realism in the risk assessment of chemicals: development of an integrated model. PhD Thesis, Ghent University, Belgium.

ISBN 978-90-5989-891-2

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

µg	microgram
CR	Concentration-response
d	Day
DEB	Dynamic Energy Budget
DEBkiss	Dynamic Energy Budget, Keep It Simple, Stupid
dw	Dry weight
EC	European Commission
ERA	Ecological Risk Assessment
EU	European Union
FOCUS	FOrum for the Co-ordination of pesticide fate models and their Use
h	Hour
HC_5	Harmful Concentration for 5% of the species
IBM	Individual-based Model
ind	individuals
K	Phytoplankton carrying capacity
L	Litre
mg	Milligram
mL	Millilitre
mm	Millimetre
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ	Risk Quotient
SCHER	Scientific Committee on Health and Environmental Risks
SSD	Species Sensitivity Distribution
TKTD	Toxicokinetic-toxicodynamic
WFD	Water Framework Directive
ww	Wet weight

1

GENERAL INTRODUCTION AND OUTLINE

1.2. Ecological risk assessment

Awareness for the anthropogenic impact on the environment has greatly increased the past decades. Human activities have been recognized as the major cause of climate change, biodiversity loss and disruption of nutrient cycles (Hooper et al. 2005; Cardinale et al. 2012). Public reports of massive animal mortality after e.g. oil spills such as the Deepwater Horizon disaster (Abbriano et al. 2011) are typically observed following accidental discharges of chemical waste e.g. the 2015 dam burst in a Brazilian mine (UN Human Rigths 2015). However, pollution also affects ecosystems at smaller spatial scales and many effects are more subtle and do not necessarily lead to mass mortality of individuals (Fleeger et al. 2003). In order to prevent such effects, it is important to quantify the risk a chemical poses to the ecosystem to make informed decisions about its production and use. Most chemicals are potential hazards for ecosystems i.e. they have inherent properties that can cause damage to the ecosystem (van Leeuwen and Vermeire 2007). The ecological risk of a chemical refers to the probability that a chemical will cause damage to the ecosystem, taking into account exposure. Quantification of the risk a chemical poses for the ecosystem is done by performing an ecological risk assessment (ERA).

In general, the goal of ecological risk assessment of chemicals is to quantify the risk that a concentration of a given chemical would impair the structure and functioning of natural ecosystems and to derive maximum environmental concentrations that prevent ecological effects (Preston 2002; De Laender and Janssen 2013). Typically, ecological risk assessment is divided in two parts: exposure assessment and assessment of the potential ecological effects (Figure 1.1; van Leeuwen and Vermeire 2007). Exposure assessment is used to determine the concentration to which the ecosystem will be exposed. In Europe, this is typically the Predicted Environmental Concentration (PEC). Chemicals in the environment undergo different processes such as (bio)degradation, absorption and evaporation (Chapman et al. 1998). All these processes will determine the final bioavailable concentration i.e. the concentration to which organisms are actually exposed. Effect assessment is used to determine the Predicted No Effect Concentration (PNEC), a threshold environmental concentration below which effects to the ecosystem are not expected to occur. Traditionally, the PEC value is divided by the PNEC to calculate the Risk Quotient (RQ). RQ values higher than 1 indicate potential risk when the chemical of concern would be released in the environment without mitigation measures.

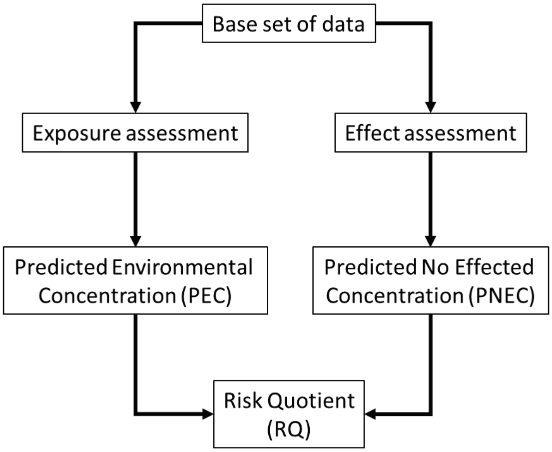


Figure 1.1: *Scheme of a traditional ecological risk assessment. Adapted from van Leeuwen and Vermeire 2007.*

Box 1.1*: Selection of important environmental legislation in the European Union and their respective environmental objectives.*

Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; EC 2007): to regulate the production and use of chemicals.

- Protection of human health and the environment
- Increased transparency
- Promotion of non-animal testing
- Integration with international efforts

Water Framework Directive (WFD; EC 2000; SCHER et al. 2013): to protect the ground and surface waters of Europe against environmental pollution

- Good ecological status of aquatic water bodies. In other words, the characteristics of aquatic ecosystems should be as close as possible to the reference conditions of natural water bodies not subject to human pressure.

Regulation 91/ 414/EEC: to regulate plant protection products (EC 2004)

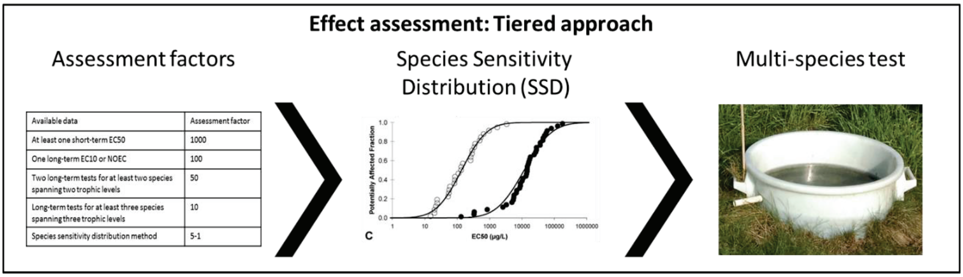
- The use of plant protection products does not have any long-term repercussions for the abundance and diversity of non-target species. This implies protection of populations and communities (not individual organisms), and the possibility of accepting some short-term effects if followed by recovery.

Legislation has been developed to prevent environmental risk by performing predictive risk assessments and specifying formal environmental protection goals. In Europe, several legislations were adopted over the years (Box 1.1). Biodiversity and ecosystem functions are usually specified as protection goals (Hommen et al. 2010). For example, the European Commission has made a commitment towards "halting the loss of biodiversity and the degradation of ecosystem services in the EU by 2020" (EU, 2010) and, according to the Water Framework Directive (WFD), all European surface and groundwater bodies should have a good ecological status by 2015 (EU, 2000). The focus on biodiversity in legislation is understandable as biodiversity is generally considered an useful descriptor of ecosystem structure and its role in ecosystem productivity and stability is generally accepted in the ecological literature (Hooper et al. 2005, 2012; Cardinale et al. 2012).

1.3. Current ERA methods and their limitations

The number of registered chemicals is approximately 100,000 and still increasing (Clements and Rohr 2009). To cope with this high number of chemicals, ERA is typically conducted using a tiered approach (Figure 1.2) i.e. chemicals that pose a higher risk at lower tiers are subjected to more extensive and complex risk assessment methods at higher tiers (Brock et al. 2006; SCHER et al. 2013). The lowest tier is primarily used for screening chemicals i.e. identification of chemicals that could pose a risk. This lowest tier is very similar in all EU directives and is based on the risk quotient i.e. the ratio of the PEC and the concentration at which effects are expected (Hommen et al. 2010). In all directives except for the plant protection directive, the Predicted No Effect Concentration (PNEC) is used as the reference concentration for effects. The PNEC is calculated by dividing the endpoint of the most sensitive test species by an appropriate assessment factor. Assessment factors, also sometimes called safety factors, are used to account for uncertainty concerning the accuracy of the selected endpoint such as intra- and interspecies differences in sensitivity, differences between acute and chronic tests and the lab-to-field extrapolation (Chapman et al. 1998). If the risk quotient is larger than 1, the chemical has a potential risk to the environment and needs to either undergo higher tier risk assessment to further assess the risk or be risk managed.

The use of the risk quotient, and especially assessment factors, has been heavily criticized in ERA. Assessment factors are a conservative method to deal with the uncertainty related to the extrapolation to real situations but are based on policy and not on science (Chapman et al. 1998; Forbes et al. 2008). Indeed, this approach relies too much on expert judgement to relate risk ratios to environmental protection goals (Forbes et al. 2009a). This often leads to an overestimation of the risk and consequently, unrealistically low "safe" concentrations (Chapman et al. 1998). Also, this approach only uses the most sensitive endpoint of the available toxicity tests (Forbes et al. 2008). Unless additional toxicity tests reduce the assessment factor e.g. a chronic test versus an acute test, they are only used to calculate a new PNEC when the measured endpoint is even more sensitive than the previous one. More information on the potential toxicity of a chemical does thus not necessarily lead to more accurate risk assessments with this approach.



Available data	Assessment factor
At least one short-term EC50	1000
One long-term EC10 or NOEC	100
Two long-term tests for at least two species spanning two trophic levels	50
Long-term tests for at least three species spanning three trophic levels	10
Species sensitivity distribution method	5-1

Figure 1.2: *The tiered approach to the risk assessment of chemicals. Chemicals that pose a higher risk may be subject to more extensive assessment methods.*

Higher tier risk assessment methods include the use of extrapolation models and multi-species test systems. The species sensitivity distribution (SSD) is the most used extrapolation model and fits a probability distribution to a set of toxicity thresholds derived in single species toxicity tests (Forbes and Calow 2002; Posthuma et al. 2002). The probability distribution is used to determine a concentration at which a certain percentage of the species is not affected. Typically, the concentration at which 95% of the species are protected is used as the PNEC value. A major advantage of this approach compared to the risk quotient technique is that it incorporates all information available from different species. The SSD approach has been compared with model ecosystem data and field data for many chemicals e.g. for endosulfan (Hose and Van den Brink 2004) and fluazinam (van Wijngaarden et al. 2005). Indeed, most SSD-derived treshold concentrations were protective for ecosystem structure and functioning (Versteeg et al. 1999). However, the properties and underlying assumptions of SSDs have been discussed in depth over the years and questions, mainly related to their underlying assumptions, have been raised about their use for ecological risk assessment (Forbes and Calow 2002).

One of the main issues is that the species included in the SSD are considered as components of a realistic community while this is rarely the case (Forbes and Calow 2002). Generally, any organism for which sensitivity data are available is included and this species is assumed to be equally important for structure and functioning of the ecosystem. This ignores the possible presence of keystone species which have a larger than average contribution to ecosystem structure and functioning. Also, SSDs assume that interactions between individuals and species will not influence the sensitivity of the community (De Laender et al. 2008c; Schmitt-Jansen et al. 2008). However, there are many examples of how ecological interactions can influence the outcome of chemical exposure and this assumption is thus unrealistic. For example, one modelling study compared a SSD approach that neglected ecological interactions with one that accounted for ecological interactions (De Laender et al. 2008c). The latter study showed that for approximately 25% of the toxicants, the SSD approach that took ecological interactions into account was more strict than the SSD approach that neglected ecological interactions. Therefore, it is impossible to determine if derived safe concentrations with a SSD are protective for real ecological communities.

Several statistical considerations need to be accounted for when applying SSDs. To calculate accurate percentiles, a sufficient number of species needs to be included and the appropriate distribution should be used. The amount of species needed differs between cases but has been estimated to range between 15 and 55 species (Newman et al. 2000). This is higher than what is required for most regulatory purposes and for most chemicals this amount of data is not available. Also, the identity of the species included in the SSD will influence the derived safe concentrations (Forbes et al. 2001; De Laender et al. 2013). Inclusion of a large number of species sensitive to a certain chemical e.g. primary producer for a photo-synthesis inhibiting herbicide, will lead to lower safe concentrations than a set of species where primary producers are under-represented (Van den Brink et al. 2006). To fit the SSD to the data, the log-normal distribution is often chosen but this distribution is often not applicable to the data (Newman et al. 2000). These statistical considerations are often neglected which leads to inaccurate predictions and a high uncertainty on the derived percentiles and thus ‘safe’ concentrations (Forbes and Calow 2002). Finally, questions can be raised about how a SSD is used and interpreted. Often the HC_5 i.e. the concentration at which 5% of all species are affected, is calculated and used as a safe environmental concentration. This assumes that 5% of the species is an appropriate protection level i.e. that the loss of 5% of the species does not affect the ecosystem structure and functioning (functional redundancy) and that biodiversity, as a measure of ecosystem structure, is a more sensitive ecosystem endpoint than ecosystem functions (Newman et al. 2000; Forbes and Calow 2002; De Laender et al. 2008a). This appears to be true for herbicides, insecticides and fungicides: comparison between SSD-derived HC_5 values and no-effect concentrations in model communities showed that HC_5 values were, in general, protective for at least short-term exposure (Van den Brink et al. 2006; Maltby et al. 2009). For insecticides, the SSD-approach was protective in 25 of the 27 cases when compared with experiments with model communities (van Wijngaarden et al. 2015). Further evaluation is however required for chemicals that have chronic toxicity or modes of action that have been rarely tested e.g. neonicotinoids.

Experiments with model communities, both small scale (microcosms) and large scale (mesocosms), are currently considered the most ecologically relevant effect assessment techniques because they expose realistic aquatic communities over a longer period of time to the chemical (Schmitt-Jansen et al. 2008). This approach can account for ecological interactions and indirect effects i.e. effects on tolerant species through interactions with sensitive species (Fleeger et al. 2003; De Laender et al. 2011). Population and ecosystem recovery can also be assessed with this method. However, micro- and mesocosms also have several disadvantages. The amount of time and resources required to perform such experiments is a major drawback (De Laender et al. 2013). Also, interpretation of the results is non-trivial, although methods like the principal response curve technique have been developed to deal with this (Van den Brink and Ter Braak 1998). Other disadvantages include problems with scaling effects, the protection of rare species, sensitivity to starting conditions and the inability to replicate every natural system (Forbes et al. 2008; Schmitt-Jansen et al. 2008). Effects occurring in the model communities do not necessarily correspond with effects at more realistic spatial scales, referred to as scaling effects (Forbes et al. 2008). For example, in realistic landscapes, migration of individuals can alter the observed effects. Moreover, model communities might miss effects on rare species not present in the sampled community. Species can also be sensitive to the starting conditions of the model community experiment, reducing the relevance of the experiments (Hjorth et al. 2007). Lastly, it is impossible to perform such experiments for each natural system. Comparison between different communities exposed to the same chemical has shown that, although the sensitive species are affected at similar concentrations, indirect effects and recovery can indeed be very different (Daam and Van den Brink 2010).

In general, all current ERA methods fail to provide an accurate and certain answer to the central question in ecotoxicology: what are the large-scale effects of chemical stress in real-world systems (Beketov and Liess 2012). As a result, decisions in risk assessment are accompanied with large uncertainty and the prescribed protective concentrations are possibly either under- or overprotective. The major problem is how to extrapolate from these, at best, simple community tests performed in a controlled environment to the protection goals set by the authorities (De Laender et al. 2008a; Forbes et al. 2008). The relationship between typical ecotoxicological endpoints such as growth, survival and fecundity and population or ecosystem dynamics is complex, non-linear and thus difficult to predict using simple techniques (Forbes et al. 2008). Therefore, ecological risk cannot be adequately assessed using procedures that disregard most of the inherent environmental and ecological complexity (De Laender et al. 2014a). However, current ERA procedures fail to consider newly developed methods such as ecological models that were specifically developed to reduce the uncertainty in ERA. Also, it is unclear that current approaches are able to accurately predict future risks, especially considering that the number of environmental stressors are increasing (Grimm and Martin 2013). Multiple stressors can refer to a combination of different chemicals but also to the combination of chemicals and other abiotic stressors such as temperature (De Laender and Janssen 2013). How these multiple stressors interact is difficult to predict (Gabsi et al. 2014b) but it is clear that the presence of multiple stressors may have potentially large implications for ERA.

1.4. Integration of ecology into the risk assessment procedure

In order to more accurately predict the effects of chemicals on communities and ecosystems, more ecology needs to be integrated in ERA approaches (Chapman 2002; Clements and Rohr 2009; Grimm et al. 2009). The call for integration of ecological processes was already brought up as early as the 1980s – "putting the eco in ecotoxicology" (Cairns 1988) – and 1990s (Baird et al. 1996). Recent notable efforts call for the integration of macro-ecology in ecotoxicology (Beketov and Liess 2012) and for the application of community ecology to ecotoxicological theory (Schmitt-Jansen et al. 2008). Scientific efforts have been done to address these concerns e.g. the use of ecological models (Galic et al. 2010). However, integration of more ecology at the regulatory level has thus far been limited to the use of micro- and mesocosm experiments as highest tier ERA tools. Criticism of the current ERA procedures was summarized in an opinion report of different Scientific Committees of the European Union (SCHER et al. 2013). The report recognizes that current ERA procedures lack ecological realism which leads to high uncertainty associated with the predictions made.

With the current advances in ecological modelling and informatics, it should be feasible to use more complex and computationally intensive techniques that are better at integrating ecological principles. Several ecological features have been identified or suggested as important to consider in ERA. These include, but are not limited to, ecological interactions, patterns in exposure, the spatial structure and scale, the presence of keystone species, functional redundancy and recovery potential (Figure 1.3). Three of these are addressed in detail in this PhD thesis: ecological interactions, spatial scale and structure and recovery. Keystone species are not the focus of this PhD because they are not present in every community. Effects on functional redundancy is related to effects on ecosystem functioning while this work focuses more on patterns in species abundances. However, the tools developed in this thesis could easily be adapted to cover these two cases e.g. by adjusting the community to incorporate a keystone species or by monitoring ecosystem functions such as primary or secondary production.

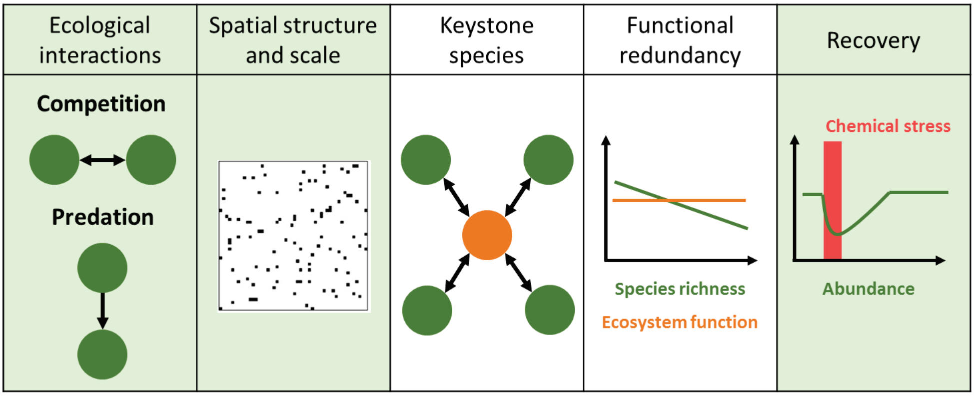


Figure 1.3: *Overview of different ecological aspects that are often overlooked with traditional ecological risk assessment approaches. Aspects covered in this PhD thesis are marked in green.*

In ecosystems, individuals exposed to a chemical are not isolated but interact with individuals of the same and/or of other species. However, traditional ERA approaches regard individuals as discrete units instead of interacting entities (Preston 2002). In more diverse communities the number and complexity of interactions is higher than in less diverse communities (Relyea and Hoverman 2006). Accurately assessing species interactions is essential to perform ecologically realistic risk assessments (De Laender et al. 2014a). In natural systems, indirect effects of chemicals - effects on more tolerant species through interactions with sensitive species - are common (Rohr et al. 2006; Clements and Rohr 2009). Because they involve multiple species, indirect effects are also intrinsically more complex and more difficult to predict (Rohr et al. 2006). Competition and predation are regarded as the most important ecological interactions when considering indirect effects of chemicals (Preston 2002).

Competition can refer to competition for the same food source but also to competition for space, light or other limiting resources. Competition can occur between individuals of different species (interspecific competition) but also within one population of the same species (intraspecific competition). Generally, when chemical stress leads to decreased population densities, the surviving individuals in the population experience less intraspecific competition (Foit et al. 2012). This decreased intraspecific competition reduces the effect of chemical stress on population densities (Liess 2002; Foit et al. 2012; Del Arco et al. 2015) and allows more rapid recovery of population density or size structure after exposure (Foit et al. 2012; Knillmann et al. 2012b). For competition between tolerant and sensitive species, tolerant species are expected to be, at least partly, relieved from competition (Foit et al. 2012). Consequently, the tolerant species perform better after exposure to stress. This was experimentally shown for *Daphnia magna* and *Culex* sp. larvae exposed to fenvalerate (Foit et al. 2012) and for *Daphnia* spp. in microcosms exposed to esfenvalerate (Knillmann et al. 2012b). Other examples include modelling studies where competition prolonged the effects of the chemical (Kattwinkel and Liess 2013) or increased the vulnerability of the population to chemical stress (Gabsi et al. 2014b). In reality, species can interact in other ways than only via competition. For example, in an experiment with *Asellus aquaticus* and *Gammarus pulex*, competition positively influenced *G. pulex* survival during exposure to carbendazim (Del Arco et al. 2015). This was attributed to predatory compensatory mechanisms by *G. pulex* under food-limited conditions, showing that reality can be much more complex and unpredictable than standard experiments would suggest.

For predation, chemical stress can either affect the prey, the predator, or both. Similarly to interspecific competition, effects on the predator may relieve the prey species from predation, allowing it to increase in abundance (Fleeger et al. 2003). For example, phytoplankton species increased in abundance after elimination of the grazers due to carbendazim toxicity (Van den Brink et al. 2000). A common observation when prey are more sensitive than predators is that prey exposed to a toxicant are more vulnerable to predation (Fleeger et al. 2003; Beketov and Liess 2006). For example, *Artemia* sp. populations went extinct after combined exposure to chemical stress and simulated predation (Beketov and Liess 2006). In this case, the predation pressure prevented density-dependent compensation for the chemical effect i.e. increased reproduction at low densities was not possible. *Chironomus* larvae were less active after cadmium exposure (Rooks et al. 2009). This increased their susceptibility to active predators but interestingly, the predation rate by ambush predators was not affected. Predator species can also be affected by chemical stress through their prey e.g. ciliates starved when their food source was eliminated by prometryn (Liebig et al. 2008). However, predation does not always lead to higher effects of a chemical. Presence of predator kairomones resulted in *Daphnia magna* producing larger offspring that were more resistant to chemicals, thus reducing the effect of carbaryl exposure (Coors and Meester 2008; Gergs et al. 2013). How chemical stress interacts with competition and predation is clearly chemical and species specific. To predict how these interactions influence the effect of chemicals, a better understanding of the underlying processes is needed.

Differences in application time, emission rates and location can result in significant differences in timing and levels of exposure, ultimately leading to different effects on local populations (Galic et al. 2012). Depending on the chemical identity, exposure patterns can differ greatly (De Laender et al. 2014a). Chemicals differ in their application time, partition to different compartments in the environment and differ in their persistence in the environment. These realistic exposure patterns can differ greatly from typical lab tests with short, constant exposure. Another important aspect is the timing of exposure in the life cycle of the exposed populations. Early life stages, especially embryonic stages, are often more sensitive to chemicals. Exposure during periods of reproduction can thus lead to larger population effects than exposure during periods of no reproduction, especially for species with long life cycles (Bridges 2000; Galic et al. 2012). The landscape structure and the presence of unexposed populations is another factor determining the outcome of chemical exposure and is especially important for the recovery of the affected populations. For example, isolated communities recovered more slowly from the application of endosulfan than less isolated communities (Trekels et al. 2011). Similarly, the presence of uncontaminated patches decreased the recovery time of mesocosm communities after lufenuron exposure (Brock et al. 2009).

“Keystone species” are species that are essential for certain ecosystem functions or that enable other species to survive in the ecosystem (Chapman 2002). Keystone species often indicate the presence of an ecological threshold and the loss of these keystone species often results in abrupt, non-linear changes in ecosystem structure and functioning that are difficult to recover from (Clements and Rohr 2009). For example, the burrowing ghost shrimp *Neotrypaea californiensis* is an important facilitator for many other species of the soft sediment benthos. Direct effects of carbaryl on this shrimp species also indirectly affected the associated benthos species (Dumbauld et al. 2001). Therefore, it is important to identify the presence and identity of keystone species in ecosystems. Closely related to this is the concept of functional redundancy. Functional redundancy occurs when the loss of some species does not result in the loss of ecosystem functioning because more tolerant species compensate for the affected species (Chapman 2002). Not all species are thus always equally important for the ecosystem structure and functioning and ecological risk assessments should take this into account.

Another often neglected characteristic of natural populations is their potential to recover after a disturbance event. In natural ecosystems, populations are regularly disturbed by environmental factors. If we consider the long-term effects of chemicals, the recovery potential of a population or ecosystem is thus an important factor (Relyea and Hoverman 2006; Clements and Rohr 2009). The recovery potential differs between species and systems e.g. short lived species will typically recover quicker from chemical stress than species with a longer life cycle, and is influenced by other environmental factors e.g. indirect effects (De Lange et al. 2010; Knillmann et al. 2012b). Ideally, ecological risk assessments should take the recovery potential of a system into account and be more stringent when the recovery potential is low.

1.5. Modelling as a risk assessment tool

A main challenge in the field of ecotoxicology is to develop tools that can take into account the ecological complexity displayed in real ecosystems (Figure 1.3) so that site-specific effects can be assessed. Ecological modelling was proposed as one of the best options to improve effect assessment, specifically to account for ecological interactions and spatial and temporal variability in exposure (SCHER et al. 2013). However, the use of models is far less accepted in effect assessment than in exposure assessment. In particular, the development of FOCUS (FOrum for the Co-ordination of pesticide fate models and their Use; FOCUS 2001) models and scenarios provided a standardized way to develop and use environmental fate models (Grimm and Martin 2013; SCHER et al. 2013). The inherent complexity of ecosystems, the apparent lack of universal ecological laws and the lack of clear protection goals have hindered the acceptance of models as tools for effect assessment of chemicals (Van Straalen 2003; Van den Brink et al. 2006). In legislation, ecological models are thus mostly ignored as possible ERA tools. Only the guidance document relating to aquatic toxicology under Directive 91/414/ EEC (SANCO 2002) lists ecological models as possible tools in higher tiered risk assessments for extrapolation from microcosm or mesocosm studies to the field (Hommen et al. 2010). It is clear that more ecological knowledge and more complex decision making is required to apply ecological models as tools for effect assessment (Dohmen et al. 2015). Indeed, modelling could be an ecology based alternative to the standard ERA approaches and can actually be used to assess the effects of chemicals on the actual protection goals i.e. biodiversity and ecosystem functioning (De Laender et al. 2008b; Forbes et al. 2009a). Potential applications of models in ERA include (i) evaluating the relevance of effects on individuals for population dynamics, (ii) extrapolation to untested exposure patterns, (iii) extrapolation of recovery processes from lab to field, (iv) assessment of indirect effects and (v) evaluation of bioaccumulation and biomagnification of chemicals (Hommen et al. 2010). Several initiatives have been taken during the past decade to explore and promote the use of ecological models in risk assessment e.g. the CREAM (Grimm et al. 2009) and ChimERA (De Laender et al. 2014a) projects, as well as through the establishment of the MemoRisk SETAC advisory group (Preuss et al. 2009b) and the organization of several workshops e.g. LEMTOX (Forbes et al. 2009b) and MODELLINK (Hommen et al. 2016).

Using models to assess the effects of chemicals on populations, communities or ecosystems has several advantages. Most importantly, models can help clarify how chemicals affect higher levels of biological organization, why certain effects are happening and what the most important drivers are (Grimm et al. 2009). Ecological models have successfully been used to comprehend and predict the effects of chemicals on populations (Galic et al. 2010; Preuss et al. 2010; Dohmen et al. 2015), communities (De Laender et al. 2014b), and ecosystems (De Laender et al. 2015). With advances in computational power and efficiency, the explicit consideration of time and space in models is becoming more feasible (Galic et al. 2010). This allows the modelling of populations in heterogeneous landscapes, where the exposure can be drastically different between different locations and different times of the year. Modelled *Asellus aquaticus* populations were predicted to recover faster when connectivity in the habitat was higher (Galic et al. 2012). Exposure during periods of reproduction resulted in slower recovery, indicating the importance of the exposure profile. Models are also ideally suited to study how effects on individuals translate to effects on population and ecosystem dynamics (Bradbury et al. 2004; Forbes and Calow 2012). For example, evaluation of different physiological modes of actions in *Daphnia magna* populations showed that direct effects on survival and reproduction had a much larger impact on population densities than effects on growth or feeding rate (Gabsi et al. 2014a). Modelling can thus help reduce the uncertainty associated with the extrapolation from ecotoxicological observations to ecological effects (Forbes et al. 2008).

Modelling approaches can also be informative for the lower tiers of risk assessment. Experiments *in silico* can help to design toxicity tests, interpret individual responses to chemical stress and identify deficits in current datasets, allowing for more focused experimental work (Galic et al. 2010; Jager et al. 2014). Moreover, the large amount of historical ecotoxicology data can be used for modelling purposes and models can reduce the need for additional ecotoxicological tests, reducing the amount of test animals needed.

1.6. Individual based modelling

Individual based models (IBMs) seem particularly suited for use in ERA. IBMs consider processes occurring at the individual level such as feeding, growth and reproduction (Martin et al. 2013b; Gabsi et al. 2014b). Population properties are not modelled directly but emerge from the individuals in the population. Since most ecotoxicological tests focus on the individual level, IBMs are ideal tools to translate these test results to the population level. Incorporating chemical effects on individuals in IBMs allows exploration of the effects of chemicals at the population level. In recent years, IBMs have been used to predict the population dynamics of a number of typical test species used in ecotoxicology e.g. *Daphnia magna* (Preuss et al. 2010; Martin et al. 2013a) and *Asellus aquaticus* (Galic et al. 2012). Similarly, the effects of a hypothetical insecticide on three populations of arthropods were modelled using individual based models (Dohmen et al. 2015). These IBM applications neglected possible interactions with other species and focused solely on a single species. However, the absence of interactions between species is one of the main criticisms on current ERA methods (Rohr et al. 2006; Clements and Rohr 2009). Considering that ecological models have been suggested as tools to extrapolate individual-level effects observed in experiments to population, food web and ecosystem level effects (Grimm et al. 2009; De Laender et al. 2014a), it is important to develop ecological models that do incorporate interactions between species. One recent modelling effort showed that adding interspecific competition to individual-based models increased recovery times following chemical stress up to three times (Kattwinkel and Liess 2013). IBMs have however not been applied yet for more complex systems e.g. food webs, where more species and more interactions between these species need to be considered.

1.7. Current limitations to using modelling in risk assessment

Modelling could be useful to overcome many of the shortcomings of current ERA procedures. However, modelling needs to improve in certain key areas before it will be fully accepted as an ERA tool. These improvements are two-fold: (1) improvements of the science underlying the models and (2) improvements of the regulatory use of models (Grimm and Martin 2013). To improve the science behind the models, validation is of key importance i.e. how can we know that developed models capture reality, that the science is sound (Forbes et al. 2008; Grimm and Martin 2013)? Validation is, however, a very broad term and unclear terminology is often an obstacle to understand what validation entails (Augusiak et al. 2014). A better defined validation process such as the evaludation process defined by Augusiak et al. 2014, will increase the acceptance of models and increase the understanding of the science behind it. Validation is closely linked to the emergence of patterns from data (Grimm and Martin 2013). Observations are considered patterns if they are unlikely to result from random processes and thus contain information about the underlying organization. By comparing model output with these patterns, we can validate the model i.e. assess if the model accurately captures the underlying processes and is thus a realistic, scientifically sound representation of the world. A related scientific question is how general does a model need to be, how much complexity does it need, to accurately answer the questions posed (Forbes et al. 2008). In general, more complex models can predict the outcome more accurately but are harder to validate. The selection of the correct level of complexity for the focus situation is thus essential.

To improve the regulatory use of models, risk assessors need to be convinced of their accuracy and potential (Forbes et al. 2009b; Grimm and Martin 2013). Risk assessors are usually not trained in modelling and cannot be expected to accurately assess the appropriateness and validity of a model. For exposure assessment, this problem was solved by developing the FOCUS framework (FOCUS 2001).

This provided risk assessors with a standard way to evaluate exposure models. A similar standardization is needed for ecological models. Some efforts have been made to promote the use of ecological models for ERA e.g. The EU-funded CREAM project (Chemical Risk Effects Assessment models; Grimm et al. 2009; Grimm and Thorbek 2014). Similarly, good modelling practices such as the ODD (overview, design concepts and details) protocol and TRACE (transparent and comprehensive ecological modelling) have been developed (Grimm et al. 2006; Schmolke et al. 2010).

1.8. Problem formulation and objectives of this PhD thesis

It is clear that ERA needs state-of-the-art tools to achieve its goal: accurately assessing the risk a chemical poses to the environment, taking into account essential ecological characteristics of real systems. One key characteristic that needs to be accounted for are interactions between individuals, both within one species and between different species. Individual based models (IBMs) are one of the most promising tools for ERA but their applicability to food webs is unclear. Also, realistic exposure scenarios, with spatial and temporal variability in exposure, need to be considered. Therefore, the objectives of this PhD thesis are:

- understand how species interactions (competition and predation) interfere with chemical stress;
- develop and apply IBMs for two species competing for the same food source;
- develop an individual-based food-web model;
- develop and apply an integrated ecological risk assessment model (ChimERA) to different environmental scenarios;

This research is presented in five main chapters (Figure 1.4), increasing the complexity from simple food webs in the laboratory to more complex food webs in an integrated exposure and effect model. The main conclusions were summarized in a final chapter.

1.9. Thesis outline

1.9.1. Species interactions and chemical stress

Interactions between individuals of the same species and of different species will alter how these individuals respond to chemical stress. To understand how intra- and interspecific competition and predation can alter the effect of chemical stress, experiments were performed with simple food webs (**Chapter 2**). Communities of the water flea *Daphnia magna*, the rotifer *Brachionus calyciflorus* and the phantom midge *Chaoborus obscuripes* larvae were exposed to pyrene. Effects of pyrene were expected to be higher in *D. magna* populations exposed to strong competition or predation. The tolerant competitor *B. calyciflorus* was expected to increase in abundance after pyrene exposure. An indirect negative effect on the predator *C. obscuripes* was expected through direct pyrene effects on its prey *D. magna*.

1.9.2. Modelling competing species under chemical stress

Models can help to understand and predict the effects of chemicals on populations and food webs. IBMs in particular are useful because effects of chemicals can be included at the level of the individual, the focal level of standard ecotoxicity tests. To make the IBMs as widely applicable as possible, they should be based on a generic theory. In **Chapter 3**, I describe the development of the DEBkiss IBM: an individual based model based on the DEBkiss theory. Chemical effects were included in the model by considering effects on survival using either concentration-response curves or toxicokinetic-toxicodynamics models.

The validity of the DEBkiss IBM framework was tested in **Chapter 4** by applying IBMs to the experiments with *D. magna* and *B. calyciflorus* described in Chapter 2. More specifically, I evaluated whether the DEBkiss IBM framework could simulate the population densities of these two grazers when exposed to pyrene, interspecific competition and both pyrene exposure and interspecific competition. This was done by comparing the model simulations with the patterns observed in the experiment.

1.9.3. Food web model development and integration with a fate model: the ChimERA model

Realistic ecosystems are not limited to two species but consist of many interacting species. In **Chapter 5**, predation was therefore added to the competition implementation presented in Chapters 3 and 4 and used to develop an individual-based food web model. Although traditionally separated, exposure and effect assessment are both integral parts of ecological risk assessment. Chapter 5 is therefore concluded with the integration of a fate model with the food web model, forming the ChimERA model: a spatially-explicit model simulating both the fate of a chemical and the effects on the food web.

1.9.4. Scenario analysis with the ChimERA model

A key feature of the integrated ChimERA model is that the effects of different environmental parameters on the food web dynamics can be evaluated, opening a whole new, prospective approach to ERA. In **Chapter 6**, the influence of trophic state, temperature, hydrodynamics and chemical exposure pattern on the resulting chemical effects were evaluated. Effects of these environmental variables on the environmental fate of the chemical and on the food web dynamics was assessed.

1.9.5. Conclusion and future directions

In the final **Chapter 7**, the findings of this dissertation are reviewed and summarized in a set of conclusions. Suggestions and possible directions for future research are provided.

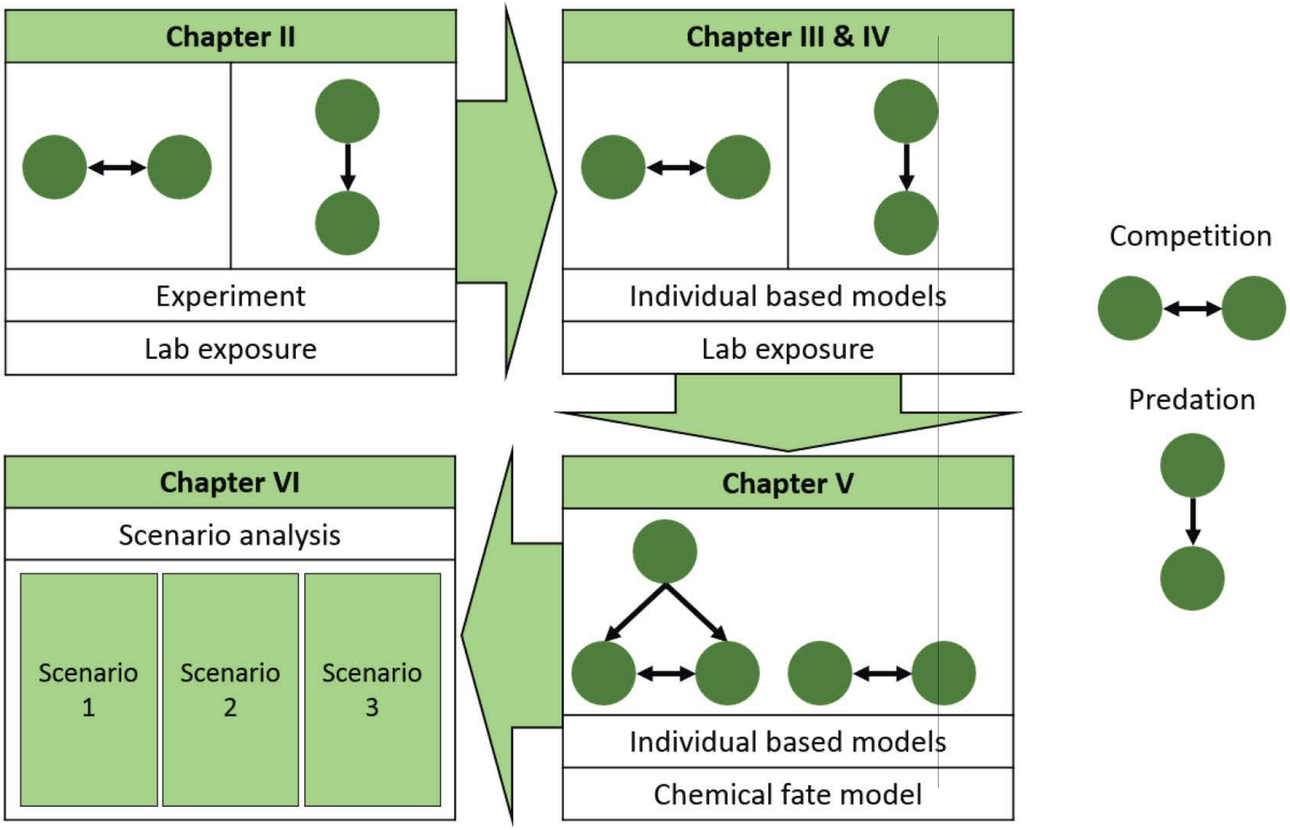


***Figure 1.4**: Schematic overview of the chapters included in this dissertation and the subjects they discuss.*

2

SPECIES INTERACTIONS AND CHEMICAL STRESS: COMBINED EFFECTS OF INTRASPECIFIC AND INTERSPECIFIC INTERACTIONS AND PYRENE ON *DAPHNIA MAGNA* POPULATION DYNAMICS

Redrafted from:

Viaene KPJ, De Laender F, Rico A, Van den Brink PJ, Di Guardo A, Morselli M, et al. Species interactions and chemical stress: Combined effects of intraspecific and interspecific interactions and pyrene on Daphnia magna population dynamics. Environ Toxicol Chem. 2015;34(8):1751–9.

Abstract

Species interactions are often suggested as an important factor when assessing the effects of chemicals on higher levels of biological organization. Nevertheless, the contribution of intra- and interspecific interactions to chemical effects on populations is often overlooked. In the current chapter, Daphnia magna populations were initiated with different levels of intraspecific competition, interspecific competition and predation and exposed to pyrene pulses. Generalized linear models were used to test which of these factors significantly explained population size and structure at different time points. Pyrene had a negative effect on total population densities, with effects being more pronounced on smaller D. magna individuals. Among all species interactions tested, predation had the largest negative effect on population densities. Predation and high initial intraspecific competition were shown to interact antagonistically with pyrene exposure. This was attributed to differences in population structure prior to pyrene exposure and pyrene-induced reductions in predation pressure by Chaoborus sp. larvae. The results presented in this chapter provide empirical evidence that species interactions within and between populations can alter the response of aquatic populations to chemical exposure. It is concluded that such interactions are important factors to be considered in ecological risk assessments.

2.1. Introduction

Current procedures for the ecological risk assessment (ERA) of chemicals are generally based on the extrapolation of individual-level responses to the whole ecosystem and often fail to integrate a sufficient level of ecological realism (De Laender et al. 2008b; SCHER (Scientific Committee on Health and Environmental Risks) et al. 2013). In ecosystems, individuals exposed to a chemical are rarely isolated but interact with individuals of the same and/or of another species. Despite being one of the key characteristics of ecosystems, interactions within and between species are rarely included in current prospective ERAs, especially for non-pesticidal chemicals (De Laender et al. 2008c). However, species interactions can alter the direct effects of a chemical on a sensitive species e.g. increased mortality after pesticide addition because of decreased predator avoidance behavior (Hanazato 2001). Alternatively, by interacting with sensitive species, tolerant species can also be affected leading to indirect effects of chemical stress e.g. starvation of the consumer species when the prey species is affected (Rohr and Crumrine 2005; De Hoop et al. 2013) or reduced competition with the affected species (Rohr and Crumrine 2005). The indirect effects of a chemical are often overlooked but can be as large or even larger than the direct effects of the chemical (Fleeger et al. 2003). Interactions with other species can either increase or decrease the susceptibility of populations and communities to a chemical (Preston 2002; Fleeger et al. 2003). For example, the no observed effect concentration (NOEC) of prometryn for ciliates was more than two orders of magnitude lower in microcosms compared with a single-species toxicity test because of the sensitivity of their food source to prometryn (Liebig et al. 2008). Also, elimination of grazers by the fungicide carbendazim allowed certain phytoplankton species to increase in abundance (Van den Brink et al. 2000) and exposure to insecticides resulted in the development of anti-predator structures in daphnids, potentially reducing the effect of predation (Hanazato 2001). Accurately assessing species interactions is thus essential to perform ecologically realistic chemical risk assessments (De Laender et al. 2014a).

Competition and predation are regarded as the most important species interactions when considering indirect effects of chemicals (Preston 2002). Competition can occur between individuals of different species (interspecific competition) but also within one population of the same species (intraspecific competition). Although several studies exist on the combined effects of interspecific competition and chemicals (Foit et al. 2012; Knillmann et al. 2012b), studies on how intraspecific competition affects the response of populations to chemical exposure are rather underrepresented in the ecotoxicological literature.

The objective of the current chapter was to investigate how initial differences in species interactions influence the response of aquatic invertebrate populations to chemical stress. To this end, *Daphnia magna* populations were initiated with different levels of intraspecific and interspecific competition and predation with pyrene as a chemical stressor. Population size and structure of *D. magna* were evaluated using generalized linear models. Higher effects of pyrene were expected in populations that are experiencing increased competition or predation pressure compared to a control population.

2.2. Materials and Methods

2.2.1. Experimental design

D. magna populations were exposed to six levels of species interactions (i.e. species interaction control, low and high intraspecific competition, low and high interspecific competition, and predation) and to five different pyrene exposure profiles (i.e. control, solvent control, and low, medium and high exposure; see Table 2.1). The experiment was performed in triplicate (n = 3). Two additional replicates were added for the species interaction control treatment without pyrene exposure (n = 5). In a follow-up experiment, referred to as experiment 2, *D. magna* populations were exposed to continuous interspecific competition and to five different pyrene exposure profiles (i.e. control and four different pyrene concentrations; see Table 2.1). The experiments were carried out in glass vessels (1.5 L) filled with 0.5 L of fresh water RT medium (Tollrian 1993). The test vessels were randomly distributed within a water bath placed in a temperature-controlled room (20.8 ± 1 °C) and exposed to low artificial light conditions (1000-1500 lux). Experiment 1 lasted for 29 days with an adaptation period of 7 days (day -7 until day 0). Pyrene was added twice, on day 0 and day 8. After the second pyrene addition, population densities were monitored for another 14 days until day 22. In the follow-up experiment, pyrene was added once after 15 days of adaptation (day -15 until day 0) and population densities were monitored for another 16 days until day 16.

The *D. magna* organisms used in the experiment were obtained from the laboratory culture of the department of Aquatic Ecology and Water Quality Management from Wageningen University (The Netherlands). *Scenedesmus obliquus* was used as a food source for the *D. magna* cultures prior to the experiment and throughout the course of the experiment. Test vessels were fed six times a week with S. obliquus (1 mg carbon · L^{-1} · day^{-1}). The rotifer *Brachionus calyciflorus*, which also feeds on *S. obliquus*, is expected to compete with *D. magna* for food and was used to simulate interspecific competition. *B. calyciflorus* cysts were obtained from MicroBioTest Inc.© (Mariakerke, Belgium) and a stock culture was set up in RT medium at 20°C. *Chaoborus* sp. larvae, which were added to simulate predation, were collected from unpolluted mesocosms at ‘de Sinderhoeve’ research station (www.sinderhoeve.org, Renkum, The Netherlands).

Table 2.1. *Overview of the different species interactions tested in Experiment 1. The columns indicate how many individuals of each species were added to the test vessels for the different species interaction treatments. Each of these treatments was exposed to five different pyrene exposure profiles in experiment 1: no pyrene, solvent control, low, medium and high pyrene exposure. In experiment 2, each of the treatments was exposed to four different pyrene exposure profiles.*

Treatment	**Number of *D. magna***	**Number of *B. calyciflorus***	**Number of *Chaoborus* sp. larvae**
Experiment 1			
Control	10	0	0
Intraspecific competition: low	20	0	0
Intraspecific competition: high	40	0	0
Interspecific competition: low	10	333	0
Interspecific competition: high	10	999	0
Predation	10	0	1
Experiment 2			
Control	10	0	0
Competition	10	200 week^{-1}	0

For both experiments, identical *D. magna* population structures were introduced in all test vessels. They were composed of 20% adults, 40% juveniles and 40% neonates. The classification of *D. magna* organisms within these three groups was based on size, and was performed by filtering the culture medium through sieves with different mesh sizes (i.e. adults > 800 µm; juveniles between 800 and 500 µm; and neonates < 500 µm) (Preuss et al. 2009a). Neonates typically correspond to individuals younger than 48 hours. By using populations composed of different life stages, I wanted to simulate realistic population structures and study the sensitivity of different life stages and its implications for *D. magna* population dynamics.

In the first experiment, the effect of intraspecific competition on *D. magna* populations was studied by using initial densities of 10 (species interaction control), 20 (low intraspecific competition) and 40 (high intraspecific competition) *D. magna* individuals per test vessel. To study how interspecific competition affected the *D. magna* population, *B. calyciflorus* was added to the test vessels at the start of experiment 1 in densities of approximately 333 rotifers · vessel^{-1} (low interspecific competition) and 999 rotifers · vessel^{-1} (high interspecific competition). In the follow-up experiment, more continuous competition was imposed by adding 200 rotifers · vessel^{-1} weekly. Predation was imposed by the addition of one *Chaoborus* sp. larva per test vessel. *Chaoborus* sp. larvae were added 3 days after the addition of daphnids to the test vessels to allow the daphnids to acclimatize. When a *Chaoborus* sp. larva died during the experiment, it was replaced to assure continuous predation pressure.

Pyrene is a polycyclic aromatic hydrocarbon consisting of four benzene rings. Pyrene was chosen as model compound for this experiment because of its non-specific, narcotic mode of action (Di Toro et al.

2000). Phototoxicity of pyrene has been reported (Bellas et al. 2008) and experiments were therefore performed under low light conditions (1000-1500 lux). Acetonitrile was used as solvent for pyrene and, therefore, a solvent control was included in the experimental design (38 and 75 µg L^{-1} added for the first and second addition, respectively). A stock solution of 0.75 g L^{-1} pyrene was prepared in acetonitrile and stirred intensively before addition to the test vessels.

In experiment 1, pyrene was applied twice to the test vessels. The first dosing was applied 7 days after the start of the experiment (day 0) at a nominal concentration of 7.5, 20 and 55 µg L^{-1} for the low (Pyrene1A), medium (Pyrene1B) and high (Pyrene1C) pyrene exposure profile, respectively. Pyrene concentrations were chosen between the EC10 and EC50 values for immobilization. An EC50,immobilization value of 68 (44-106) µg L^{-1} was estimated based on a 48 hours toxicity test with *D. magna* (OECD 2004) (See Appendix A Figure A1 for the concentration response curve). Using a similar protocol, no mortality effects were observed for *B. calyciflorus* and the *Chaoborus* sp. larvae at pyrene concentrations up to 150 µg L^{-1}. Because the first pyrene addition had no observable effects on population densities, pyrene was added a second time at higher concentrations. The second application was performed 15 days after the start of the experiment (day 8) with a nominal pyrene concentration of 15, 40 and 110 µg L^{-1}, corresponding to the low, medium and high pyrene exposure profile, respectively. In experiment 2, pyrene was applied only once after 15 days in nominal pyrene concentrations of 20 (Pyrene2A), 50 (Pyrene2B), 100 (Pyrene2C) and 150 µg L^{-1} (Pyrene2D).

2.2.2. Biological monitoring

In experiment 1, *D. magna* and *B. calyciflorus* abundances in the test vessels were monitored on day -4, 0, 2, 4, 7, 10, 15 and 22. For experiment 2, abundances were counted on day -15, -12, -8, -5, -1, 2, 6, 9, 13 and 16. *D. magna* were counted and divided into the size classes adult, juvenile and neonate by filtering the test medium over sieves with mesh sizes of 800 µm, 500 µm and 200 µm, respectively. *B. calyciflorus* abundances in the test medium of the interspecific treatments were monitored by taking two 6 mL sub-samples per test vessel and counting swimming rotifers using an inverted microscope (magnification 10x).

2.2.3. Chemical analyses

Samples for pyrene analysis were taken after the first pyrene application, before the second pyrene application and two, four and twelve days after the second pyrene application. Pyrene samples were stored in the dark at -20 °C in glass tubes. The chemical analysis was performed with gas chromatography–mass spectrometry (Trace GC 2000 series, Thermoquest, DSQ, Finnigan/Thermoquest). An apolar Zebron ZB 5-ms column (Phenomenex) was used for the analysis, and extraction and elution were performed by solid-phase extraction according to the manufacturer's instructions (Waters and Phenomenex). An internal standard (fluoranthene-d10) at a concentration of 10-50 µg L-1 (depending on expected pyrene concentration) was used to control and correct for extraction losses. The method's recovery was always >75%. Immediately before injection of the sample, a recovery standard was also applied to control for the injection itself.

2.2.4. Fate model analysis

The recently developed dynamic water-sediment organism model EcoDyna (Morselli et al. 2014) was used to predict the temporal fate of pyrene during the experiments. The model was calibrated using the nominal water volume (500 mL) of the experiment and water-sediment interaction was minimized to simulate negligible exchange, given the lack of a sediment phase in the vessels used. In order to calculate potential algal uptake, a daily contribution of 1 mg carbon L^{-1} was assumed, while organism biomass was calculated using length-weight relationships (Dumont et al. 1975; Dumont and Balvay 1979). Physical-chemical properties for pyrene were obtained from literature (Mackay et al. 1992).

2.2.5. Statistical analyses

All analyses were performed using the statistical software package R (version 3.1.1; (R Core Team 2012)). For each sampling time, generalized linear models (GLMs) were constructed. Total, adult, juvenile and neonate *D. magna* densities were considered as response variables, allowing for the examination of population structure. The effect of intraspecific competition (control, low, high), interspecific competition (control, low, high) and predation (non-predation, and predation) was assessed for each point in time by constructing a GLM with pyrene and the species interaction considered as the predictor variables. Time itself was not included as a predictor variable because the effect of time is non-linear and the effects of the other predictor variables will change over time. The full model is given by:

$$E(Density_t|SI, Pyr) = \alpha + SI + Pyrene + Pyrene \cdot SI$$

The expected density ($E(Density)$) at time t is the result of the sum of the intercept α, the species interaction being considered (SI), the pyrene concentration ($Pyrene$) and their interaction ($Pyrene \cdot SI$).

GLMs were initially constructed assuming a Poisson distribution (Zuur et al. 2009) but this led to unsatisfactory model validation. I therefore opted to perform GLM analyses with a normal distribution on the log10-transformed *D. magna* abundance data. The solvent control treatment was not included in the GLM analysis as preliminary tests showed no significant differences between the control and the solvent control treatments. Backwards model selection was used, dropping predictor variables based on the Akaike's Information Criterion (AIC), hypothesis testing and model validation analysis (Zuur et al.

2009). As model validation analysis, I (1) inspected if patterns in the data were present using predicted versus observed plots, (2) inspected if patterns in the residuals were present using predictor versus residuals plots, and (3) tested the normality of the residuals using QQ-plots (Zuur et al. 2009).

2.3. Results

The effects of the different explanatory variables and their interactions are discussed below. I only included the results for the total *D. magna* abundance in this chapter, results for the different size classes are included in Appendix A.

2.3.1. Pyrene fate

Measured pyrene concentrations in water were lower than expected from the nominal values (Table 2.2). Nevertheless, there was a clear difference between the three pyrene exposure profiles at any given point in time. The EcoDyna model was used to simulate pyrene concentration variations in water. The model was run to fit actual water concentrations, and the importance of the main fluxes dominating the change in concentration with time after the spikes. As a result of the fitting procedure, it was found that a chemical half-life in water of 30 h was necessary to reproduce the observed concentrations (no distinction could be made between biotic and abiotic processes), while volatilization accounted for about 20% of losses. Simulations confirmed that pyrene uptake in algae and animal biomass was negligible.

2.3.2. Population dynamics in absence of pyrene and interactions

Independent of the explanatory variables, a clear trend in the model intercept value could be observed. In experiment 1, there was an increase in the intercept until day 7, afterwards the intercept slowly decreased (Table 2.3-2.5). Similarly, in experiment 2 there was a strong population increase the first 14 days, followed by a slow decrease (Table 2.4). The intercept is the value estimated by the GLMs without any effect of the predictor variables (pyrene and species interactions) and thus reflects the population dynamics of *D. magna* without stress. The initial increase and then the decline of the intercept indicated that the population was growing until the carrying capacity was reached (Figure 2.1).

Table 2.2: *Measured pyrene concentrations (µg L-1) for the used pyrene exposure profiles. Measured pyrene concentrations are shown with standard deviations. Nominal concentrations (µg L-1) are shown between brackets when pyrene was added (day 0 and day 8). The letters A, B, C and D refer to the used pyrene treatments.*

Experiment 1						
Time (d)	**0**	**7**	**8**	**10**	**14**	**22**
Pyrene1A	5.2 ± 0.8 (7.5)	0.2 ± 0.1	10.6 ± 1.4 (15)	(3.9 ± 1	0.6 ± 0.2	0.3 ± 0.2
Pyrene1B	13.0 ± 2.2 (20)	0.6 ± 0.3	22.9 ± 4 (40)	12.8 ± 2.1	3.2 ± 0.5	0.3 ± 0.2
Pyrene1C	38.6 ± 11.9 (55)	2.3 ± 0.5	62.8 ±26.6 (110)	44.5 ± 16.9	13.0 ± 2.5	1.5 ± 1.3

Experiment 2				
Time (d)	**0**	**2**	**6**	**13**
Pyrene2A	9 ± 1.4 (20)	4.7 ± 0.5	1.3 ± 0.1	0.3 ± 0.0
Pyrene2B	19.9 ± 2.0 (50)	10.9 ± 0.3	3.2 ± 0.6	0.6 ± 0.0
Pyrene2C	27.4 ± 3.0 (100)	21.4 ± 6.7	6.1 ± 1.6	11 ± 0.5
Pyrene2D	33.9 ± 4.1 (150)	24.5 ± 5.1	8.0 ± 0.9	1.1 ± 0.2

Table 2.3: *GLM estimates of pyrene exposure and intraspecific competition for log10-transformed total D. magna abundance after backwards model selection. For each time point, the significant estimates (*$p < 0.05$*) of explanatory variables and their interactions are shown. Positive and negative values indicate a higher or lower abundance than the control treatment, respectively. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	1.03	1.18	1.64	1.91	2.13	2.03	1.97	1.88
Low pyrene	/	/	/	/	-0.12	/	/	0.10
Medium pyrene	/	0.19	/	/	-0.09	/	/	/
High pyrene	/	/	/	/	/	-0.07	-0.56	/
Low intraspecific	0.26	0.22	/	-0.12	-0.17	-0.09	/	-0.07
High intraspecific	0.50	0.39	0.12	-0.11	-0.11	-0.13	/	-0.11
Low pyrene X Low intraspecific	/	/	/	/	0.17	/	/	/
Medium pyrene X High intraspecific	/	/	/	0.18	/	/	/	/
High pyrene X High intraspecific	/	/	/	/	/	/	0.30	/

Table 2.4: *GLM estimates of pyrene exposure and interspecific competition for log10-transformed total D. magna abundance after backwards model selection for experiment 1 and 2. For each time point, the significant estimates ($p < 0.05$) of explanatory variables and their interactions are shown. Positive and negative values indicate a higher or lower abundance than the control treatment, respectively. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Experiment 1								
Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	1.06	1.20	1.70	1.90	2.13	2.02	1.97	1.88
Low pyrene	/	/	/	/	-0.12	/	/	/
Medium pyrene	/	0.17	/	/	/	/	/	/
High pyrene	/	/	/	/	/	-0.09	-0.63	/
Low interspecific	/	0.13	/	/	-0.15	-0.12	-0.11	/
High interspecific	/	/	/	-0.16	-0.24	-0.12	-0.13	/
Low pyrene X High interspecific	/	/	/	/	0.17	/	/	-0.18
Experiment 2								
Time (days)	**-8**	**-5**	**-1**	**2**	**6**	**9**	**13**	**16**
(Intercept)	1.19	1.57	1.95	2.02	2.03	1.97	1.86	1.57
PyreneC1	/	/	/	/	/	/	/	/
PyreneC2	/	/	/	/	/	/	-0.38	-0.71
PyreneC3	/	/	/	/	/	-0.10	-0.34	-0.72
PyreneC4	/	/	/	/	-0.18	-0.25	-0.51	-0.88
Competition	-0.15	/	/	/	/	0.09	0.25	0.58

Table 2.5: *GLM estimates of pyrene exposure and predation for log10-transformed total D. magna abundance after backwards model selection. For each time point, the significant estimates ($p < 0.05$) of explanatory variables and their interactions are shown. Positive and negative values indicate a higher or lower abundance than the control treatment, respectively. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	1.07	1.27	1.69	1.90	2.08	2.01	1.97	1.88
High pyrene	/	/	/	/	/	/	-0.56	/
Predation	/	/	-0.32	-0.40	-0.38	-0.28	-0.25	-0.22
High pyrene X Predation	/	/	/	/	/	/	0.37	/

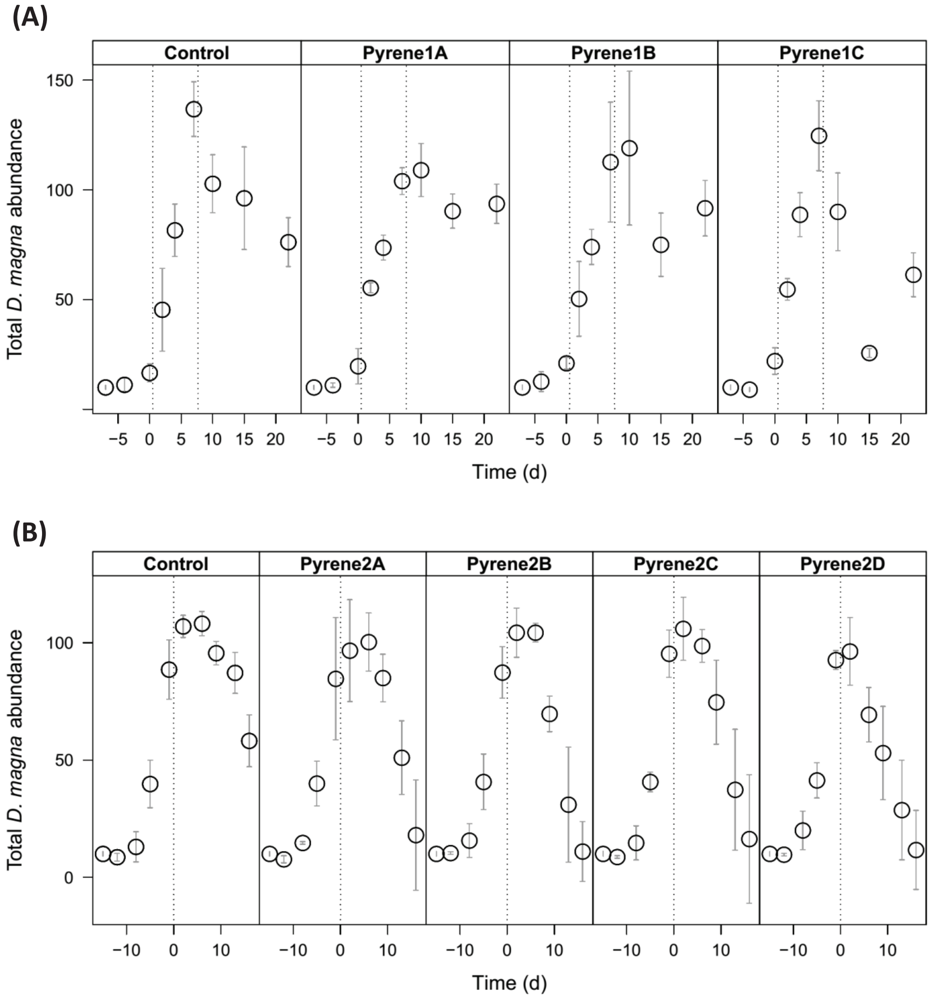


Figure 2.1. *Total D. magna abundances over time for experiment 1 (A) with four pyrene exposure profiles and for experiment 2 (B) with five pyrene exposure profiles. Data shown are the D. magna population densities with no additional species interactions. Average values with standard deviations (error bars) are depicted. Dashed lines indicate pyrene applications.*

2.3.3. Effects of pyrene

For the first experiment, the estimated direct effects of pyrene were almost identical between the different treatments of species interactions (Table 2.3-2.5). For experiment 1, I will therefore only refer to Table 2.3 here. The first pyrene addition did not significantly affect *D. magna* population densities (Figure 2.1 and Table 2.3). However, the highest pyrene exposure did reduce total population densities 7 days after the second pyrene addition (day 15). The description and discussion of the experiment results will therefore focus on the observed effects after the second pyrene addition. Effects of the medium and low pyrene exposure profiles on total *D. magna* abundance were absent or negligible (< 0.13; Table 2.3). The variance of the total population densities explained by pyrene exposure at day 15 was >45% (Appendix A: Table A1-A3). Fourteen days after the second pyrene addition, *D. magna* populations were recovering (day 22): no differences in total population densities were observed between pyrene exposure profiles. However, at that time, the abundances of neonates were higher in the high pyrene exposure profile than in the control treatment (Appendix A: Table A6 and Figure A2). Also, the negative effect of high pyrene exposure on the abundances of adults persisted on day 22, although this effect was smaller than on day 15 (Appendix A: Table A4). Although the total population densities had recovered, differences in population structure were thus still observed between pyrene treatments (Figure 2.1 and 2.2). In experiment 2, a similar delay in pyrene effect was observed (Figure 1B). Significant negative effects were only observed 6 days after the pyrene addition (Table 2.4). Contrary to experiment 1, significant negative effects were observed at the lower nominal pyrene concentrations. Also, no recovery was observed at the end of experiment 2 and pyrene effects were visible at all size classes (Appendix A: Table A13-A15).

2.3.4. Effects of interactions: competition and predation

During the first 9 days of the experiment, the populations with the higher initial population density of 40 individuals (and therefore a higher degree of intraspecific competition) remained more abundant but this effect decreased with time (Figure 2.3 and Table 2.3). For the populations with an initial population density of 20 individuals, this effect only persisted during the first 7 days. The variance explained by intraspecific competition also decreased from 71% to 22% over this period (Appendix A: Table A1). A high initial density resulted in lower future population densities (starting from day 4), although this effect was limited (Table 2.3). The population with the lowest initial density (10 Daphnia per test vessel) reached the highest total *D. magna* abundance (135 individuals). The initial positive effect of a high initial density persisted longer for adult *D. magna* (until day 10; Appendix A: Table A4) than for the other size classes (day 2 and -4 for juveniles and neonates, respectively; Appendix A: Table A5-A6). High initial densities resulted in a higher and more constant abundance of adults in the second half of the experiment compared to low initial densities (Figure 2.2).

In experiment 1, *B. calyciflorus* population densities decreased sharply after one week and *B. calyciflorus* completely disappeared by day 10 (Appendix A: Figure A14). Although *B. calyciflorus* disappeared, significant but limited differences were observed between population densities of *D. magna* of the different interspecific competition treatments starting from day 4 until day 15 (Figure 2.4 and Table 2.4). At the end of the experimental period, differences in population density were no longer observed between the different degrees of interspecific competition. Abundances of adult *D. magna* were never negatively affected by interspecific competition during the whole experiment (Appendix A: Table A7) while abundances of juvenile and neonate individuals were (Appendix A: Table A8-A9). The effect of interspecific competition on the abundance of neonates was only significant up to day 10 because almost no neonates were observed in either of the pyrene exposure treatments the following sample days.

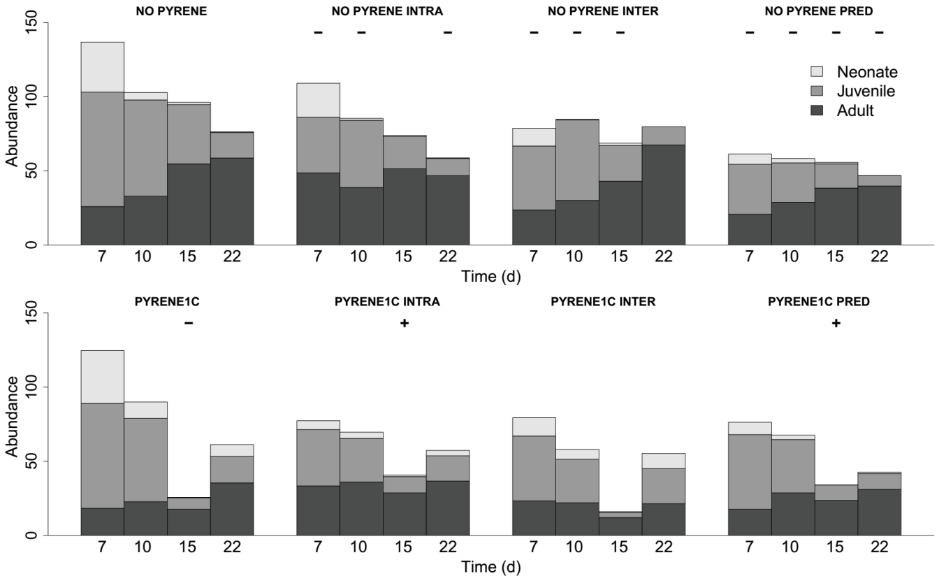


Figure 2.2. *Average population structure of D. magna just before and after the second pyrene application (day 8) for different treatments. Data shown are the average abundances of adults (dark grey), juveniles (medium grey) and neonates (light grey) of the specific treatments. "–" and "+" indicate a significant negative and positive effect, respectively, of that treatment or combination of treatments on total population density, compared to the control treatment ("no pyrene"). High pyrene high pyrene exposure; INTRA = high intraspecific competition treatment; INTER = high interspecific competition treatment; PRED= predation treatment.*

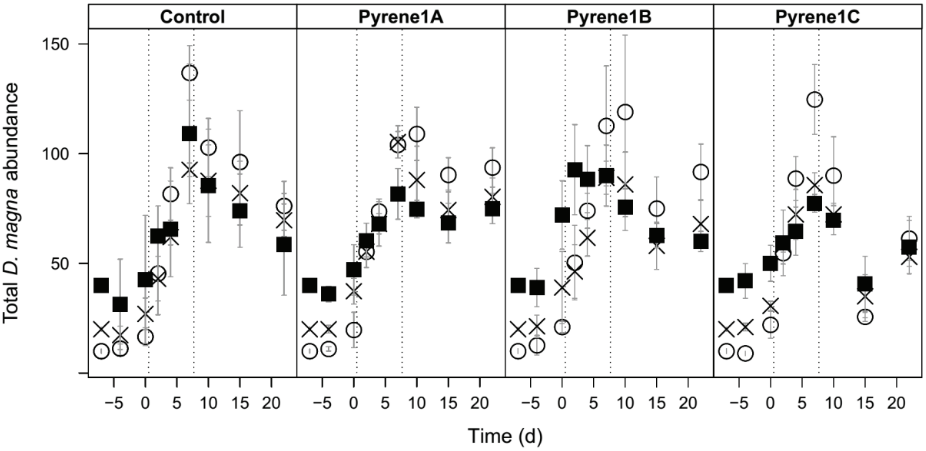


Figure 2.3. *Total D. magna abundance over time for four pyrene exposure profiles. Data shown are the D. magna population densities for the treatment with no additional species interactions (points), low intraspecific competition (crosses) and high intraspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

In experiment 2, *B. calyciflorus* individuals were added weekly to ensure more continuous competition. *B. calyciflorus* population dynamics were similar to experiment 1, with a sharp population decline after one week (Appendix A: Figure A15). The weekly addition of *B. calyciflorus* did not result in notable rotifer densities, although *D. magna* was significantly affected by pyrene from day 6 onward (Figure 2.4B and Table 2.4). Interspecific competition with rotifers had a negligible negative effect on total *D. magna* abundances only on day -8 and even resulted in higher population densities from day 9 onwards (Figure 4B and Table 2.4).

Of all species interactions studied, predation had the largest negative effect on population densities (Figure 2.2, 2.5 and Table 2.5). Predation had a continuous negative effect on total *D. magna* abundance. The explained variance was always higher than 42%, except on day 15 when most variance was explained by pyrene exposure (Table 2.5). Because *Chaoborus* sp. larvae were added 3 days after the start of the experiment, predation was not significant at day -4. A negative effect of predation was first observed for adults (at day 0) but the largest effects were observed for the abundances of neonates and juveniles (Figure 2.2 and Appendix A: Table A10-A12).

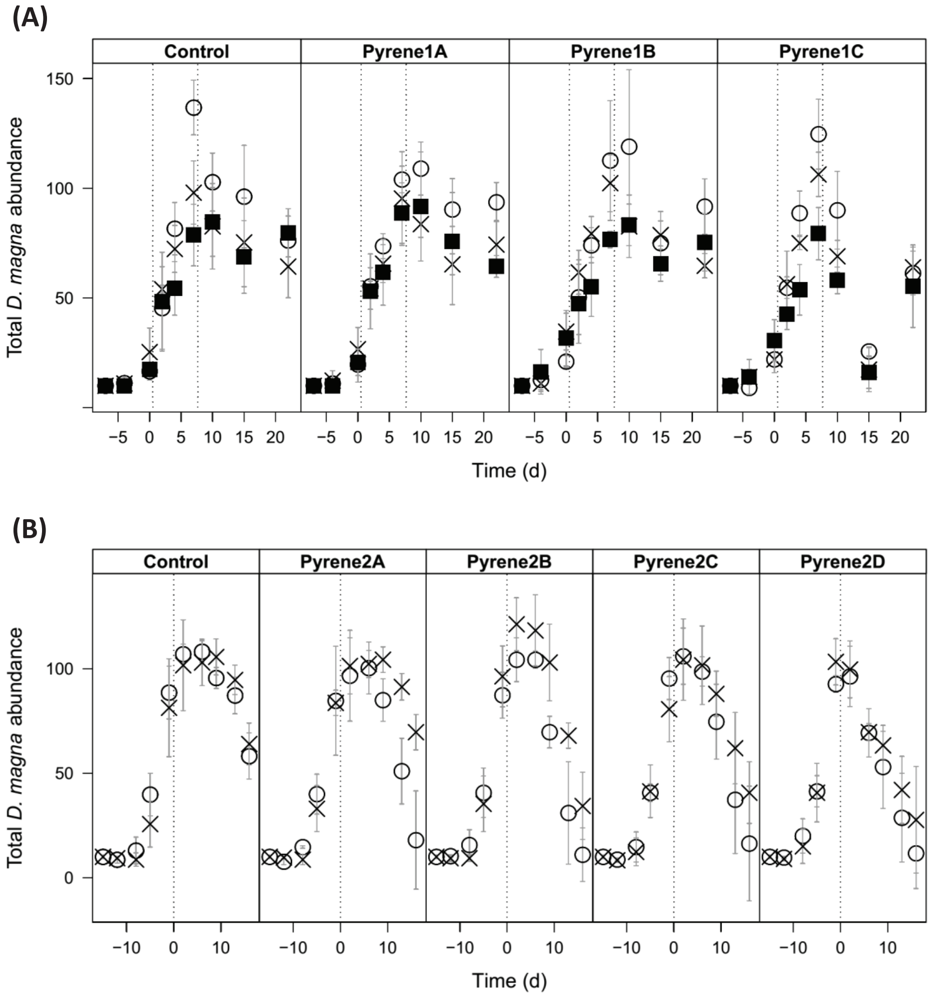


Figure 2.4. *Total D. magna abundance over time for experiment 1 (A) with four pyrene exposure profiles and for experiment 2 (B) with five pyrene exposure profiles. (A) Data shown are the D. magna population densities for the treatment with no additional species interactions (points), low interspecific competition (crosses) and high interspecific competition (black squares); (B) Data shown are the D. magna population densities without interspecific competition (points) and with continuous B. calyciflorus competition (crosses). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the pyrene applications.*

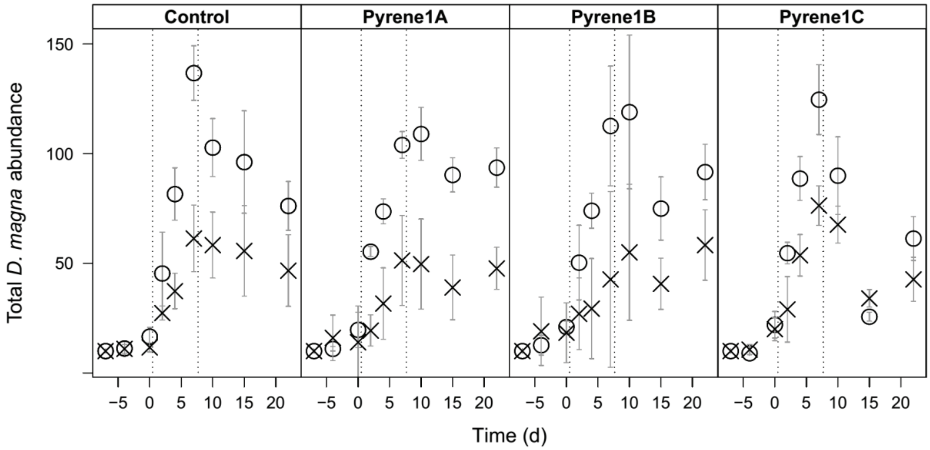


Figure 2.5. *Total D. magna abundance over time for four pyrene exposure profiles. Data shown are the D. magna population densities for the treatment without (points) and with predation (crosses). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

2.3.5. Combined effects of pyrene and species interactions

Significant interactions between pyrene and predation or between pyrene and competition were rare and most of the times changed inconsistently with increasing pyrene exposure (Tables 2.3-2.5). However, on day 15, the interaction between high pyrene exposure and predation and between high pyrene exposure and intraspecific competition positively affected the total *D. magna* abundance. These positive interactions indicated that the negative effect of high pyrene exposure was less pronounced when the population was already exposed to predation or had experienced high intraspecific competition at the start of the experiment, suggesting antagonism between each of these two types of species interaction and chemical toxicity. The variance of the total abundance explained by these two interactions on day 15 was 8.4% and 16.8%, respectively (Appendix A: Table A1 and A3).

2.4. Discussion

2.4.1. Pyrene toxicity

Short-term effects of pyrene were limited and the highest effects occurred 7 days after the second pyrene addition in experiment 1 and 6 days after the pyrene addition in experiment 2 (Table 2.3-2.5). It is unclear why the first pyrene addition in experiment 1 had no observable effects on population densities. The highest concentration measured after the first pyrene addition (71 µg/L) was similar to the $EC_{50,immobilization}$ (68 µg/L), determined in the toxicity test performed on neonate *D. magna* (<500 µm) from the same clone. However, even neonate *D. magna* – often considered the most sensitive individuals (Muyssen and Janssen 2007) - were not affected by the first pyrene addition (Appendix A: Figure A10 and Table A6). The results of the pyrene toxicity test did thus not seem applicable to experiment 1.

It was unclear if the negative effects of pyrene on the abundances of adults after the second pyrene addition resulted from direct mortality or from a combination of direct mortality and reduced survival or growth of smaller life classes. Reduced survival and growth of earlier life stages will reduce the number of juveniles that reach the adult stage (Liess and Foit 2010). The negative effect of pyrene was largest on abundances of juveniles (Appendix A: Table A4-A6). Adult *D. magna* were the only size class still affected by pyrene at the end of the experiment (Appendix A: Table 2.4). Probably, the negative effect of pyrene on the abundances of adults was thus, at least partly, attributable to effects on earlier life stages. Neonates were almost absent after the second pyrene addition, even in the control treatment (Appendix A: Figure A10), which explains the absence of significant pyrene effects for neonates. A low abundance of small individuals is not uncommon in similar experiments with Daphnia (Preuss et al. 2009a) and can be attributed to the strong competition which reduces the energy available for reproduction.

Interestingly, abundances of neonates were significantly higher on day 22 in the high pyrene exposure profile than in the control treatment (Figure 2.2 and Appendix A: Table A6). As a result, total population densities were not significantly different between the different pyrene treatments at the end of the experiment (Table 2.3), leading to the conclusion that total population density was recovered. However, it should be noted that the final *D. magna* populations in the high pyrene exposure profile, consisting mainly of neonates, were probably more susceptible to new chemical stress events than those in the other pyrene treatments. This illustrates that population structure needs to be accounted for when assessing the response and recovery of a population to (chemical) stress (Foit et al. 2012).

In the follow-up experiment, measured pyrene concentrations were similar to the measured concentrations of the first pyrene addition in the original experiment (Table 2.2). Nevertheless, this time significant effects were observed, even in the lower pyrene concentrations (Table 2.4). It is unclear whether this is the result of natural variation or of other factors such as the time of pyrene application. The follow-up experiment was performed six months after the original experiment and it is possible variations in environmental or biological conditions resulted in the observed differences in pyrene effect. However, it is likely that the time of application was also an important factor: in the first experiment, the pyrene was added when the population was growing and competition for resources between individuals was limited. In the follow-up experiment, pyrene was added after 15 days, when the population growth phase was already over. Competition for resources between *D. magna* individuals was strong at this moment, possibly making the individuals less resistant to pyrene stress.

Pyrene is a substance with low water solubility (0.132 mg L^{-1}). Acetonitrile was used here as a solvent to ensure that pyrene was dissolved in the water. Solvents are often used with pesticides to ensure a solution that can easily be applied in the field. Solvents can possibly contribute to the toxicity of a pesticide and it is therefore important to test the toxicity of the solvent itself. Acetonitrile was not toxic to *D. magna* at the concentrations used here.

2.4.2. Species interactions

Of all species interactions studied, predation had the largest effect on total *D. magna* abundance. Effects were visible six days after the addition of *Chaoborus* sp. larvae (starting from day 2) and the highest effects were observed for the abundances of juveniles and neonates (Figure 2.2 and Appendix A: Table A10-A12). This indicated a feeding preference: *Chaoborus* sp. larvae preferred to prey on smaller juvenile and neonate *D. magna* than on adult *D. magna*. Size selective feeding by *Chaoborus* sp. larvae has been observed before (Swift 1992). Surprisingly, however, a significant negative effect of predation was first observed for adults (on day 0) and not for juveniles or neonates. Abundances of juveniles and neonates were very low (juveniles) or zero (neonates) until day 0, so probably the *Chaoborus* larvae were forced to feed on the larger *D. magna* adults. At later time points, neonates and juveniles were more abundant and *Chaoborus* larvae fed on these size classes, leading to a reduced or absent effect of predation on adults. These data show that feeding preferences depend on the ecological context shaped by the prey's population structure.

It is difficult to assess the effects of interspecific competition for the full duration of experiment 1 because *B. calyciflorus* were reduced to low densities (<10%) after day 7 and completely disappeared after day 10 (Appendix A: Figure A14). The effects of interspecific competition on total population densities were therefore limited (Table 2.3). Posterior tests performed with the same conditions showed that even in the highest pyrene concentration, *B. calyciflorus* was able to survive for at least 24 days (Appendix A: Figure A16). The rotifers were thus outcompeted by *D. magna*, as previously observed in interaction experiments between *B. calyciflorus* and *D. pulex* without chemical stress (Gilbert 1985). Gilbert et al. (1985) observed limited to no effects of the competition with *B. calyciflorus* on population densities of *D. pulex*, similar to the results of the current chapter. In the follow-up experiment, *B. calyciflorus* was added weekly but, even when *D. magna* was negatively affected by pyrene, notable population densities of *B. calyciflorus* were not observed (Appendix A: Figure A15). Clearly, *D. magna* was the superior competitor, even when exposed to the pyrene concentrations applied here. Unexpectedly, weekly addition of rotifers had a positive effect on total *D. magna* population density in the second half of experiment 2 (Table 2.4). To avoid the addition of additional algae, rotifers were taken from the rotifer culture at least 24 hours after feeding. Nevertheless, it seems that algae and possibly other nutrients were still present in high enough concentrations to positively influence *D. magna* population abundances, masking any possible competition effect.

Both intraspecific and interspecific competition seemed to result in effects on reproductive output in experiment 1. Negative effects of different initial densities on the abundances of neonates and juveniles were observed starting from day 2 while these were absent for adults (Appendix A: Table A4-A6). Similarly, negative effects of interspecific competition were observed for juveniles and neonates from day 4 onward while adults were not affected (Appendix A: Table A7-A9). High initial competition thus mainly affected early life stages at later time points, suggesting competition-induced effects on *D. magna* reproduction over direct competition effects. According to the dynamic energy budget (DEB) theory, the competition with *B. calyciflorus* or other *D. magna* individuals could reduce the amount of energy that could be allocated to reproduction, resulting in less offspring (Kooijman 2010). However, it is probable that direct competition, through starvation, also contributed to the results. Young *D. magna* life stages are more prone to starvation than adults (Preuss et al. 2009a). Under high competition conditions, less food is available per capita, possibly leading to starvation of smaller individuals and contributing to the lower proportion of young life stages in the population.

2.4.3. Reduced effect of pyrene when combined with predation and competition

On day 15, when the pyrene effect was largest, predation and intraspecific competition reduced the negative effect of pyrene on population densities (Figure 2.2). Contrary to the antagonism observed in the current chapter, species interactions often lead to greater effects of chemical stress. For example, the combination of predation by *Notonecta maculata* and exposure to nonylphenol led to loss of resilience in *Daphnia magna* populations while individual stressors failed to affect population densities (Gergs et al. 2013). Synergistic effects of competition and chemical stress have been reported for *D. magna* (Foit et al. 2012) and other Daphnia species (Knillmann et al. 2012a). Next to synergistic effects, antagonistic effects have been reported as well. For example, exposure to predator kairomones led to antagonistic interactions with carbaryl exposure on reproduction of *Daphnia magna* (Coors and Meester 2008). This was attributed to larger-sized and thus more tolerant offspring when predation cues were present. Two mechanisms are proposed to explain the antagonism observed in the current chapter: differences in population structure and pyrene-induced alterations in species interactions. First, the structure of the populations exposed to predation or to high intraspecific competition differed from that of the populations experiencing low intraspecific competition and populations not exposed to predation. On day 7, immediately before the second pyrene addition, a large negative effect of intraspecific competition and predation on the abundances of juveniles and neonates was observed while the abundances of adults were less affected (Figure 2.2, Appendix A: Table A4-A6;A10-A12). Differences in sensitivity for different *D. magna* size classes have been shown before e.g. for four metals (Hoang and Klaine 2007) or carbaryl (Coors and Meester 2008). Because of the lower proportion of small-sized and thus more sensitive life stages in populations with predation or high (initial) intraspecific competition, pyrene effects were smaller. Second, the feeding rate of *Chaoborus* sp. larvae was possibly inhibited by the pyrene exposure, leading to reduced predation losses. Indeed, the estimated effect of predation (Table 2.5) was lower on day 15. The effect of pyrene on the feeding rates of *Chaoborus* sp. larvae was not tested in the current chapter but chemicals have been shown to alter feeding behaviour of fish (Weis et al. 2001) and invertebrates (Maltby and Hills 2008).

Contrary to a similar study with *D. magna* populations exposed to fenvalerate (Liess and Foit 2010), we observed no prolonged dominance of smaller-sized organisms after chemical stress in the treatment with predation: after high pyrene exposure, the proportion of small individuals was higher in the populations not exposed to predation (Figure 2.2). These contradicting results can be explained by differences in how predation was applied in the two studies. While Liess and Foit (2010) simulated predation by removing individuals non-selective on size, *Chaoborus* sp. larvae preferred to prey on smaller individuals, leading to lower abundances of neonates in the predation treatments at the end of the experiment. This highlights the complexity of assessing how ecological interactions alter the response of a population to chemical stress and the need for ecologically realistic tools (De Laender and Janssen 2013; Gabsi et al. 2014b).

The results presented in the current chapter present an example of how species interactions can lead to *a priori* unpredictable effects of chemicals. Predation and intraspecific competition were shown to interact antagonistically with pyrene when the effect of pyrene was most pronounced. The current chapter also highlights the need to not only consider the effects of a chemical on population density but also on population structure when assessing the risk of chemicals for populations and communities. Although the complexity of the interactions studied in this chapter was limited to interactions between two species (competitors or predator-prey), this can yield significant insights that are applicable to more complex food webs. Studies such as this chapter, in combination with approaches such as mechanistic ecological models (De Laender et al. 2008b; Bontje et al. 2009; Galic et al. 2010), could be used to integrate species interactions while assessing the long-term ecological risk of a chemical. Using mechanistic ecological models will increase our understanding on how and when species interactions influence the effects of a chemical. This will help identify in which situations species interactions are an important factor for the ecological risk of a chemical and thus when special attention from the regulators for species interactions is required.

3

DEVELOPMENT OF THE DEBKISS IBM

Redrafted from:

Viaene KPJ, De Laender F, Janssen CR, Baveco JM, Van den Brink PJ, Focks A. DEBkiss IBMs as a novel tool to assess the population-level effects of chemicals on competing species. Submitted

Abstract

Ecological models, in particular individual-based models (IBMs) have been suggested as a way forward for the ecological risk assessment of chemicals. Most ecotoxicological tests focus on the individual level and IBMs are ideal tools to translate these test results to the population and community level but need to be based on a sound theoretical basis. Dynamic energy budget theory based on the keep it simple, stupid principle (DEBkiss) offers a good compromise between complexity and the amount of data required to parameterize the model. In this chapter, DEBkiss theory is presented and implemented with an IBM to describe the life cycle of a generic organism. To account for the effects of chemicals, two possible toxicity models were implemented: concentration-response curves and toxicokinetic toxicodynamic models.

3.1. Introduction

In Chapter 2, I have shown that interactions with other species can be important for population-level effects in a community context. In the current chapter I will lay the foundations of a food-web model based on IBMs. To this end, I present an novel and parameter sparse approach to individual-based modelling based on the DEBkiss theory (Figure 3.1).

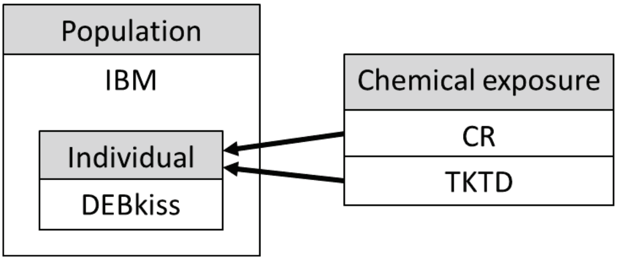


Figure 3.1: *Overview of the DEBkiss IBM implementation. DEBkiss theory is used to model individual-level processes (see 3.3). An IBM is used to model all individuals in the population and derive population properties (see 3.4). The effects of chemical exposure are modelled using either concentration-response (CR) curves or toxicokinetic-toxicodynamic (TKTD) models (see 3.5).*

3.2. Modelling species interactions under chemical stress

The complexity of species interactions makes it difficult to predict population-, community-, and ecosystem-level effects of chemicals from single-species ecotoxicological tests. Therefore, ecological models have been used to understand and predict the effects of chemicals on populations (De Laender et al. 2008b; Galic et al. 2010), communities (De Laender et al. 2014b), and ecosystems (De Laender et al. 2015). Models can help to test hypotheses regarding the relationship between effects occurring at lower and higher levels of biological organisation. When based on solid mechanistic understanding, they can be used to perform experiments *in silico*. Individual based models (IBMs) seem particularly suited for use in ERA because population parameters and dynamics emerge from the mechanisms at the individual level on which most ecotoxicological tests focus (Martin et al. 2013b). IBMs thus allow exploring how individual-level effects extrapolate to higher levels of organisation (Galic et al. 2014; Martin et al. 2014). In recent years, IBMs have been used to predict chemical effects on the population dynamics of a number of typical test species used in ecotoxicology e.g. *Daphnia magna* (Martin et al. 2013a) and *Asellus aquaticus* (Galic et al. 2012). However, these IBM applications have focused solely on the population level and not at higher levels of organisation.

IBMs need a theory describing the underlying individual-level processes. The dynamic energy budget theory (DEB) was proposed as a suitable underlying theory for the individual level energy balance in IBMs because this theory provides mechanistic understanding of patterns in growth, reproduction and mortality of individuals, and enables determination of species-specific parameters (Martin et al. 2013b). One of the main challenges of standard DEB theory is the number of parameters and the type of experiments required to accurately parameterize the model (Jager et al. 2013). For many species, these are not available and the standard DEB model can thus not be parameterized. Also, DEB has some shortcomings considering its use in a regulatory context: the equations in the standard DEB model can be difficult to grasp and implementation in software is not straightforward. Therefore, a simplified version of DEB, DEBkiss, following the paradigm of “keep it simple, stupid” and using less equations and parameters, has been proposed to allow more transparency, reduce the amount of data required and increase the user-friendliness (Jager et al. 2013). In DEBkiss, the parameters are more easy to interpret and they can be more readily related to easily measured endpoints such as the maximum length or the individual growth rate (Jager et al. 2013). Therefore, DEBkiss IBMs could be a useful tool for species for which data are sparse.

When IBMs are used to predict the effects of chemicals, the individual-level (physiological) processes that are sensitive to chemical stress need to be defined. Chemicals can interfere with physiological processes in many ways. Even in a simplified DEB model, possible physiological modes of action include effects on assimilation, maintenance and reproduction (Jager and Zimmer 2012). To determine the actual physiological mode of action of a chemical, ideally full life-cycle studies are used (Jager and Zimmer 2012). However, when data availability is limited, one is often forced to focus solely on the effects of a chemical on individual survival. This drastically simplifies the model but also eases its parameterization because data are more abundant. For example, the General Unified Threshold model for Survival (GUTS) can be parameterized based on the results of simple acute toxicity tests (Jager et al. 2011). GUTS is a generalization of a large range of existing toxicokinetic-toxicodynamic (TKTD) models i.e. models that simulate the time course of processes leading to toxic effects. These models have the advantage over the often-used concentration-effect equations that they can predict toxic effects even when exposure is not constant. This allows them to be used in more realistic conditions, when exposure to a chemical is not constant but changes over time.

3.3. DEBkiss theory

The dynamic energy budget (DEB) theory was originally developed in the ‘80s (Kooijman and Metz 1984) but only became widely applied for making sense of ecotoxicological studies during the past decade (Kooijman 2010; Jager and Zimmer 2012). In DEB theory, all processes and states of an individual are expressed as energy (or mass). Next, an energy (or mass) balance for the individual is created (Kooijman 2010). DEB models describe processes at the level of the individual because, compared to sub- and supra-individual levels of biological organization, it is relatively easy to make energy and mass balances at the individual level (Kooijman 2010). DEB models use differential equations to describe how the energy from food is used by the individual for maintenance, growth and reproduction (Nisbet et al. 2000). A general overview of the mass fluxes considered in the standard DEB model can be found in Figure 3.2.

The DEBkiss model is based on the standard DEB model but the number of parameters and processes is reduced by making additional assumptions relating to the life cycle and energy metabolism of the organism (Jager et al. 2013). The main assumption in DEBkiss is that the energy taken from food is immediately used to fuel other metabolic processes such as growth, maintenance or reproduction. A reserve compartment where the energy taken up from food is temporarily stored, is thus not considered in the DEBkiss theory. The mass fluxes (dry weight per unit of time) in the DEBkiss model are depicted in Figure 3.2. A concise description of the DEBkiss model used is provided in this chapter. For a more in depth description of the processes and assumptions in the model, I refer to (Jager et al. 2013). Abbreviations and symbols for all DEBkiss IBM parameters are provided in Table 3.1.

The typical DEBkiss life cycle consists of three stages: embryo, juvenile and adult. Three compartments are considered for each individual: the egg buffer W_B, structural body mass W_V and reproduction buffer W_R. The changes of energy in these compartments over time is described by energy fluxes and are given by

$$\frac{d}{dt}W_B = -J_A \quad \text{until} \quad W_B = 0 \tag{3.1}$$

$$\frac{d}{dt}W_V = J_V \tag{3.2}$$

$$\frac{d}{dt}W_R = J_R \tag{3.3}$$

The egg buffer W_B is only relevant for embryos and once depleted, the embryo will hatch and become a juvenile. Once an individual reaches a critical body mass, the individual is considered mature (puberty) and starts allocating energy to the reproduction buffer.

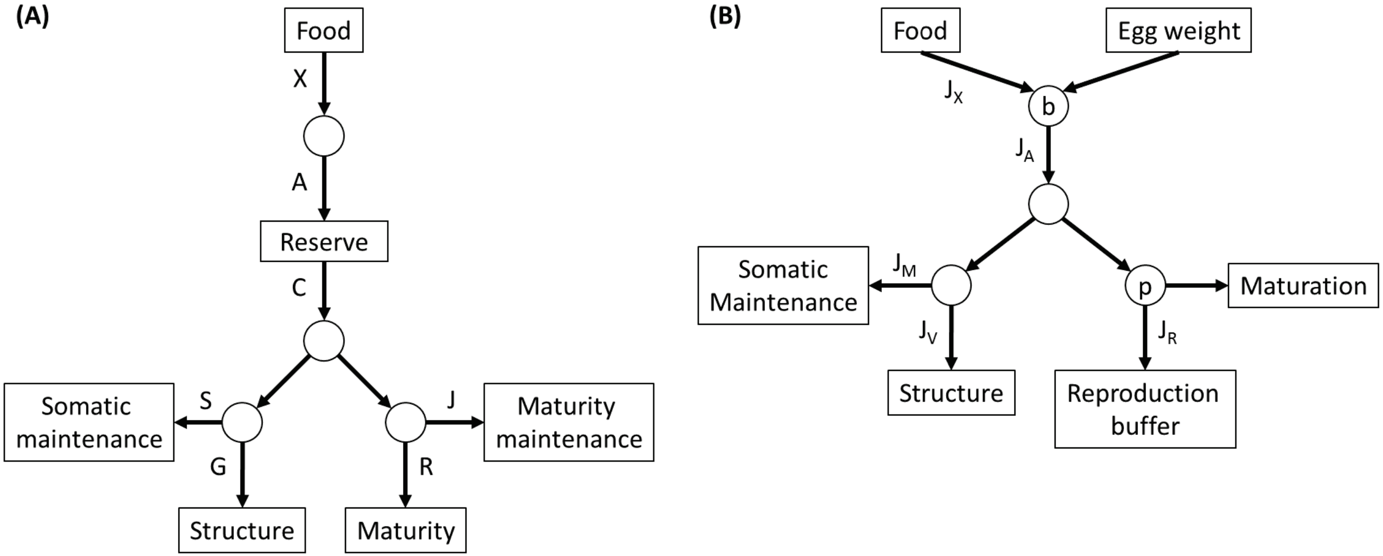


Figure 3.2: *General scheme of the energy fluxes considered in the standard DEB model (A) and in the DEBkiss model (B). (A) In the standard DEB model, energy from food is taken up (X) and assimilated in the reserve compartment (A). Energy from the reserve is mobilised (C) to fuel somatic maintenance (S), growth (G), maturity maintenance (J) and reproduction (R). (B) In the DEBkiss model, energy enters the individual via the feeding flux (JX) or from the egg buffer if birth (b) has not yet occurred. The assimilation flux JA is divided in a fraction for growth (flux JV to structure) and somatic maintenance (JM) and a fraction for maturation and reproduction (flux JR to the reproduction buffer), dependent on whether puberty (p) has occurred.*

Table 3.1: *DEBkiss IBM model parameters. Primary parameters refer to parameters that are directly linked to the physiological processes and that do not depend on other parameters. Secondary parameters are determined by one or more primary parameters.*

Symbol	Parameter	Unit
Primary parameters		
F_m^a	Maximum specific searching rate	L mm^{-2} d^{-1}
J_{Am}^a	Maximum specific assimilation rate	mg dw mm^{-2} d^{-1}
J_M^v	Volume-specific maintenance costs	mg dw mm^{-3} d^{-1}
W_{BO}	Assimilates in a single freshly laid egg	mg dw
W_{Vp}	Structural body mass at puberty	mg dw
y_{VA}	Yield of structure on assimilates (growth)	-
y_{AV}	Yield of assimilates on structure (starvation)	-
y_{BA}	Yield for conversion of reproduction buffer to eggs	-
y_{AX}	Yield of assimilates on food	-
κ	Fraction of assimilates for growth and maintenance	-
Conversions		
d_V	Dry-weight density of structure	mg mm^{-3}
δ_M	Shape correction coefficient	-
Fluxes and states		
J_A	Mass flux for assimilation	mg dw d^{-1}
J_M	Mass flux for maintenance	mg dw d^{-1}
J_R	Mass flux for reproduction buffer	mg dw d^{-1}
J_V	Mass flux for structure	mg dw d^{-1}
J_X	Mass flux of food	mg dw d^{-1}
W_B	Mass of egg	mg dw
W_R	Mass of reproduction buffer	mg dw
W_V	Mass of structural body	mg dw
X	Food biomass	mg dw L^{-1}
Secondary parameters		
f	Scaled functional response (0-1)	-
J_{Xm}^a	Maximum area-specific feeding rate	mg dw mm^{-2} d^{-1}
K	Half-saturation food density	mg dw L^{-1}
L	Volumetric body length	mm
L_m	Maximum volumetric length	mm
L_w	Physical body length	mm
r_B	Von Bertalanffy growth rate constant	d^{-1}
ΔR	Number of eggs in a clutch	number of eggs

Food biomass is taken up by the organism (feeding flux J_X) and assimilated by juveniles and adults (assimilation flux J_A) to fuel different metabolic processes. Embryos do not assimilate food but consume their egg buffer W_B until birth. A fraction κ of the assimilation flux J_A is used to support maintenance (J_M) and for somatic growth (J_V) of the structural biomass W_V. A fraction 1 - κ of the assimilation flux J_A is used by juveniles for maturation (not explicitly tracked). After puberty, the 1 - κ fraction of the assimilation flux describes the mass flux J_R towards the reproduction buffer W_R. At reproduction events, the available reproduction buffer is converted to eggs. The number of eggs is determined by the size of the reproduction buffer. Stochasticity is included in the model in two ways (Martin et al. 2013a). First, all mortality processes are probabilistic. Second, variation between individuals in DEBkiss parameter values was included by multiplying the maximum specific assimilation rate with a log-normally distributed scatter multiplier.

In DEBkiss, food biomass (X) is assumed to be instantly assimilated with a conversion efficiency of y_{AX}. This conversion efficiency can be used to reflect the quality of the food and a higher efficiency indicates food of a higher quality. The assimilation flux J_A is equal to

$$J_A = f\, J_{Am}^a\, L^2 \quad \text{with } f = \frac{X}{X+K} \text{ with } K = \frac{J_{Xm}^a}{F_m^a} \tag{3.4}$$

where *f* is the scaled functional response that adjusts the assimilation flux to the available food biomass; J_{Am}^a is the maximum specific assimilation rate (mg DW mm^{-2} d^{-1}); *L* is the volumetric body length (mm); K is the half-saturation food density (mg DW L^{-1}); J_{Xm}^a is the maximum area-specific feeding rate (mg DW mm^{-2} d^{-1}) and F_m^a the specific searching rate (L mm^{-2} d^{-1}). Embryos do not consume an external food source but their egg buffer W_B and *f* is set to 1 for this life stage. Food dynamics can be included in multiple ways e.g. by daily adding a fixed amount of food or by using a differential equation that takes into account losses due to feeding.

The change in structural body mass is the result of the amount of energy that is assimilated minus the amount of energy needed for maintenance. The flux in structural body mass is thus equal to

$$J_V = y_{VA}(\kappa J_A - J_M) \text{ where } J_M = J_M^V\, L^3 \tag{3.5}$$

where J_V is the mass flux to structure (mg DW d^{-1}); y_{VA} is the yield of structure on assimilates; κ is the fraction of assimilates for growth and maintenance; J_A is the mass flux for assimilation (mg DW d^{-1}); J_M the mass flux for maintenance (mg DW d^{-1}); J_M^V the volume-specific maintenance costs (mg DW mm^{-3} d^{-1}) and L the volumetric length (mm).

The growth of an organism can be simplified to a von Bertalanffy equation (Jager et al. 2013) equal to

$$\frac{d}{dt}L = r_B(f L_m - L) \quad \text{with} \quad r_B = \frac{y_{VA}}{3 d_V} J_M^v \quad \text{and} \quad L_m = \kappa \frac{J_{Am}^a}{J_M^v} \tag{3.6}$$

where L is the volumetric length (mm); r_B the von Bertalanffy growth constant (d^{-1}); y_{VA} is the yield of structure on assimilates; d_V the dry-weight density of structure; J_M^V the volume-specific maintenance costs (mg DW mm^{-3} d^{-1}); L_m the maximum volumetric length an individual can obtain (mm); κ is the fraction of assimilates for growth and maintenance; $J_{Am}^a$is the maximum specific assimilation rate (mg DW mm^{-2} d^{-1}) and J_M^V the volume-specific maintenance costs (mg DW mm^{-3} d^{-1}).

The (1 – κ) fraction of the assimilation flux J_A is used for maturation when the individual is juvenile ($W < W_{Vp}$) or for the reproduction buffer when the individual is mature. The dynamics of the mass in the reproduction buffer J_R (mg DW d^{-1}) are described by

$$J_R = (1-\kappa) J_A \tag{3.7}$$

where κ is the fraction of assimilates for growth and maintenance and J_A the mass flux for assimilation.

At reproduction events, the available reproduction buffer is transformed into a number of eggs (ΔR) equal to

$$\Delta R = floor(\frac{y_{BA}W_R}{W_{BO}}) \tag{3.8}$$

where y_{BA} is the yield for conversion of reproduction buffer to eggs; *floor* indicates that the lowest whole number made possible by the division is selected; W_R the mass of the reproduction buffer (mg DW) and W_{B0} the egg mass (mg DW).

A special set of rules was used in the case of starvation (Jager et al. 2013). Initially, the energy shortage is taken from the reproduction buffer (if any). In a second phase of starvation, the energy needed is translated from the structural biomass and the organism shrinks. If an organism shrinks to a critical fraction of its maximal attained weight, it has a high chance (0.35 d-^{1}) of dying.

first stage: if $\kappa J_A < J_M$ but $J_A > J_M$ or $W_R > 0$

$$J_V = 0 \tag{3.9}$$

$$J_R = J_A - J_M \tag{3.10}$$

second stage: if $\kappa J_A < J_M$ and $W_R = 0$ and $J_A \leq J_M$

$$J_V = \frac{J_A - J_M}{y_{AV}} \tag{3.11}$$

$$J_R = 0 \tag{3.12}$$

where κ is the fraction of assimilates for growth and maintenance; J_A the mass flux for assimilation (mg DW mm^{-2} d^{-1}); J_M the mass flux for maintenance (mg DW mm^{-2} d^{-1}); W_R the mass of the reproduction buffer (mg DW); J_V the mass flux for structure (mg DW mm^{-2} d^{-1}) and y_{VA} the yield of assimilates on structure (starvation).

3.4. The DEBkiss individual based model

A general feature of an IBM is that at every time step, all individuals obey a series of sequential rules (Figure 3.3). A specific feature of the DEBkiss IBM developed here is that these rules are based on DEBkiss theory (see 3.2). First, the individual takes up energy from food. Next, mortality of the individual is evaluated: does the individual die from stochastic processes, starvation, toxicity or aging? If the individual survives, the growth of the individual is calculated. If the individual is not yet adult, no more rules are considered this time step and the individual continues to the next time step. If the individual is adult, the next rule is to evaluate whether reproduction will occur. If not, the reproduction buffer is updated and the individual continues to the next time step. If reproduction occurs, the number of offspring is determined and afterwards the reproduction buffer is updated. New-born individuals undergo the same rules in the next time step.

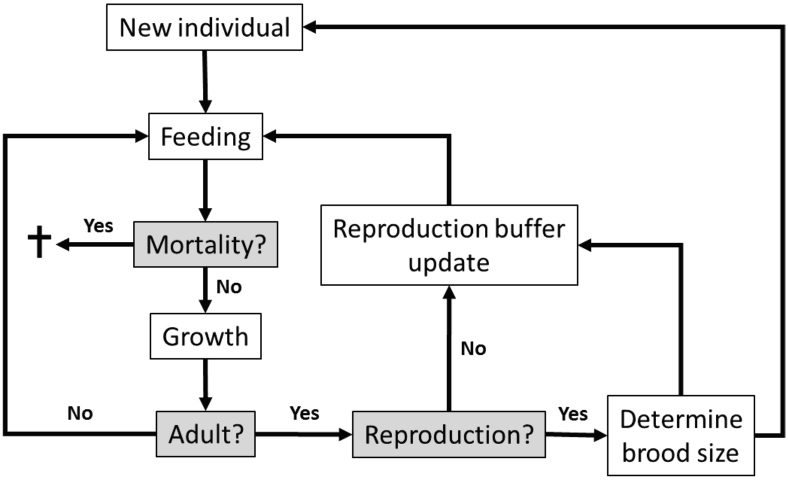


Figure 3.3: *Rules that an individual in the IBM goes through during a time step. Rules that change their outcome depending on the state of the individual are marked in grey.*

3.5. Toxicity implementation

Toxicity can be included in multiple ways in DEB model simulations. Often, toxic effects in DEB models are described by impairment of one, or a combination of, different physiological modes of action, where effects on survival, assimilation, growth, maintenance and reproduction are the most common (Kooijman 2010; Jager and Zimmer 2012). In this modelling framework, chemicals can thus have a wide range of effects on organisms and their physiology, and it can be very difficult to discern which modes of action are relevant for a specific chemical. Moreover, it is unclear how sub-lethal effects translate to the population level. Consistent with the philosophy of DEBkiss, a simplified approach was implemented where only effects on survival were considered. Two approaches to predict the effects of a chemical on survival were evaluated: concentration-response (CR) relationships and toxicokinetic-toxicodynamic (TKTD) models.

3.5.1. Concentration-response relationships

CR relationships give a straightforward relationship between the concentration of the chemical in the water and the survival probability of an individual, based on standard toxicity test results. The survival probability for a given time period (e.g. one day) can be predicted from the water concentration by

$$p(survival) = \frac{1}{1+\left(\frac{C}{LC_{50}}\right)^{\alpha}} \tag{3.13}$$

which only includes the concentration of the chemical in the water (C) and two chemical-specific parameters: the slope parameter α and the LC_{50} of the chemical.

3.5.2. Toxicokinetic-toxicodynamic models

The TKTD model used is based on the general unified threshold model for survival (GUTS) which offers a general framework for the wide variety of TKTD models available (Jager et al. 2011). In short, toxicokinetic-toxicodynamic models predict the internal concentration over time from a given external concentration (toxicokinetics) and link this internal concentration to a toxic effect at the level of the individual over time (toxicodynamics) (Jager et al. 2011). The implemented TKTD model is based on the reduced GUTS-SD model, assuming stochastic death and using a scaled internal concentration (Figure 3.4). The GUTS-IT model, assuming the individual tolerance concept, was also implemented but was sub-optimal for the species and chemical used here when applied to standard toxicity experiments.

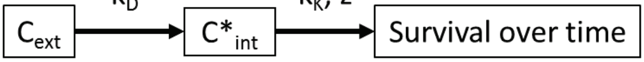


Figure 3.4: *The implemented TKTD model. The dominant rate constant (kD1) describes the rate at which a chemical is taken up by the individual (C*int) from the environment (Cext). The killing rate constant (kk) and the internal threshold for effects (z) are used to link the scaled internal concentration (C*int) to a survival probability over time.*

The TKTD model is described by three parameters: the dominant rate constant k_D, the killing rate constant k_K and the internal threshold for effects z. To calculate the internal concentration of a chemical (toxicokinetics), a first-order one-compartment model can often be used:

$$\frac{dC_{int}(t)}{dt} = k_I C_{ext}(t) - k_E C_{int}(t) \tag{3.14}$$

where C_{int} is the internal concentration (µg kg^{-1}); t is the time (d), k_I is the uptake rate constant (L kg^{-1} d^{-1}); C_{ext} the external concentration (µg $^{-1}$) and k_E the elimination rate constant (d^{-1}). However, in ecotoxicological studies, the internal concentration is mostly not measured. The GUTS model can still be parameterized then by rescaling equation (3.14) and dividing both sides by k_I/k_E. This way, the rescaled internal concentration C^*_{int} is directly proportional to the actual internal concentration and has the dimensions of the external concentration:

$$\frac{dC^*_{int}(t)}{dt} = k_D (C_{ext}(t) - C^*_{int}(t)) \tag{3.15}$$

where C^*_{int} is the rescaled internal concentration (µg L^{-1}); t is the time (d); k_D is the dominant rate constant and C_{ext} the external concentration (µg $^{-1}$). The elimination rate constant can be estimated from the time-course of survival data even if internal concentrations are unknown. However, it is not guaranteed that this estimated elimination rate constant represents only whole-body elimination. When the elimination rate is estimated from only external concentration and survival data over time, the slowest compensatory process, which can be either toxicokinetic (i.e. elimination from the body) or toxicodynamic (i.e. recovery from damage), will dominate the dynamics of toxicity. Therefore, we refer to the dominant rate constant k_D instead of the elimination rate constant k_E for the rescaled internal concentration (Equation 3.15). To translate this rescaled internal concentration to effects on survival over time, the cumulative hazard was used:

$$S(t) = e^{-H_Z(t)} \text{ with } H_Z(t) = k_K\, max(0, C^*_{int}(t) - z) + h_B(t) \tag{3.16}$$

where S(t) is the survival probability of an individual at time t; t is the time (d); H_Z the cumulative hazard of an individual at time t; k_K the killing rate (L µg^{-1} d^{-1}); max selects the maximum of 0 and $C^*_{int}(t) - z$; C^*_{int} is the rescaled internal concentration (µg L^{-1}); z the internal threshold for effects (µg L^{-1}) and h_B the background mortality rate (d^{-1}).

Both toxicity approaches provide the probability, based on the chemical exposure, that an individual will survive one more day. This survival probability is then evaluated for each individual in a population to determine if it survives until the next day. The main difference between the two toxicity sub-models is that CR relationships only consider the current chemical concentration and not earlier exposure while chemical effects can still occur after exposure when using TKTD-SD.

3.6. Software

The DEBkiss IBM framework was implemented in NetLogo (Wilensky 1999), which was specifically designed for IBMs and has been used before to implement DEB IBMs (Martin et al. 2012).

4

APPLICATION OF THE DEBKISS IBM FRAMEWORK TO ASSESS THE EFFECTS OF COMPETITION AND CHEMICAL STRESS ON THE POPULATION DYNAMICS OF *BRACHIONUS CALYCIFLORUS* AND *DAPHNIA MAGNA*

Redrafted from:

Viaene KPJ, De Laender F, Janssen CR, Baveco JM, Van den Brink PJ, Focks A. DEBkiss IBMs as a novel tool to assess the population-level effects of chemicals on competing species. In preparation.

Abstract

To increase the ecological relevance of chemical risk assessment, the potential influence of species interactions such as competition need to be accounted for. Ecological models have been identified as a possible approach to consider species interactions, however, high data requirements often impair the use of modelling approaches. Individual-based models based on a simplified dynamic energy budget theory (DEBkiss IBMs) provide transparent and readily applicable models. In the current chapter, I tested the capacity of an integrated model composed of two DEBkiss IBMs to predict the effects of pyrene on two competing consumers, the cladoceran Daphnia magna and the rotifer Brachionus calyciflorus. Both IBMs were calibrated using only data available in the literature and their performance was evaluated using data from competition experiments. To predict the effects of pyrene, two possible toxicity model implementations – concentration-response curves and toxicokinetic-toxicodynamic models – in the IBMs were compared. Population dynamics of both species in isolation were reasonably well predicted, although the population structure for D. magna was less well predicted. Agreeing with the results of the experiments, D. magna outcompeted B. calyciflorus in the integrated model with both species. The toxicokinetic-toxicodynamic model captured short-term effects of pyrene better than the concentration-response model but overpredicted the chronic effects. Simulated rotifer population dynamics exposed to both pyrene and competition were higher than those observed because of the overprediction of pyrene effects on daphnid populations. Despite its limitations, the tested DEBkiss IBM approach can be a useful tool for assessing the risk to species with short life cycles in case of low data availability.

4.1. Introduction

Ecological models and especially individual-based models (IBMs) have received increasing attention in ecotoxicology during the past decade. IBMs have been developed for several species such as *Asellus aquaticus* (Galic et al. 2012), *Daphnia magna* (Preuss et al. 2009a) and *Chaoborus* (Dohmen et al. 2015). However, these models are still met with much scepticism by industry and regulators that are unfamiliar with using models for effect assessment. In order to improve their use and acceptance, models need to be validated (Grimm and Martin 2013; Grimm and Thorbek 2014). One common way to validate models is to compare model output with patterns in available data. Patterns in this context are defined as characteristics of the observations that are unlikely to be the result of random processes (Grimm and Martin 2013). For complex systems, it is critical to evaluate multiple patterns because single patterns can often be reproduced in multiple ways and it is then unknown whether the underlying mechanisms are correctly captured. Key characteristics of complex ecological systems are interactions between individuals of different species. Ecological models in general and IBMs more specifically that can accurately predict how these interactions affect the population dynamics of the species involved, especially in the context of chemical stress, could become essential tools in future ecological risk assessment approaches.

The main objective of this chapter was therefore to test whether the DEBkiss IBM framework described in Chapter 3 is suitable to simulate and understand patterns observed for two interacting populations under chemical stress. To reach this objective, DEBkiss IBMs were implemented and parameterized for two grazers (the water flea *Daphnia magna* and the rotifer *Brachionus calyciflorus*) and a framework was developed to allow multiple DEBkiss IBMs to compete for a shared resource (the algae *Scenedesmus obliquus*). Simulations were performed for the control treatment, for exposure to pyrene, for interspecific competition, and for a combination of pyrene exposure and interspecific competition. To evaluate the performance of the model for predicting the combination of ecological (competition) and chemical stress, simulation results were compared to the patterns observed in the experiments described in Chapter 2.

4.2. Materials and Methods

4.2.1. Model parameterization

Available literature data were used to determine a range of possible DEBkiss IBM parameter values (Table 4.1). The available literature for *Daphnia magna* and *Brachionus calyciflorus* was queried for DEB-related parameters such as maximum body lengths, growth rates, ingestion rates, egg weights and length-weight relationships. Parameter values from other DEB models were avoided because these have been fitted for more complex DEB models which differ from DEBkiss (e.g. by including a reserve compartment) and were therefore incomparable to the DEBkiss parameters. If not found, DEBkiss parameters were calculated from the available information. For example, if the von Bertalanffy growth constant (r_B) was known and standard values of 0.1 mg DW mm^{-3} and 0.8 were used for the dry-weight density of structure (d_V) and the yield of structure on assimilates (y_{VA}), respectively, the volume-specific maintenance costs were calculated using equation (3.6). This approach assumed that (1) the available literature data were applicable to the individuals used in the current chapter, which, given the large variation within a species, is not always certain and (2) the assumptions made in the original DEBkiss paper also applied here. Both the concentration-response curves and the TKTD model were parameterized using a standard 48-hour toxicity test performed with *D. magna* (See Chapter 2).

4.2.2. Species-specific adjustments to the DEBkiss-IBM framework

The DEBkiss IBM framework offers a very general description of an organism's life cycle. This is very useful when specifics about the life cycle and physiology are not available. In the current chapter, two species are considered that have been widely used and studied in ecotoxicology. Species-specific details are thus known and species specific traits can be accounted for in the DEBkiss IBM.

Daphnia magna

Because of their carapace, *D. magna* individuals cannot physically shrink when starving (Martin et al. 2013a). So, although their weight decreases, their length stays constant. This has implications for the assimilation of food because the amount of food daphnids obtain from the water depends on the length of the appendages. Therefore, instead of using the actual length of an individual, the maximum obtained length was used to calculate the assimilation flux (Equation 3-4).

Under starvation, daphnids can convert body mass to energy until a critical weight is attained and mortality occurs (Rinke and Vijverberg 2005). To simulate this in the current chapter, individuals that shrink to 40% of their previously attained maximum weight have a high probability of dying (per capita death rate of 0.35 d^{-1} (Martin et al. 2013a)). Similar starvation rules were found insufficient in an earlier DEB IBM implementation for *D. magna* (Martin et al. 2013a). Indeed, preliminary simulations with these standard starvation rules showed shortcomings similar to those reported in Martin et al. 2013a study: densities of individuals in the medium size class were overpredicted and no increase of individuals in the largest size class was predicted when food becomes limiting.

Table 4.1: *Parameter value ranges for D. magna and B. calyciflorus. a Calculated using equations (3.4) and (3.6). b Suggested values*

Symbol	Parameter	***Daphnia magna***	***Brachionus calyciflorus***
W_{BO}	Assimilates in a single freshly laid egg	[7-10] * 10^{-3} mg dw (Dumont et al. 1975; Tillmann and Lampert 1984; Glazier 1992)	[0.02 – 0.12] * 10^{-3} mg dw (Dumont et al. 1975; Saunders III and Lewis Jr 1988)
W_{Vp}	Structural body mass at puberty	[0.027 – 0.0315] mg dw [a]	[0.15 – 0.21] * 10^{-3} mg dw [a]
J^{a}_{Am}	Maximum specific assimilation rate	[0.06 – 0.121] mg dw mm^{-2} d^{-1} [a]	[0.088 – 0.195] mg dw mm^{-2} d^{-1} [a]
F^{a}_{m}	Specific searching rate	[0.0265 – 0.6526] L mm^{-2} d^{-1} [a]	[0.337 – 0.747] L mm^{-2} d^{-1} [a]
J^{v}_{M}	Volume-specific maintenance costs	[0.04125 – 0.051] mg dw mm^{-3} d^{-1} [a]	0.7647 mg dw mm^{-3} d^{-1} [a]
r_B	Von Bertalanffy growth rate constant	0.137 d^{-1} (Pieters et al. 2006; Jager and Zimmer 2012)	2.0393 d^{-1} [a]
L_m	Maximum volumetric length	1.9 mm [a]	[0.092 – 0.204] mm [a]
K	Half-saturation food density	[0.122 – 2.238] mg dw L^{-1} (Mulder and Hendriks 2014)	0.3263914 dw L^{-1} (Hansen and Bjornsen 1997)
y_{VA}	Yield of structure on assimilates (growth)	0.8 [b]	[0.6-0.95] [b]
y_{AV}	Yield of assimilates on structure (starvation)	0.8 [b]	0.8 [b]
y_{BA}	Yield for conversion of reproduction buffer to eggs	0.95 [b]	0.95 [b]
y_{AX}	Yield of assimilates on food	0.8 [b]	[0.6-0.95] [b]
κ	Fraction of assimilates for growth and maintenance	[0.5 – 0.9] [b]	[0.4 – 0.9] [b]
d_V	Dry-weight density of structure	0.1 mg dw mm^{-3} [a]	0.1 mg dw mm^{-3} [a]
δ_M	Shape correction coefficient	0.38 [a]	0.5097 [a]
-	Time between reproduction events	2.8 d [a]	0.5 d (Halbach 1970b)
-	Body mass fraction at which starvation occurs	0.4 [a]	*0.8* [a]
-	Stochastic mortality rate	0.02 d^{-1} [a]	*0.01* d^{-1} [a]
-	Maximum age of an individual	96 d (MacArthur and Baillie 1929)	10 d *(Halbach 1970b)*

Therefore, I evaluated if model fit could be improved by additional starvation rules. In Martin *et al.* (Martin et al. 2013a), this problem was mitigated by including an additional mortality term based on the amount of energy in the reserve compartment. In DEBkiss, a reserve compartment is missing (Jager et al. 2013) and therefore a mortality rate based on the reserve density cannot be included. However, this additional mortality can be viewed as a time-weighted average of the feeding history (Martin et al. 2013a), summarized in the functional response f in DEBkiss. Therefore, a time-weighted average of the functional response values of the last five days is calculated (fH) and combined with a mortality coefficient m to provide a starvation-dependent additional mortality probability:

$$\text{Probability(mortality)} = m\,(1 - fH) \qquad (4.1),$$

Earlier work has shown that only considering this starvation mortality for juveniles led to the best simulations (Martin et al. 2013a). In the current implementation, starvation mortality was therefore only implemented for juveniles and values between 0 and 0.2 were evaluated for the mortality coefficient m.

Brachionus calyciflorus

B. calyciflorus is known to have a post-reproductive period of 1-2 days (at 20°C) where individuals still feed but don't reproduce (Jensen and Verschoor 2004). Given that the typical life duration of *B. calyciflorus* is 10-11 days at 20°C (Jensen and Verschoor 2004), this is quite significant. This post-reproductive period was included by limiting the reproduction to individuals younger than 9 days. Also, a maximum age of 11 days was included for rotifers. Post-reproduction individuals were not eliminated from the simulated populations because they still feed and thus influence other *B. calyciflorus* and *D. magna* individuals.

4.2.3. Coupling

Species that are in competition need to be able to interact with one another. Instead of implementing both species in one NetLogo instance, we opted to write a central interface in Java that calls separate NetLogo instances. This allows the whole modelling framework to be more flexible, making it easier to e.g. adjust the food input, use species-specific DEBkiss IBMs and extending the framework to more than two species. This implementation also made it easier to the expand the food web further, as described in chapter 5-6.

4.2.4. Comparison with data from experiments

DEBkiss IBM simulations were compared to the experimental results described in Chapter 2. In these experiments, isolated populations of *B. calyciflorus* and *D. magna* and the two species in competition were exposed to a pyrene pulse. Using the population dynamics of the control populations (without competition and pyrene stress), parameter values were optimized. For *D. magna*, control population dynamics were available from two experiments (see Chapter 2) and both were used for parameter optimization. To account for variation in the parameters, 100 simulations were performed with random sampling from the range of possible DEBkiss parameter values given in Table 4.1. 100 simulations were chosen because this was deemed a good compromise between computation time and accounting for the variation on the DEBkiss parameters. Optimization was done by likelihood optimization (Jager and Zimmer 2012) with the likelihood *l* of the parameter set *θ* given the data *Y* calculated as

$$l(\theta|\mathrm{Y})=-\frac{\mathrm{N}}{2}ln\,SSQ(\theta;\mathrm{Y}) \tag{4.2}$$

where N is the total number of data points and SSQ the sum of squared residuals. The SSQ is given by

$$SSQ(\theta;Y)=\sum_{i=1}^{k}(\hat{Y}_i(\theta)-Y_i)^2 \tag{4.3}$$

where *i* is the time point of the observation (from *i* to *k*), $\hat{Y}_i$ the estimated value for observation *i* using parameter set *θ* and Y_i the observed value for observation *i*.

Population dynamics of the control treatments were used to select the 10 best (out of 100) parameter combinations for each species based on the calculated likelihoods (Table 4.2). Because the size structure of the population was important for the response to stress (Chapter 2), likelihoods for *D. magna* were calculated for both the total density and the densities of the size classes. The likelihoods of the total density and of the three size classes were then multiplied to derive one value to be optimized. By doing so, the parameter combinations were selected that best described both the total population density and the population structure. Initial exploration of the likelihoods showed that the maximum area-specific assimilation rate (J^a_{Am}) was the dominant factor determining the likelihood, with the lowest likelihood for high values (Appendix B Figure B1). Therefore, the range for $J^a_{Am}$was limited in the simulations to values between 0.06 mg mm^{-2} d^{-1} and 0.121 mg mm^{-2} d^{-1}. Extinction of *B. calyciflorus* in the control treatment was observed by the end of the experiment (31 days), most likely due to abiotic causes e.g. decreased water quality. Therefore, only the population densities of the control treatment of the first 22 days were used to fit the model.

Table 4.2: *Steps, parameters and experiments used for the simulations in this Chapter.*

Step	Parameters used	Related Chapter 2 experiment
1	100 random combinations of literature parameter values (Table 4.1)	Experiment 1: Control Experiment 2: Control
2	10 optimal parameter combinations from step 1	Experiment 2: Interspecific competition
3	10 optimal parameter combinations from step 1	Experiment 2: Pyrene
4	10 optimal parameter combinations from step 1	Experiment 2: Interspecific competition + Pyrene

The 10 optimal parameter combinations for both species were then used to predict the dynamics for exposure to competition, exposure to pyrene and exposure to both competition and pyrene (Table 4.2). When simulating two competing species, each of the 10 optimal parameter combination of *D. magna* were combined with each of the 10 optimal parameter combination of *B. calyciflorus*, resulting in a total of 100 simulations. To evaluate the effects of pyrene, both the concentration-response (CR) and the toxicokinetic-toxicodynamic (TKTD) models were tested. Simulations were only compared to the data of experiment 2 because it was unclear how exposure to two pyrene pulses, as was the case in experiment 1, affected the daphnids and if the toxicity models could capture this. In experiment 2, only one pyrene addition was performed and the applicability of both toxicity models should be more clear. 100 simulations were run for the pyrene exposure to account for stochasticity. Simulations for exposure to both competition and pyrene were also simulated 100 times. For all these treatments, the calculated likelihoods were used again to select the 10 simulations (out of 100) closest to the observed densities.

4.3. Results

4.3.1. *Daphnia magna*

Figures 4.1-4.3 in the current chapter show the optimal fit of the DEBkiss IBMs i.e. the 10 best simulations of the 100 simulations performed per treatment, based on the likelihood. Simulations of isolated *D. magna* populations without chemical stress showed a population growth phase followed by a decline as the carrying capacity of the system was reached (Figure 4.1). Despite differences in the observed maximum population densities of the two experiments used for parameter optimization (150 individuals versus 110 individuals), the population trends in both experiments were similar (Figure 4.1). The maximum density of the first experiment was predicted by some of the 100 performed simulations but since the optimization was performed for all counted abundances, the best simulations shown in Figure 4.1 are more in line with the maximum density counted in the second experiment. The maximum population density in the simulations was lower and occurred earlier than in the experimental observations. In the second half of the experiments, the simulated population density was therefore lower than the observed density. The population densities at the end of the experiment were more comparable between simulations and experiments (Figure 4.1).

The largest discrepancy between the control treatment of experiments and simulations was observed for the abundance of large individuals at the end of the experiment (Appendix B Figure B2). While a steady increase of large individuals was observed until the end of the experiment, a constant, low abundance of large individuals was predicted. Related to this, the abundance of intermediate individuals strongly decreased in the experiments while this was not observed in the simulations. A similar trend was observed in an earlier study and was alleviated by including additional starvation mortality related to the reserve density of an individual (Martin et al. 2013a). Because DEBkiss IBMs lack a reserve compartment, additional starvation mortality based on the history of the functional response parameter *f* was used as an alternative. Adding this functional response based starvation mortality did, however, not decrease the deviation for large individuals nor increase the fit for total abundances (Appendix B Figure B2-B3). Therefore, this additional starvation mortality mechanism was not used in other simulations shown in the current chapter (Figure 4.2-4.3). Similar to the experiments, the abundance of small individuals, indicating reproduction, was highest in the first half of the simulation period. However, while reproduction was not observed after day 10 in the experiments, low abundances (<20 individuals) of small individuals were still observed in the simulations.

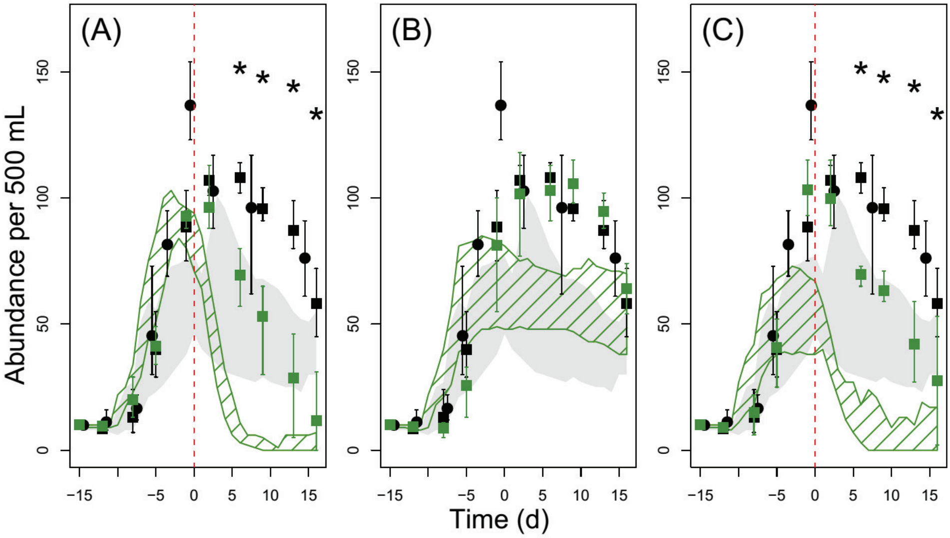


Figure 4.1: *Observed and predicted population dynamics for D. magna using a DEBkiss IBM. D. magna populations were exposed to (A) pyrene; (B) the competitor B. calyciflorus and (C) the competitor B. calyciflorus and pyrene. The grey area indicates the 10 best simulations out of 100 for the control treatment (no pyrene exposure or competition). Green shaded areas indicate the 10 best simulations out of 100 for populations under stress. Black bullets and squares show the observed dynamics for the control treatment in the two experiments. Green squares show the observed dynamics for populations under stress (competition and/or pyrene exposure). The red dashed line indicate the time of pyrene addition. Asterisks indicate significant differences ($p < 0.05$) between control and treatment in the experiment.*

Two toxicity models were evaluated, concentration-response curves (CR) and toxicokinetic-toxicodynamic models with stochastic death (TKTD-SD; Figure 4.2). Effects of pyrene were only considered for *D. magna* as no effects were observed for *B. calyciflorus*, neither in the acute toxicity test (Chapter 2) nor in the population experiment (Figure 4.3). Using CR curves, pyrene effects occurred immediately while a delay of a week was observed in the experiments (Figure 4.1). Also, *D. magna* populations recovered after two weeks in the simulations while this was not observed in the experiment. Using TKTD-SD, effects were more delayed (1-2 days) but still too fast. Also, the effects of pyrene were larger, potentially even leading to extinction of the daphnid population, and persisted longer than with the CR toxicity model (Figure 4.2). Both considered toxicity models were thus unable to fully capture the effects of pyrene on *D. magna* population dynamics. Although the predicted effects of pyrene were too high, the TKTD-SD model was preferred over the CR model because the slight delay in pyrene effects and the absence of population recovery matched the observations most closely.

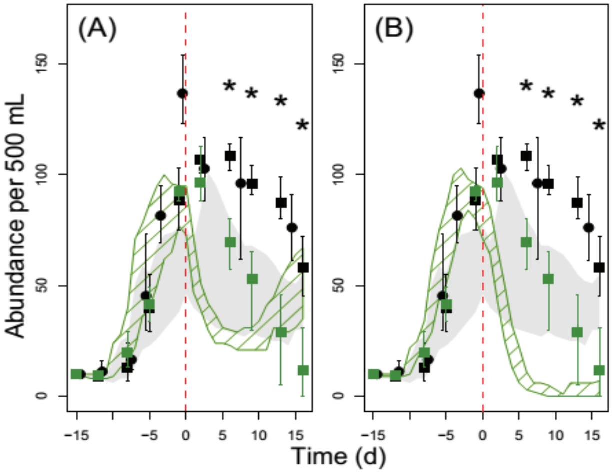


Figure 4.2: *Comparison of the DEBkiss simulations for D. magna with concentration-response curve (A) or TKTD-SD (B) as toxic effect sub-model. The grey area indicates the 10 best simulations out of 100 for the control treatment (no pyrene exposure or competition). Green shaded areas indicate the 10 best simulations out of 100 for populations under stress. Black bullets and squares show the observed dynamics for the control treatment in the two experiments. Green squares show the observed dynamics for populations under stress (competition and/or pyrene exposure). The red dashed line indicates the time of pyrene addition. Asterisks indicate significant differences (p < 0.05) between control and treatment in the experiment.*

4.3.2. Brachionus calyciflorus

Simulations for the isolated *B. calyciflorus* populations reflected the pattern of population increase and decrease observed in the experiment (Figure 4.3). For *B. calyciflorus*, the simulated maximum total population density was comparable to that observed in the experiments. Because pyrene effects were not predicted for individuals at the measured pyrene concentrations, exposure to pyrene did not alter the *B. calyciflorus* population dynamics.

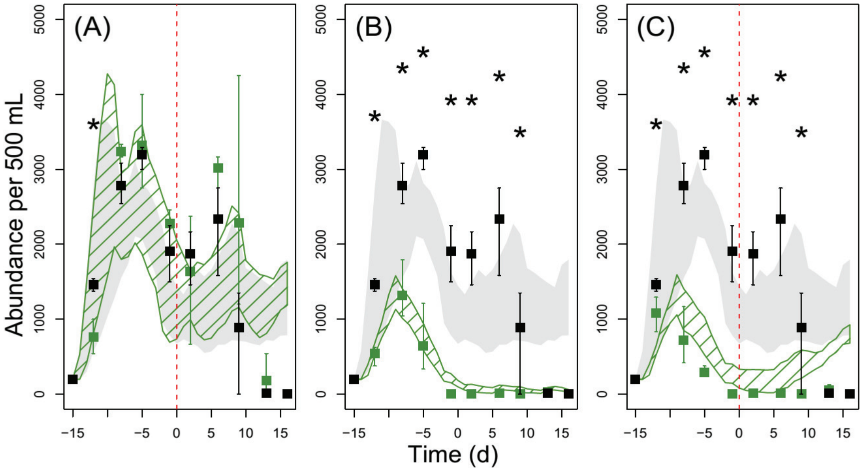


Figure 4.3: *Simulated population dynamics of B. calyciflorus using a DEBkiss IBM. B. calyciflorus populations were exposed to (A) pyrene; (B) competition with D. magna and (C) both competition with D. magna and pyrene exposure. The grey area indicates the 10 best simulations out of 100 for the control treatment (no pyrene exposure or competition). Green shaded areas indicate the 10 best simulations out of 100 for populations under stress. Black squares show the observed dynamics for the control treatment in the two experiments. Green squares show the observed dynamics for populations under stress (competition and/or pyrene exposure). The red dashed lines indicates the time of pyrene addition. Asterisks indicate significant differences ($p < 0.05$) between control and treatment in the experiment.*

4.3.3. Competition

As observed in the experiments, daphnids were also the superior competitors in the simulations (Figure 4.1 and Figure 4.3). Simulated daphnid population dynamics were largely unaffected by competition with rotifers, which corresponds to the experimental results. The only effects of competition on daphnid populations were a 23% lower maximum population density and an earlier cessation of population growth (five days earlier) in the competition treatment simulations than in the simulations of the control treatment. However, differences between isolated populations and populations in competition with rotifers were not observed in the experiment. Simulated population dynamics of *B. calyciflorus* competing with *D. magna* corresponded well with experimental results (Figure 4.3): a population increase when food was not yet limiting – but lower than for isolated *B. calyciflorus* populations – and a very low population abundance or extinction when food was scarce, although the population persisted longer than observed.

4.3.4. Competition and chemical stress

When *D. magna* was exposed to both pyrene and competition, the effect of pyrene on *D. magna* was unaltered: daphnid populations decreased, potentially leading to extinction (Figure 4.1). This was similar to that observed in the experiments where the effect of pyrene showed no interaction with the effect of interspecific competition (see Chapter 2). Contrary to the experiments, rotifers increased in abundance after pyrene exposure (Figure 4.3).

4.4. Discussion

4.4.1. Predictive capability of DEBkiss IBM for *Daphnia magna* and *Brachionus calyciflorus*

In general, the effects of food shortage on *D. magna* populations appeared sooner in the DEBkiss IBM than in reality. Several deviations between the experiments and the simulations could be ascribed to the absence of a reserve compartment in the *D. magna* DEBkiss IBM: compared to the experiments, the simulations showed lower maximum population densities, earlier population decline, lower abundance of large individuals and higher effects of competition with *B. calyciflorus.* The implementation of an additional starvation mechanism similar to another *D. magna* DEB IBM implementation (Martin et al. 2013a) proved unsuccessful. The absence of a reserve compartment thus limits the application of the DEBkiss IBM for conditions when the population is starving. The starvation recovery rules were also lacking: while reproduction was not observed after day 10 in the experiments, low abundances (<20 individuals) of small individuals were still observed in the simulations. In the DEBkiss implementation, starving individuals shrink to meet their energy needs. When food is available again, they immediately spend a fixed part of the assimilated energy on reproduction. However, it seems unlikely that the proportion of assimilated energy spent on reproduction is identical for well-fed individuals and individuals recovering from starvation. Implementing starvation recovery rules that favour recovery of weight over growth and reproduction could improve the fit of the simulations. However, many different starvation recovery strategies have been proposed for *D. magna* and a general consensus is lacking (Vanoverbeke 2008).

The instantaneous effects of pyrene on *D. magna* populations when modelling toxicity with CR curves is not surprising given that only the current chemical concentration is used to predict pyrene effects. There is thus no delay between exposure and effects possible, although a delay of about a week was observed for pyrene. Similarly, effects of pyrene disappear quickly with the CR approach, explaining the predicted recovery. The TKTD approach can account for a delay in effects and recovery but effects were still too fast for the experiments used. A possible cause for the deviation between the effects predicted by the toxicity models and the observed effects is that the parameterization of these models was done using the result from an acute toxicity test performed with *D. magna* juveniles. Chronic toxicity can differ from acute toxicity e.g. because of alternative modes of action or toxicity of the biotransformation products (Dom et al. 2012). Also, the endpoint used in the *D. magna* toxicity test was immobilization and it can take a few days for immobilized individuals to die. Moreover, the presence of different size classes can influence the susceptibility of a population to toxic stress: larger *D. magna* individuals have been shown to be more resistant to e.g. carbaryl (Coors and Meester 2008), possibly explaining the observed delay in effect of pyrene at concentrations close to the acute EC_{50}. Alternatively, due to its assumed narcotic mode of action (Di Toro et al. 2000), it is possible that pyrene causes sub-lethal effects in daphnids. Including such sub-lethal effects might explain the delayed effect of pyrene on total population density. More complex toxicity models which e.g. consider effects on maintenance costs or other modes of action were not tested because (1) the aim of the chapter was to test the efficacy of a simple (toxicity) model and (2) accurate information about the mode of action of pyrene or different sensitivity of life stages to pyrene is lacking.

The observed population decline of rotifers at the end of the experiment was most likely due to unsuitable environmental conditions e.g. decreased water quality, waste accumulation and/or changed physico-chemical parameters of the water, which are not considered in the IBMs. For example, the pH decreased from 7.7 ± 0.1 at the start to 6.5 ± 0.3 at the end of the second experiment and the oxidation-reduction potential increased over the same period from -61 ± 5 mV to 9 ± 16 mV. Other water quality parameter and waste products were not measured in the experiments.

Competitive exclusion has been observed frequently for rotifers competing with daphnids (MacIsaac and Gilbert 1989). We were able to simulate this exclusion and its effects on both species, although the disappearance of rotifers was slower in the simulations than in the experiments. Possibly, other mechanisms not considered here accelerated the extinction of rotifers e.g. rotifers can be damaged by mechanical interference – rejection after accidental ingestion – with feeding daphnids (Gilbert 1988). It is also plausible that such low abundances of *B. calyciflorus* were simply not detected in the experiments. Because of the transparency and low complexity of the DEBkiss IBMs, it would be easy to implement additional competition mechanisms for species when this is needed.

The predicted increase in rotifers after pyrene addition did not correspond with the experiments but is logical when looking at the other simulations. This difference was caused by the rotifers still being present in the simulations at the time of pyrene addition contrary to the experiments. Moreover, the effect of pyrene on daphnids occurred much faster in the simulations than in the experiments, increasing the amount of algae available to rotifers. Also, some daphnid populations were completely eliminated by pyrene, which resulted in improved *B. calyciflorus* growth conditions i.e. without competition for food. In fact, if a similar pyrene effect had occurred on *D. magna* in the experiments as in the simulations, an increase in *B. calyciflorus* in the experiments would be expected.

In general, population dynamics were accurately predicted using a DEBkiss IBM and the DEBkiss IBM approach is especially applicable for organisms with short life cycles. Despite the limitations of the DEBkiss IBM to capture the observed population dynamics and structure of *D. magna* exactly, the endpoints that are most focused on in traditional ERA – population growth, total population density and reproduction – were reasonably well predicted. Also, the toxicity models considered here do not account for size-dependent effects, which limits the impact of a faulty population structure on the population dynamics after toxic stress.

4.4.2. Applicability of the DEBkiss IBM framework

Without conducting the experiments often deemed necessary for DEB model parameterization (Jager et al. 2013), the population dynamics of two competing species were simulated reasonably well using available literature data and universal parameter values (Table 4.1). DEB parameters are often difficult to measure and interpret. When not available, these were derived from more easily available parameters using simple equations. For example, the area-specific searching rate of *D. magna* was derived from the half-saturation constant for ingestion using Equation (3.4). Although the use of these simple formulas entails acceptance of certain assumptions (Jager et al. 2013) - e.g. for the example above we assume that the functional response is a Holling type II - they also offer an easy way to calculate parameter values that are difficult to determine. Therefore, despite its limitations, DEBkiss IBMs can be useful to predict population-level effects for species where the large data requirements of typical DEB models is problematic. Given the limited complexity of DEBkiss IBMs and the problems encountered with simulating the size structure of the relatively simple *D. magna* in the current study, their usefulness for predicting population dynamics of more complex organisms such as fish or large macro-invertebrates with fluctuating food conditions seems limited. However, they might be used to simulate key life processes for these organisms such as growth in well-described situations e.g. when food is abundant, as was done for the pond snail *Limnae stagnalis* (Jager et al. 2013).

Calibration of a model to only one dataset is not without risk. The validity of the model is only tested for a specific situation and the parameters can be adjusted to fit the observations without necessarily capturing the underlying processes(Forbes et al. 2008; Grimm and Martin 2013). In this chapter, the DEBkiss IBMs were calibrated using one experiment in the case of *B. calyciflorus* and two experiments in the case of *D. magna*. These calibrated models were then applied to three different treatments: pyrene stress, competition and a combination of both. Although calibrated on a limited dataset, these models were thus applied successfully to very different situations, increasing their acceptability and credibility.

In conclusion, the tested DEBkiss IBMs were able to capture the general patterns of the population dynamics observed in experiments with *D. magna* and *B. calyciflorus*, especially for *B. calyciflorus*. Two areas in particular need improvement: the simulated population structure of *D. magna* and the toxicity model of pyrene. Improving the modelling of the population structure might be a challenge because of the absence of a reserve compartment in DEBkiss theory. The toxicity of pyrene could be better captured using more complex toxicity models that consider other modes of action. This information is, however, currently lacking for pyrene. For chemicals that have clear acute effects on survival, the TKTD-SD model implemented here might already prove sufficient.

5

DEVELOPMENT OF THE INTEGRATED CHIMERA MODEL

Abstract

Ecological risk assessment does not aim to protect one or two species in the laboratory but to protect realistic communities in the field. Two of the most important interactions in a food web are competition and predation. In this chapter, a DEBkiss IBM implementation for predation was developed and combined with the earlier developed competition implementation to form the ChimERA$_{foodweb}$ model. The ChimERA$_{foodweb}$ model included two grazers (Brachionus and Daphnia), their predator (Chaoborus) and two detritus feeders (Asellus and Gammarus). To perform simulations for realistic environmental conditions, ChimERA$_{foodweb}$ was coupled with ChimERA$_{fate}$ to form the integrated ChimERA model. ChimERA$_{fate}$ is a dynamic and spatially-explicit fate model that predicts environmental concentrations based on environmental variables (hydrodynamics, temperature and trophic state). The integrated ChimERA model is able to provide spatially-explicit predictions of food web dynamics and the effects of chemical exposure over time, based on the environmental conditions provided.

5.1. Introduction

Ecological risk assessment (ERA) procedures were developed to quantify the risk that a chemical poses in the environment. Ecological risk assessment is typically divided in environmental exposure assessment and assessment of the potential ecological effects, with distinct procedures for exposure and effect assessment. Current ERA methods have however received considerable criticism for being unrealistic, both on the exposure and effect assessment side (Forbes et al. 2008; SCHER (Scientific Committee on Health and Environmental Risks) et al. 2013; De Laender et al. 2014a). Effect assessment procedures have been criticized for neglecting important ecological processes such as species interactions (Fleeger et al. 2003; De Laender et al. 2014a) and spatio-temporal variation in effects of chemicals (Beketov and Liess 2012). Exposure assessment procedures need to be improved to account more explicitly for variability in time and space (De Laender et al. 2014a).

Interactions between species, such as competition and predation, can influence the outcome of chemical stress at the population level by increasing or decreasing the effect of the chemical. For example, competition with *Culex* larvae increased the recovery time of *Daphnia magna* after exposure to fenvalerate (Foit et al. 2012). Alternatively, in Chapter 2 of this thesis, the effects of pyrene were found to be lower in *D. magna* populations experiencing predation because of a higher proportion of large, more tolerant individuals. Accurately predicting the effects of chemical exposure on higher levels of biological organization can thus not be done without taking these interactions into account. This has been recognized in many opinion and review papers and in (regulatory) advisory documents (Fleeger et al. 2003; Forbes et al. 2009a; SCENIHR et al. 2012).

The landscape structure, the location of exposure within the landscape and the presence of unexposed populations in the vicinity are other factors determining chemical effects and these are especially important for the recovery of the affected populations. For example, isolated communities recovered more slowly from the application of endosulfan than less isolated communities (Trekels et al. 2011). In another study, the presence of uncontaminated patches increased the recovery time of communities after lufenuron exposure in mesocosms (Brock et al. 2009). Similarly, the timing of exposure can greatly influence population dynamics (Relyea and Hoverman 2006). Early life stages, especially embryonic stages, are often more sensitive than “older” individuals to chemicals. Exposure during periods of reproduction can thus lead to larger population effects compared to exposure during periods without reproduction (Bridges 2000; Galic et al. 2012). Also, the exposure pattern of the chemical is important i.e. where the exposure occurs, how frequently and in what concentrations. Exposure patterns can differ greatly between chemicals and this leads to different effects on population dynamics (De Laender et al. 2014a). For example, pesticides are typically applied during brief periods of the year. When pesticides have a rapid degradation rate, species will be exposed for only a short period to a peak concentration.

Pesticides that are more persistent will stay in the environment longer and species will thus be exposed for a longer time. Other chemicals may have no distinct application time and emission to the environment will hence be more constant (e.g. industrial chemicals or personal care products).

Many factors, both abiotic and biotic, can thus influence the outcome of exposure to a chemical and it is impossible to account for all these factors in experiments. Models are a good alternatives and have been successfully applied in both exposure and effect studies. The dynamic and spatially-explicit ChimERA$_{\text{fate}}$ model, for example, has been developed to predict the chemical concentration in the different environmental compartments of a shallow pond (Morselli et al. 2015). Similarly, population models have been used to predict the effects of chemicals on populations. The effect of the insecticide modelmethrin to populations of three different arthropods (*Chaoborus crystallinus*, *Daphnia magna* and *Gammarus pulex*) was modelled using individual based models and a mechanistic effect model (Dohmen et al. 2015). In another study, the influence of where and when a stress event occurred on the recovery of the isopod *Asellus aquaticus* was studied using an individual based model (Galic et al. 2012). Ecological models have typically been applied to predict the effects of chemicals on populations of one species and thus neglecting possible effects of interspecies interactions. Considering that ecological models have been suggested as good tools to extrapolate individual-level effects to population, food web and/or ecosystem level effects (Grimm et al. 2009; De Laender et al. 2014a), it is important to incorporate interactions between species in these models. One recent modelling study showed for example that adding interspecific competition to individual based models increased recovery times after chemical exposure up to three times (Kattwinkel and Liess 2013).

In Chapters 3 and 4, we developed and successfully applied DEBkiss IBMs for two grazers (*D. magna* and *B. calyciflorus*) competing for a shared food source and successfully simulated the effects of a chemical (pyrene) on the population dynamics of these two species. However, a second key interaction between species was not yet implemented within the DEBkiss IBM framework: the predator-prey relationship. Additionally, the DEBkiss IBMs were applied only for populations in a lab environment which lacked a spatial structure. As mentioned above, the spatial and temporal dimension of chemical exposure is, however, essential to perform a realistic ecological risk assessment. Also, the two components of ecological risk assessment – exposure and effect assessment – are closely linked and interact. For example, detritus and phytoplankton are two key aspects of food webs that also affect the bio-availability of a chemical (Morselli et al. 2015). In the models presented in the previous chapters we have neglected the role of environmental fate on the subsequent effects.

The first objective of this chapter was therefore to develop a DEBkiss IBM implementation for predation and, by combining this with the earlier implementation of competition, to develop a complete food web model. The second objective was to implement an environmental fate model to predict where and when exposure will occur. Finally, both submodels were combined to form the ChimERA model, i.e. a spatially-explicit model that can predict the dynamics of a food web exposed to the realistic emission pattern of a chemical.

5.2. General architecture

The ChimERA model consists of two submodels: the ChimERA$_{foodweb}$ model and the ChimERA$_{fate}$ model (Figure 5.1). The ChimERA$_{foodweb}$ model is used to predict the densities of the different species in a specified food web and the possible effects of chemical(s) on these densities. The ChimERA$_{fate}$ model predicts, based on the emission pattern of a chemical and environmental variables (temperature, trophic state and the hydrodynamics of the system), the concentration of a chemical in the environment as well as the phytoplankton and detritus concentration. Both submodels exchange information during simulations: the ChimERA$_{fate}$ model provides the (chemical) exposure concentration, phytoplankton concentration and detritus concentration to the ChimERA$_{foodweb}$ model. The ChimERA$_{foodweb}$ model provides the amount of phytoplankton and detritus biomass consumed by the food web to the ChimERA$_{fate}$ model. The ChimERA model is a dynamic spatially-explicit model: chemical fate and food web dynamics take into account the spatial structure of the system and can provide predictions for environmental variables that are dynamic over time.

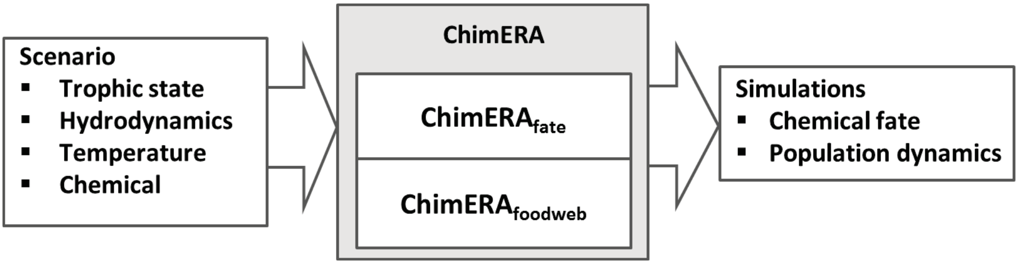


Figure 5.1: *Overview of the integrated ChimERA model: based on the trophic state, the hydrodynamics, and the temperature of a system and the chemical exposure present in a system, the integrated ChimERA model provides predictions of the chemical fate and food web dynamics.*

5.3. ChimERA$_{foodweb}$ model

A simple pond food web consisting of five species was considered in the current food web model: two grazers predated by a predator and two detritus feeders (Figure 5.2). Model genera for the two grazers were *Brachionus* and *Daphnia*, *Chaoborus* was selected for the predator and *Asellus* and *Gammarus* were used as the two detritus feeders. All five species were modelled using DEBkiss IBMs (Chapter 3). The advantages of these DEBkiss IBMs over other IBM approaches were their transparency and simplicity, which allowed easier interpretation of the simulations compared to more complex DEB approaches. Also, the limited complexity allowed for faster calculations. Parameterization of the DEBkiss IBMs was done using the 'add my pet' database (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet) and literature sources (Table 5.1). Certain DEBkiss parameters, mainly feeding-related (maximum specific feeding and searching rate), were adjusted to allow stable population dynamics using the food levels provided by the fate model. The applicability of DEBkiss IBMs has not been tested for *Asellus* and *Gammarus*. However, the parameters used were based on the validated 'add my pet' database and the application of DEBkiss IBMs for competition has been tested in Chapter 4. The competition between *Asellus* and *Gammarus* is therefore expected to be realistically captured by DEBkiss IBMs.

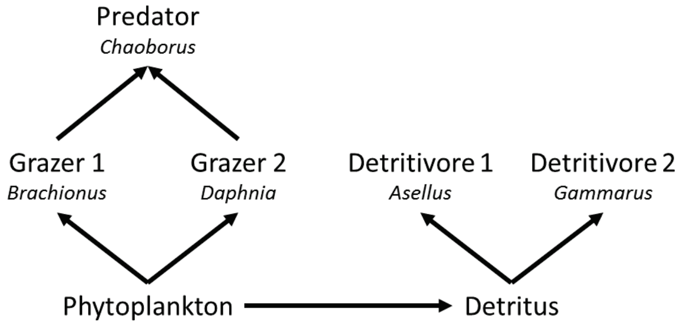


Figure 5.2: *Configuration of the pond food web used in the current chapter. Full arrows indicate the mass fluxes between the different food web components.*

Chemical effects on survival were modelled using a toxicokinetic toxicodynamic (TKTD) approach, based on the generic universal threshold model for survival (GUTS) assuming stochastic death (Jager et al. 2011). A detailed description of the DEBkiss IBM and GUTS implementation is given in Chapter 3. Exposure to a mixture of different chemicals was also implemented in the ChimERA model. Different mixture toxicity models exist (e.g. concentration addition or independent action) and these are mostly based on the mode of action of the chemicals concerned (Jonker et al. 2005).

Table 5.1: *DEBkiss IBM parameter values used for the five species considered in the ChimERAfoodweb model. Parameter values were taken from the 'add my pet' database (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet) and from literature sources (Appendix C).*

Symbol	Parameter	Asellus	Brachionus	Chaoborus	Daphnia	Gammarus
J_{Am}^{a}	Maximum specific assimilation rate (mg dw mm^{-2} d^{-1})	5.32E-02	2.35E-02	1.15E-02	1.06E-02	4.65E-02
F_{m}^{a}	Specific searching rate (L mm^{-2} d^{-1})	3.25E+00	1.96E+00	6.50E-02	4.64E-01	3.59E+00
J_{M}^{v}	Volume-specific maintenance costs (mg dw mm^{-3} d^{-1})	4.50E-03	1.40E-03	8.65E-03	4.08E-03	1.91E-01
W_{BO}	Assimilates in a single freshly laid egg (mg dw)	9.96E-03	3.40E-02	5.00E-04	1.19E-03	8.55E-05
W_{Vp}	Structural body mass at puberty (mg dw)	5.21E-01	1.08E+00	2.60E-03	5.40E-03	1.39E-04
d_V	Dry-weight density of structure (mg mm^{-3})	1.00E-01	1.00E-01	1.00E-01	1.00E-01	1.00E-01
y_{VA}	Yield of structure on assimilates (growth,, -)	8.00E-01	8.00E-01	8.00E-01	8.00E-01	8.00E-01
y_{AV}	Yield of assimilates on structure (starvation, -)	8.00E-01	8.00E-01	8.00E-01	8.00E-01	8.00E-01
y_{BA}	Yield for conversion of reproduction buffer to eggs (-)	9.50E-01	9.50E-01	9.50E-01	9.50E-01	9.50E-01
y_{AX}	Yield of assimilates on food (-)	8.00E-01	8.00E-01	8.00E-01	8.00E-01	8.00E-01
δ_M	Shape correction coefficient (-)	4.20E-01	5.10E-01	1.37E-01	3.80E-01	4.80E-01
κ	Fraction of assimilates for growth and maintenance (-)	9.00E-01	6.00E-01	9.00E-01	8.00E-01	9.00E-01
-	Body mass fraction at which starvation occurs (-)	4.00E-01	8.00E-01	6.00E-01	4.00E-01	4.00E-01
-	Maximum age of an individual (d)	3.65E02	5.00E01	1.60E02	9.60E01	3.65E02
-	Movement probability (d^{-1})	1.00E00	1.00E-01	1.00E00	1.00E00	1.00E00
-	Stochastic mortality rate (d^{-1})	1.00E-03	2.00E-02	1.00E-03	1.00E-02	1.00E-03
-	Time between reproduction events (d)	8.00E01	5.00E-01	9.00E01	2.8E00	4.00E01

Often, *a priori* knowledge on how chemicals interact is not available and an informed choice of the correct mixture toxicity model is therefore not possible. Hence, as an alternative approach, the hazard of each chemical was calculated (see equations 3.14-3.16) and summed, assuming that the hazard of a chemical was not influenced by other chemicals. The summed hazard was then used to calculate the survival over time using

$$S(t) = exp\left(-\sum_i^n H_{z,i}(t)\right) \tag{5.3}$$

with S(t) the survival probability of an individual at time t (d); H_Z the cumulative hazard caused by chemical i of an individual at time t (Equation 3.16) and *n* the number of chemicals to which the individual is exposed.

Some species-specific adjustments were done to enhance the realism of the DEBkiss IBMs. For *Asellus*, *Daphnia* and *Gammarus*, i.e. species with carapaces and appendices which are unable to shrink, the feeding flux was determined using their maximum attained structural length. All species were assumed to follow the general DEBkiss life cycle: individuals start their life cycle as an embryo, hatch as juveniles and grow to the adult life stage, where reproduction can occur. This is roughly applicable for all species except for *Chaoborus*. The *Chaoborus* present in the food web are actually the larval stage and emergence of the free-flying stage is needed for reproduction (Van Wijngaarden et al. 2006). This latter, free-flying life stage was ignored in the DEBkiss IBM implementation because its duration is small compared to that of the waterborne life stages (Von Ende 1982). So, when reproduction occurred, the *Chaoborus* individual was removed from the population and its energy available in the reproduction buffer was converted into eggs.

Competition between grazers and detritus feeders was modelled using resource competition only, as described in Chapters 3 and 4 for experiments with *Brachionus* and *Daphnia*. With each time step, the biomass consumed by each species was summed and, if the theoretical consumption was higher than the available food, rescaled to the available biomass. Although density-dependent effects on mortality can be expected for e.g. the detritivores *Asellus* and *Gammarus* (Galic et al. 2012; Dohmen et al. 2015), they were not considered in our hypothetical scenarios. The outcome of competition was thus solely determined by the rate and the efficiency of food uptake and the ability to cope with low food conditions. Predation on the two grazers by *Chaoborus* was modelled following a multi-species predation approach (Krylov 1992; Rose et al. 1999):

$$C_j = C_{max} W_i f \text{ with } f = T_{hj} \frac{N_j a_j^{'}}{1+\sum_{k=1}^{n} N_j a_j^{'} T_{hj}} \tag{5.4}$$

with C_j the biomass of prey *j* eaten by predator i (mg dw d^{-1}); C_{max} the maximum ingestion by the predator (mg dw d^{-1}); W_i the weight of predator *i*; *f* the functional response parameter (-); T_{hj} the handling time of prey *j* (d); a_j' the attack rate on prey *j* (L d^{-1}); N_j the prey density of prey *j*; and *n* the amount of prey species. A detailed description of the derivation of the predation equations can be found in Appendix C. The used attack rate and handling time were 0.2 L d^{-1} and 0.76 d for *Brachionus* (Krylov 1992), respectively and 0.11 L d^{-1} and 0.07 d for *Daphnia* (Moore 1988), respectively. Similar to the adjustment of the feeding-related DEBkiss parameters, these attack rates and handling times were adjusted literature values to avoid overexploitation of the prey species.

The landscape implemented in the ChimERA model consisted of two connected ponds and inflow and outflow streams. The spatial landscape was implemented in the ChimERA$_{\text{foodweb}}$ model by dividing the two pond system into 92 equal-sized patches of 10 m² (Figure 5.3). All patches have individual phytoplankton, detritus and population dynamics. Movement between patches was implemented as random movement: a species-specific movement parameter defined the probability to move to a neighbouring water patch (Table 5.1). All species except *Brachionus* were assumed to move on average 1 patch per day. *Brachionus* movement was considered lower and individuals only moved 0.1 patch per day, on average.

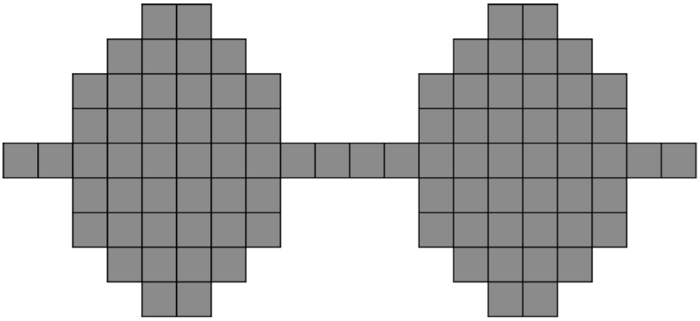


Figure 5.3: *Spatial discretization of the two-pond system in the ChimERA$_{foodweb}$ model. The spatial landscape is divided in 92 patches of 10m².*

5.4. ChimERA$_{\text{fate}}$ model

Because the focus of this PhD thesis is on the ecological effects of chemical exposure, only a brief description of the chemical fate model is given here. For a detailed description of the ChimERA$_{\text{fate}}$ model, see Morselli et al. 2015 (Morselli et al. 2015). In short, the ChimERA$_{\text{fate}}$ model was based on the fugacity approach (Mackay 2001) and modelled the water-sediment system in two connected 450 m² ponds (Figure 5.4). Pond depth and sediment layer depth were assumed to be 50 cm and 1 cm, respectively. Phytoplankton was assumed to be the dominant autotrophic group in the water column and macrophytes were therefore not considered as a compartment. Different compartments and sub-compartments were taken into account to predict the concentration of a chemical: water (water and suspended solids), sediment (pore water and sediment solids), dissolved organic carbon, particulate organic carbon and phytoplankton. Spatial discretization was obtained by dividing the two pond system in 20 slices and describing the one-dimensional water flow between these slices. A hydrological module was used to compute water volumes (m^3) and fluxes ($m^3\ h^{-1}$) on an hourly basis in the slices. Slices reflect areas with identical hydrodynamic properties but are not necessarily equal in area (Figure 5.4). All calculations in the fate model were performed for each of these 20 slices.

Because the model was dynamic, time-varying environmental variables could be used as an input for the model. Important environmental variables included the temperature, hydrodynamics and trophic state of the system. Temperature is not constant throughout the year and varies significantly between regions. The temperature influences partitioning constants and rates in the fate model e.g. the Henry law constant and the dissolution rate of detritus. Trophic state refers to the nutrient conditions in the system and is often described qualitatively as either oligotrophic, mesotrophic or eutrophic. The nutrient conditions will determine the phytoplankton concentrations in the system. The hydrodynamics of the systems describe the water flow. An often used measure for the water flow is the water residence time: the time needed for all water in a location to be replaced. The model was also capable of using time-varying chemical emissions as input to predict chemical concentrations in the different compartments at different time points.

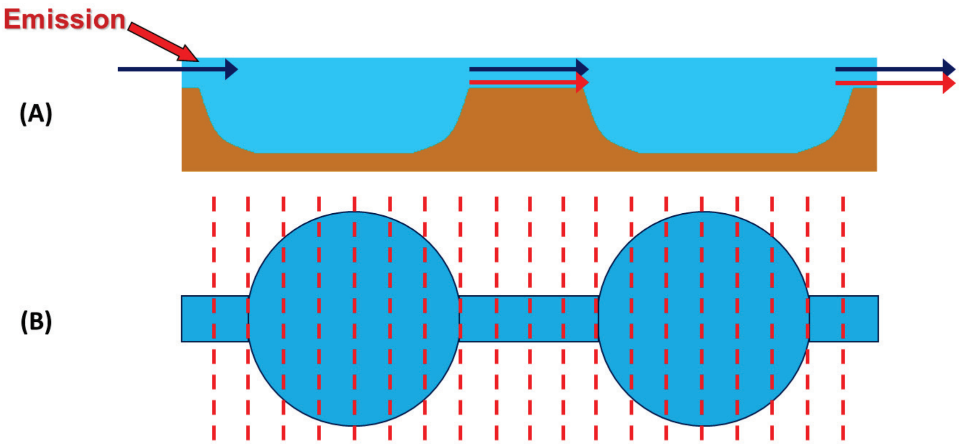


Figure 5.4: *The spatial configuration in the ChimERA$_{fate}$ model. (A) Side view of the two connected ponds. Blue arrows indicate the direction of the water flow, red arrows represent the flow of the chemical. (B) Top-down view of the two connected ponds. Red dashed lines indicate the division of the system in 20 slices by the ChimERA$_{fate}$ model.*

The compartments included in the ChimERA$_{fate}$ model that were relevant for the ChimERA$_{foodweb}$ model were the phytoplankton, detritus and water concentration of the chemical. Phytoplankton and detritus dynamics were described in the ChimERA$_{fate}$ model using ordinary differential equations (Equations 5.1 and 5.2). The phytoplankton and detritus concentrations determined the energy available for the individuals in the food web. The food web model in turn provided the phytoplankton and detritus losses due to consumption. The water concentration of the chemical was used to determine possible effects on the food web.

Phytoplankton growth was not constant throughout the year and was described by

$$\frac{dPhy}{dt} = Phy\left[\left(1 - \alpha\ \cos\frac{2\pi t}{365}\right) Gpp(1 - Resp - Excr)\left(1 - \frac{Phy}{K}\right) - Mort\right] - GrazL \qquad (5.1)$$

with $\frac{dPhy}{dt}$ the change in phytoplankton concentration over time (mg ww d^{-1} L^{-1}); Phy the phytoplankton concentration (mg ww L^{-1}); t the time (days); Gpp the gross primary production (d^{-1}); $Resp$ the fraction of Gpp spend on respiration (-); $Excr$ the fraction of Gpp spend on excretion (-); K the carrying capacity (mg ww L^{-1}); $Mort$ the mortality rate of phytoplankton (d^{-1}) and $GrazL$ the phytoplankton losses due to grazing (mg ww L^{-1} d^{-1}).

Detritus concentration was closely linked to the phytoplankton dynamics and was described by

$$\frac{dDet}{dt} = Mort\ Phy - Dis\ Det - Cons \qquad (5.2)$$

with $\frac{dDet}{dt}$ the change in detritus concentration over time (mg ww d^{-1} L^{-1}); $Mort$ the phytoplankton mortality rate (d^{-1}); Phy the phytoplankton concentration (mg ww L^{-1}); Dis the detritus dissolution rate (d^{-1}); Det the detritus concentration (mg ww L^{-1}) and $Cons$ the detritus losses due to consumption by detritus feeders (mg ww d^{-1} L^{-1}).

Gpp and *K* were two parameters that are specific for a given situation and were chosen on a case by case basis. Standard values were taken for the other phytoplankton and detritus model parameters (De Laender et al. 2015): *Resp* = 0.1; *Excr* = 0.1; *Mort* = 0.2 d^{-1}; *Dis* = 0.01 d^{-1}.

5.5. Technical implementation

The main technical challenge was coupling the fate model and the food web model which differed greatly in their architecture and internal communication. Instead of implementing direct communication between the two models, the ChimERA$_{fate}$ model and the ChimERA$_{foodweb}$ model were coupled by coordinating the communication via a server (Figure 5.5). The advantage of this approach is that it is very flexible i.e. different models can easily be plugged in, regardless of the encoding language since only simple text strings with the necessary information are exchanged with the server. As long as they can communicate with the server, new models can be connected to the server. This makes it easy to e.g. switch to a different food web model without needing to recode the server or fate model.

A detailed description of how the communication between the two models was achieved is given in the communication protocol provided in Appendix C. In short, at each time step both the fate client and the food web client send the requested data to the server. The server then selects which data are needed by individual clients and forwards it to the respective clients. The server then asks the connected models to proceed to the next time step using the provided information. For example, at the start of a time step, the server requests the phytoplankton concentrations in all slices from the fate model. The fate model sends the requested data to the server. The server then sends these phytoplankton concentrations to the food web model and asks for the grazing losses. The food web model uses the phytoplankton concentrations to calculate the biomass of phytoplankton grazed in each slice. These grazing losses are then sent to the server, which communicates it back to the fate client to calculate the phytoplankton concentrations for the next time step.

The server was developed using Pharo v4.0 (http://pharo.org). The ChimERA$_{\text{fate}}$ model was developed in Microsoft Visual Basic 6.0. DEBkiss IBMs were available in NetLogo (Wilensky 1999), as described in Chapter 3. To limit the communication necessary between server, fate model and NetLogo models, a Java framework was developed. This Java framework calls a NetLogo client per species and summarizes the information that needs to be exchanged e.g. the grazing losses provided by the *Daphnia* and *Brachionus* NetLogo clients are summed by the Java framework before being communicated to the server.

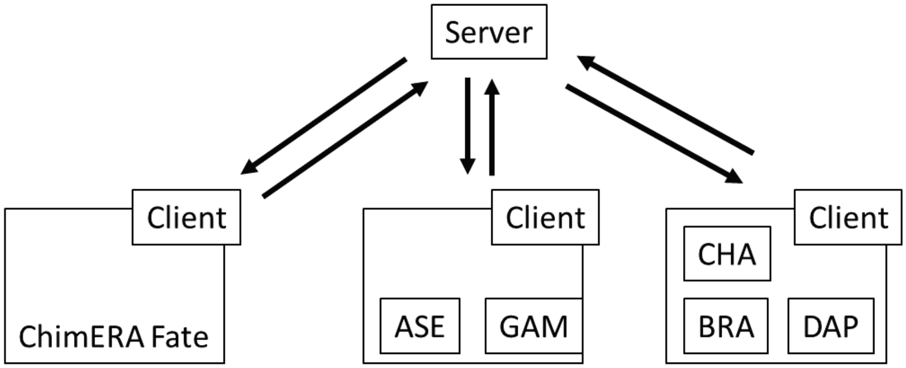


Figure 5.5: *Technical implementation of the ChimERA model. A server coordinates the communication between the different models. A client translates the server request and commands for the individual models. The food web model is divided in two parts, a detritus-based part (with ASE = Asellus and GAM = Gammarus) and a phytoplankton based part (with BRA = Brachionus, CHA = Chaoborus and DAP = Daphnia).*

Phytoplankton and detritus concentrations at the start of the year are too low to support the food web. In reality, species have different strategies to overwinter. Therefore, we assumed that food web dynamics only started when a critical food concentration of 0.025 mg ww L^{-1} was reached. Simulations were started with 100 ind L^{-1}. Early simulations showed that populations of *Daphnia* and *Brachionus* can reach high densities (> 10^6 individuals in the whole system) which increased the simulation times considerably. For the two grazers, a superindividual approach was therefore implemented to limit the computation times. Following the kiss principle, an implementation with limited complexity was chosen. Superindividuals were considered as one constant unit and were simulated in the same way as normal individuals (Grimm and Railsback 2013). The only adjustment made was that the amount of food eaten by the grazers was scaled to the superindividual level i.e. 100 individuals per superindividual for *Daphnia* and 1000 individuals per superindividual for *Brachionus*. Although this approach limits the variability in a population, it requires few additional assumptions and allows for an easy interpretation of the simulations.

5.6. Applications

The ChimERA model developed here is an innovative method because it immediately links realistic exposure predictions of chemicals to effects on food web dynamics. The flexibility and modularity of the model make it possible to apply the ChimERA model to any environmental and ecological scenario. Scenarios can differ, among others, in their spatial structure, identity of the emitted chemical, emission strength and frequency, species in the food web and food web structure. Scenarios can be simulated by supplying the currently used submodels with the necessary information or, if the currently implemented submodels are insufficient, by plugging in a more suitable fate or food web model. To demonstrate its potential, the use of the ChimERA model as a scenario analysis tool is explored in the next chapter. The ChimERA model will be used to study how differences in the environmental variables of a scenario lead to differences in the effects of the applied chemicals on food web dynamics.

6

THE CHIMERA MODEL AS A SCENARIO ANALYSIS TOOL

Abstract

The integrated ChimERA model was developed as a tool to perform more realistic ecological risk assessments. To test its performance, the ChimERA model was applied to a two-pond system connected by a stream in which 15 hypothetical scenarios – differing in water residence time, temperature, trophic state and applied chemical (carbendazim, chlorpyrifos, pyrene and the mixture of the three chemicals) – were evaluated. The predicted effects of the applied chemicals were clearly different for the selected scenarios. Densities of species were generally highest in scenarios with a high trophic state temperature and water residence time. Daphnids were the dominant grazers in the ponds while rotifers dominated in the streams. Concentrations of applied chemicals were highest in scenarios with a high water residence time. The physico-chemical properties of the chemical determined the spatial and temporal pattern of the exposure i.e. where the concentrations were highest and how fast the chemical disappeared. As expected from their sensitivities to the chemicals, direct effects were predicted on Chaoborus, Daphnia and Gammarus. These effects were, however, heterogeneously distributed in space and time and reflected the predicted differences in exposure. The most notable indirect effects were shifts in dominance from Daphnia and Gammarus to Brachionus and Asellus, respectively. Also, the predator Chaoborus was affected indirectly through effects on its prey species Daphnia. For the mixture of three chemicals, the effects of pyrene dominated for Daphnia and the effects of carbendazim and chlorpyrifos for Gammarus. These simulations demonstrate how much the outcome of chemical exposure is determined by environmental conditions, which cannot be captured with traditional risk assessment methods. Modelling tools like the ChimERA model can prove essential to answer the current challenges of ecological risk assessments.

6.1. Introduction

The ChimERA model was developed (Chapter 5) to demonstrate (proof of principle) how available knowledge and models can be combined into a predictive risk assessment tool applicable in a range of environmental conditions (De Laender et al. 2014a). To evaluate and demonstrate how such a type of model could be used to perform scenario analysis, the ChimERA model was applied to 15 hypothetical scenarios that differed in their environmental conditions and applied chemical(s). Although the main focus of this chapter is on the ecology and food web dynamics, a short discussion of the chemical fate model is also included.

6.2. Methods

6.2.1. Scenario description

Three environmental variables can be manipulated in the ChimERA model: temperature, trophic state and hydrodynamics. Trophic state refers to the amount of nutrients available in the system: oligotrophic, mesotrophic or eutrophic. Hydrodynamics refer to how fast the water flows and thus the retention time of the water in the ponds. As numerous combinations of these environmental variables and thus environmental scenarios are possible, we selected three different 'example' combinations of these variable to evaluate the potential applications and limitations of the ChimERA model (Table 6.1): Scenario I is a high velocity system with a low average annual temperature and an oligotrophic nutrient status. Scenario II is characterized by intermediate water velocity, medium average annual temperature and a mesotrophic nutrient status. Scenario III has a low water velocity, high average annual temperature and eutrophic nutrient conditions.

Table 6.1: *Description of the selected scenarios. Scenarios differ in their temperature, trophic state and hydrodynamics. Annual minimum and maximum temperatures are shown. K = phytoplankton carrying capacity; Gpp = gross primary production; RT = residence time.*

Scenario	**Temperature** (Di Guardo et al.)	**Trophic State** (Håkanson and Peters 1995)	**Hydrodynamics** (Peretyatko et al. 2007)
I	[4.2 - 20.1] °C	K = 0.3 mg ww L-1; Gpp = 0.36 d-1	RT = 5 d
II	[4.3 - 22.0] °C	K = 0.5 mg ww L-1: Gpp = 0.42 d-1	RT = 25 d
III	[6.9 - 26.3] °C	K = 1 mg ww L-1: Gpp = 0.71 d-1	RT = 100 d

These three selected environmental scenarios were combined with five exposure scenarios: no exposure, exposure to carbendazim, exposure to chlorpyrifos, exposure to pyrene and finally exposure to all three chemicals simultaneously. The three selected chemicals differ greatly in their physico-chemical properties and their exposure pattern (Appendix D: Table D1). Carbendazim is a fungicide used to control plant diseases (van Wijngaarden et al. 1998) and chlopyrifos is a widely-used insecticide (van der Hoeven and Gerritsen 1997). Pyrene is a polycyclic aromatic hydrocarbon (PAH) that is mostly formed as a by-product of anthropogenic activities (e.g. fossil fuel combustion). Pyrene mainly enters the aquatic environment accidentally through e.g. petroleum spills, wastewater or surface run-off (Nikkilä et al. 1999). Actual emission rates for these chemicals are difficult to find and are highly site-specific. The current study is a proof of principle assessment and the performance of the ChimERA model can best be discussed when effects are expected to occur. Therefore, emission strengths were chosen to result in maximum water concentrations close to the acute LC_{50}-values of the most sensitive species in the intermediate scenario II (Appendix D: Table D2). Both pesticides were considered to have a highly seasonal emission pattern, with short application periods in spring (day 180) and late summer (day 260; Figure 6.1). The length of the application periods was ten days: emission concentrations increased during the first five days and then decreased again. Maximum emission strengths were $3.7 \cdot 10^{-4}$ mol L^{-1} h^{-1} and $6.7 \cdot 10^{-7}$ mol L^{-1} h^{-1} for carbendazim and chlorpyrifos, respectively. Pyrene was considered to have a continuous ambient exposure pattern, with stable emission concentrations of $2 \cdot 10^{-6}$ mol L^{-1} h^{-1} throughout the year (Figure 6.1).

The five species in the food web (Chapter 5) differed in their sensitivity to these three chemicals (Appendix D: Table D2). *Gammarus* and *Daphnia* were most sensitive to carbendazim: the acute LC_{50} was 55 µg L^{-1} and 91 µg L^{-1}, respectively (van Wijngaarden et al. 1998). No effects on *Chaoborus* were observed in toxicity tests at concentration of up to 3435 µg L^{-1} (van Wijngaarden et al. 1998). For chlorpyrifos, *Gammarus*, *Chaoborus* and *Daphnia* were considered the only sensitive species: the acute LC_{50} was 0.23 µg L^{-1}, 0.3 µg L^{-1} and 0.8 µg L^{-1}, respectively. *Gammarus* and *Daphna* were also the most sensitive of the five species when exposed to pyrene: the acute LC_{50} was 27.1 µg L^{-1} and 68 µg L^{-1}, respectively (Chapter 2). No pyrene effects were observed at nominal concentrations of up to 2000 µg L^{-1} for *Brachionus* and *Chaoborus* (Chapter 2). *Brachionus* toxicity test results were not available for carbendazim and chlorpyrifos but rotifers are often more tolerant to chemicals than cladocerans (Girling et al. 2000) and no effects of these chemicals on rotifers were therefore assumed at the used exposure concentrations.

Because mortality and organism mobility in the DEBkiss IBMs are stochastic processes, variability is expected between simulations. To account for this variability, simulations are typically iterated multiple times. However, in this case, simulation times for one scenario were as high as 3 hours, limiting the number of iterations possible. As a compromise, each scenario was run five times. Ideally, model performance is assessed by comparing model simulation with observations. However, these observations were not available for the system and exposure scenarios modelled here. As an alternative approach, the scenario expectations were defined based on *a priori* knowledge and compared with the simulations. The expected environmental fate of each chemical was defined based on the physico-chemical characteristics,. For the food web, the interactions between species and the sensitivity of each species to the chemical were taken into account to define a priori expected population dynamics.

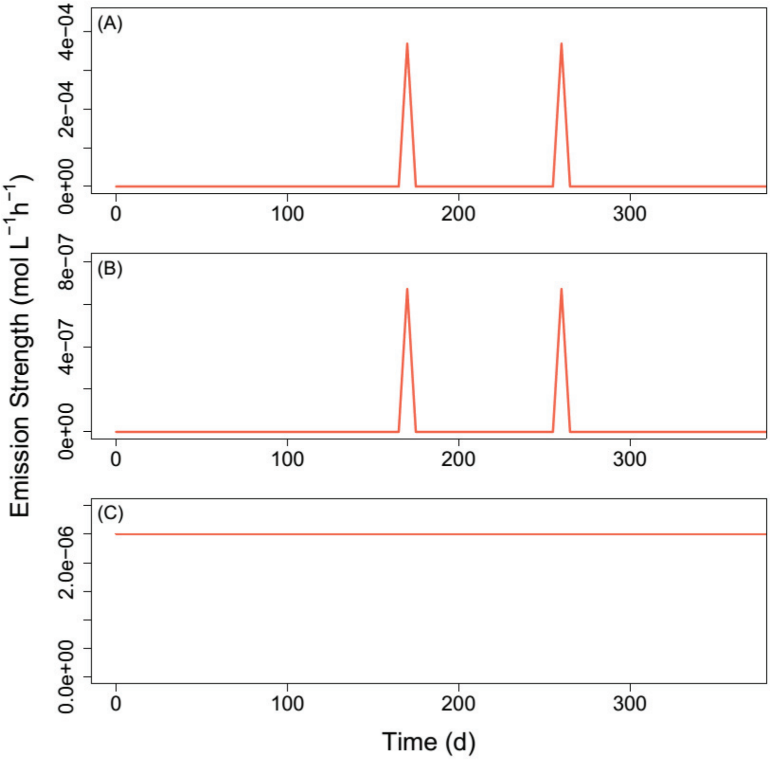


Figure 6.1: *Used emission patterns for (A) carbendazim, (B) chlorpyrifos and (C) pyrene.*

6.2.2. Expectations regarding the outcome of the scenario analyses

6.2.2.1. ChimERA$_{\text{fate}}$

In this section, a short description is given for each chemical of how their physico-chemical properties (Appendix D: Table D1) influence their environmental fate and how this is altered by changes in temperature, trophic state and water retention time. At the end of this section, a summary is given of the expectations for each chemical per scenario.

Carbendazim is a hydrophilic substance ($\log K_{ow} = 1.52$) and will thus not accumulate in the sediment. Moreover, degradation is slow ($HL_{water} = 720$ h). Carbendazim will thus mainly disappear from the system with the water outflow and, because of the low sediment accumulation, the short peaks in emission are expected to be reflected by short peaks in water concentrations. The water retention time will thus be an important factor for its environmental fate. Chemical concentrations will be higher in systems with high water retention time: for the same amount of chemical emitted over an hour, the chemical is diluted less when the water is flowing slowly. Carbendazim will thus be present in the water longer and in higher concentrations in systems with high water residence times. Because of the limited sediment accumulation and degradation, carbendazim concentrations are not expected to be influenced much by temperature or the trophic state of the system, which mainly influence degradation and accumulation.

Chlorpyrifos is a more hydrophobic substance ($\log K_{ow} = 4.96$) and degrades quickly ($HL_{water} = 24$ h). Accumulation in the sediment and degradation are thus important environmental processes for this chemical. As a result of the high sediment accumulation, a long period of reduced but constant water concentrations are expected after a peak in emission. More organic material is available in eutrophic systems compared to oligotrophic systems, which leads to, among others, more accumulation in the sediment. Temperature increases the phytoplankton growth and biomass, further increasing the amount of organic material available in the system. Also, the temperature positively influences the degradation rate of the chemical. Temperature and trophic state will thus determine the water concentrations together with the water retention time. As described for carbendazim, higher water concentrations are expected for systems with high water retention time. However, high water retention times also increase the time available for accumulation to the sediment and for degradation, both decreasing the water concentration of the chemical. Which process is dominant is hard to predict *a priori* because this depends on the relative strength of the environmental fate processes.

Pyrene is a hydrophobic substance ($\log K_{ow} = 5.18$) that degrades slowly ($HL_{water} = 17 \cdot 10^3$ h). Pyrene will thus accumulate in the sediment and persist. Because of the continuous emission pattern, constant water concentrations are expected once equilibrium between all environmental compartments is reached. As was the case for chlorpyrifos, temperature and trophic state are expected to increase the accumulation in the system, decreasing the water concentrations of pyrene. The effect of water retention time is more predictable than for chlorpyrifos: a high water retention time will increase the water concentrations because of the lower dilution of the chemical. Effects of the water retention time on accumulation and degradation are negligible because of the continuous emission of pyrene.

In summary, for the three selected environmental scenarios, carbendazim concentrations in scenario III are expected to be higher than in scenario II and I because of the longer residence times (100 days versus 25 and 5 days, respectively; Table 6.1). Peaks in carbendazim concentration are expected to disappear quickly in all scenarios because of the limited accumulation, with the narrowest peak in scenario I. For chlorpyrifos and pyrene, it is harder to predict *a priori* how the concentrations will differ between the scenarios. Water concentrations are expected to decrease with temperature and trophic state but the opposite is expected for water retention time. The differences between scenario I, II and III will thus reflect the relative importance of these three environmental variables. Chlorpyrifos peaks are expected to persist longer in scenario III than in scenario II and I because of the longer residence time and, as a result of the higher temperature and trophic state, the increased accumulation in the sediment. As was the case for chlorpyrifos, differences in pyrene concentrations between the three scenarios are hard to predict. Pyrene concentrations are expected to become constant once equilibrium between the compartments has been reached.

6.2.2.2. ChimERA$_{\textbf{foodweb}}$

This section starts with a description of how the food web dynamics are expected to be influenced by the environmental conditions. Next, the expected effects of the applied chemical(s) on the food web dynamics are discussed. Finally, a summary of the expected outcomes of the scenarios is given.

The phytoplankton carrying capacity and gross primary production, indicators for the trophic state in the fate model, will determine the phytoplankton biomass and growth. A higher phytoplankton biomass means more energy is available for the grazers. The densities of the grazers *Daphnia* and *Brachionus* and their predator *Chaoborus* are thus expected to be higher when the trophic state of the system is high. Phytoplankton is the main source for detritus and detritus dynamics are thus closely linked to the phytoplankton dynamics. In the absence of phytoplankton grazing, a high trophic state is expected to lead to high detritus concentrations, which allows the densities of the detritus feeders *Asellus* and *Gammarus* to be higher. Phytoplankton grazing will limit the biomass of phytoplankton being converted to detritus, making it harder to predict how the detritus concentrations will differ between trophic states. Because of the close link between phytoplankton and detritus, patterns in the phytoplankton dynamics are expected to be reflected in the detritus dynamics. Because of the strong relation between their food sources, patterns in grazers and detritus feeders are also expected to be linked. *Chaoborus* dynamics in turn are expected to reflect the dynamics of its food source, the grazers. Phytoplankton drift with the water is not considered in the fate model and the water retention time thus should not influence the food web dynamics.

Gammarus and *Daphnia* are the most sensitive species to the three chemicals considered here. Exposure to these three chemicals is therefore expected to lead to decreased abundances of *Gammarus* and *Daphnia*. Direct effects of chlorpyrifos are also expected for *Chaoborus* but not for the other two chemicals. As indirect effects, the densities of the more tolerant competitors *Asellus* and *Brachionus* are expected to increase. C*haoborus* feeds on both *Daphnia* and *Brachionus* and because both prey species are differently affected by carbendazim and pyrene, it is difficult to predict how these two chemicals will affect the predator dynamics. In the case of chlorpyrifos, direct effects are expected for both *Chaoborus* and *Daphnia*. Indirect effects on *Brachionus* are thus expected to be especially strong because of both reduced competition and predation by chlorpyrifos. Since chlorpyrifos and carbendazim are applied only during short periods, (partial) recovery of the *Gammarus* and *Daphnia* densities should be possible after the application periods. Pyrene exposure is constant throughout the year and consistently different food web dynamics from the control scenarios are therefore expected. When exposed to the mixture of all three chemicals, the continuous exposure to pyrene is expected to affect the food web the most because the exposure starts earlier: *Daphnia* and *Gammarus* densities will decrease and indirect effects on *Asellus*, *Brachionus* and *Chaoborus* will occur. The application of the other two chemicals will further increase the effects of pyrene, leading to higher effects than observed in the individual exposures.

In summary, the densities of all species in the food web are expected to be higher in scenario III than in scenario II and I because of the higher trophic state and temperature in that scenario. Direct effects of the chemicals are mainly expected for *Daphnia* and *Gammarus* while *Asellus*, *Brachionus* and *Chaoborus* will be indirectly affected through reduced competition or reduced prey availability. The magnitude of the predicted effects of the chemicals on the food web is closely linked to the expected exposure patterns (See 6.2.2.1). For carbendazim, higher concentrations are expected in scenario III than in scenario II and I, and higher effects on the food web dynamics are thus also expected. For chlorpyrifos and pyrene, the expected concentration differences between the scenarios are less clear and it is thus hard to predict differences in effects between the scenarios.

6.3. Results

For a clear presentation of the simulations, four slices (see 5.4) were chosen that were representative for the whole system (Figure 6.2): slice 1 was located at the inflow of the first pond i.e. where the chemicals were emitted into the system, slice 5 was located in the middle of the first pond, slice 10 was located in the stream between the first and second pond and slice 15 was located in the middle of the second pond. The observed patterns in the simulations are described in this section for the ChimERA$_{\text{fate}}$ (6.3.1) and ChimERA$_{\text{foodweb}}$ (6.3.2) model and compared to the expected patterns. The evaluation of the underlying processes is described in section 6.4.

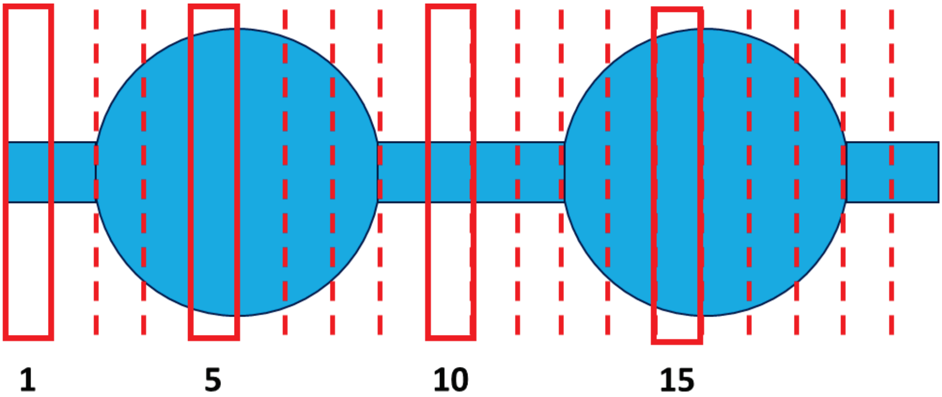


Figure 6.2: *Location of slices 1, 5, 10 and 15, for which the population dynamics are shown in Figures 6.3-6.13.*

6.3.1. ChimERA$_{fate}$

In this section, the expected results of the ChimERA$_{fate}$ model are compared with the performed simulations for each chemical. The dominant fate processes for each chemical are also provided. For a discussion of the observed patterns and the underlying processes, see 6.4.1.

As expected because of the higher water residence time, carbendazim concentrations in slice 1 were higher in scenario III (971.87 µg L^{-1}) than in scenario II (255.54 µg L^{-1}) and scenario I (51.92 µg L^{-1}). Because of the increased water retention time, the decrease in carbendazim concentrations throughout the two-pond system was also higher in scenario III than in scenario II and scenario I (Figure 6.3): the carbendazim concentration in the outflow stream (slice 20) was 50.9%, 11.1% and 2.5% of the concentration in the inflow stream (slice 1) for scenario I, II and III, respectively. Because of the limited sediment accumulation, concentrations of carbendazim are expected to decrease sharply when emission stops. This was indeed observed for scenario I and scenario II but not for scenario III. In scenario III, the carbendazim concentration only decreased sharply after emission in slice 1, where emission occurred. Further downstream, peaks were much less pronounced (slice 5) or not observed (slice 15). Outflow with water, diffusion to sediment pore water and degradation were the most important fate processes for carbendazim.

Chlorpyrifos concentrations were higher in scenario III (1.55 µg L^{-1}) than in scenario II (0.42 µg L^{-1}) and I (0.09 µg L^{-1}), indicating that water retention time was more important than temperature and trophic state (see 6.2.2.1). As expected, a reduced but constant chlorpyrifos concentration was predicted after the application period because of the accumulation in the sediment (Figure 6.3). The peak chlorpyrifos concentrations decreased sharply further downstream and this was more pronounced with higher water residence times: peak chlorpyrifos concentrations in slice 20 were 21.8%, 0.3% and <0.01% of the peak concentrations in slice 1 for scenario I, II and III, respectively. Outflow with water and degradation were the most important processes that determined the chlorpyrifos water concentration.

Pyrene concentrations were higher for scenario III (6.79 µg L^{-1}) than in scenario II (1.73 µg L^{-1}) and scenario I (0.35 µg L^{-1}) and, as expected, concentrations stayed constant once equilibrium was reached (Figure 6.3). This equilibrium between all environmental compartments was reached later further away from the emission source. Pyrene concentrations decreased further downstream and this was more pronounced in scenarios with a high water retention time: pyrene concentrations in the outflow stream were 87.0%, 52.5% and 9.9% of the concentrations in the inflow stream for scenario I, II and III, respectively. For pyrene, the dominating fate processes were the outflow with water and the particle deposition to the sediment.

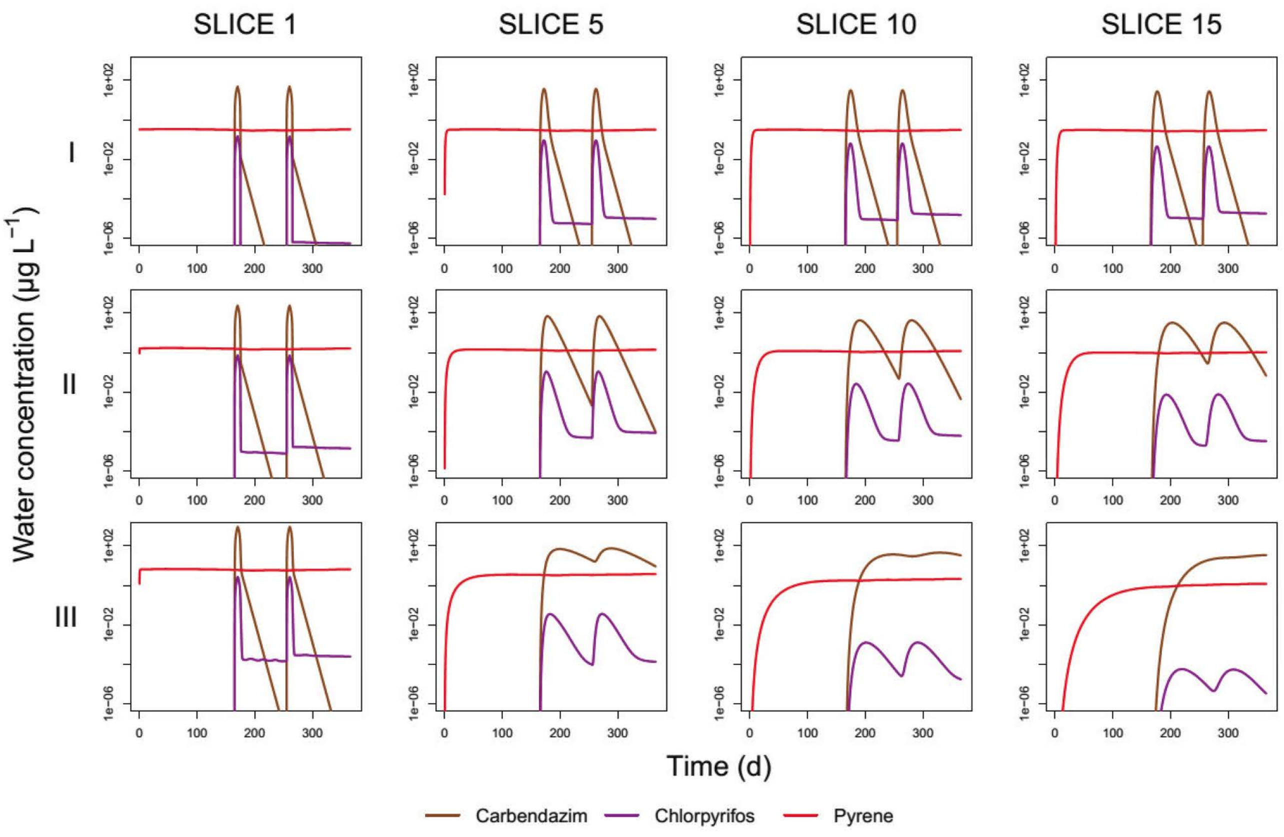


Figure 6.3: *Dissolved water concentrations of the three model contaminants as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system exposed for one year: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15).*

6.3.2. ChimERA$_{foodweb}$

In this section, the simulated food web dynamics are described and compared with the expectations. First, the scenarios with no exposure are discussed, next the carbendazim, chlorpyrifos and pyrene exposure and finally the exposure to the mixture of all three chemicals. The underlying processes are discussed in section 6.4.2. and 6.4.3.

6.3.2.1. No exposure

As expected, food web dynamics of the three environmental scenarios (I, II and III) differed greatly (Figures 6.4 and 6.5). In general, following expectations, population densities increased with an increase in the trophic state of the scenario e.g. the maximum population density of *Brachionus* was $101 \cdot 10^3$ ind L^{-1}, $224 \cdot 10^3$ ind L^{-1} and $1467 \cdot 10^3$ ind L^{-1} for scenario I, II and III, respectively. This pattern was influenced by interactions between species: *Daphnia* was the dominant grazer in scenario I and II, preventing the *Brachionus* density to increase. The dominance pattern shifted in the highest trophic level (scenario III): *Brachionus* was able to benefit more from the increased phytoplankton concentrations than *Daphnia* and became the dominant grazer. Because of the strong competition with *Brachionus*, *Daphnia* densities in scenario III ($733 \cdot 10^2$ ind L^{-1}) were not higher than the *Daphnia* densities in scenario II ($740 \cdot 10^2$ ind L^{-1}). *Chaoborus* can predate on both grazers. However, *Chaoborus* densities were highest in scenario II (403 ind L^{-1}), when *Daphnia* was dominant and not in scenario III (244 ind L^{-1}), when *Brachionus* densities were high. The population dynamics of *Chaoborus* were closely linked with the *Daphnia* dynamics but not so much with the *Brachionus* dynamics.

The higher population densities of the grazers also led to a more dynamic system in scenario III, with a typical cyclical pattern of phytoplankton growth, increase in grazer densities, phytoplankton decline and decrease in grazer densities. This pattern was also visible but with fewer peaks per year in scenario I and II where *Daphnia* followed the phytoplankton dynamics. Because of the grazing pressure, the phytoplankton was not able to grow until the carrying capacity of the system (K): maximum phytoplankton concentrations were 0.16 mg ww L^{-1} (K = 0.30 mg ww L^{-1}), 0.31 mg ww L^{-1} (K = 0.50 mg ww L^{-1}) and 0.79 mg ww L^{-1} (K = 1.00 mg ww L^{-1}) for scenario I, II and III, respectively. The detritus concentration reflected the phytoplankton concentration (Figure 6.5): more fluctuations and higher maximum detritus concentrations in scenario III (0.58 mg ww L^{-1}) than in scenario II (0.22 mg ww L^{-1}) and I (0.10 mg ww L^{-1}).

For the detritus feeders, *Gammarus* was the most dominant species in all scenarios while *Asellus* densities remained low (Figure 6.5). The highest densities of both detritus feeders were observed in scenario II, despite the higher detritus concentration in scenario III. Unexpectedly, the population dynamics of the detritus feeders differed greatly of those of the grazers: the densities of the detritus feeders mainly increased in the second half of the year while periods of population growth were observed for grazers throughout the year.

Unexpectedly, clear spatial differences were observed between the species: population densities of *Daphnia* and *Chaoborus* were much higher in the ponds (Slice 5 and 15) than in the inflow and connecting streams (Slice 1 and 10). Based on the *Brachionus* dynamics in scenario III, spatial differences are much less prominent for this species. Similar to *Daphnia* and *Chaoborus*, *Asellus* and *Gammarus* population densities were highest in the ponds and lower in the inflow and connecting streams. Contrary to the ponds, the reduced competition with *Gammarus* in the streams allowed short periods of population increase for *Asellus* e.g. slice 1 and 15 in scenario I around day 230.

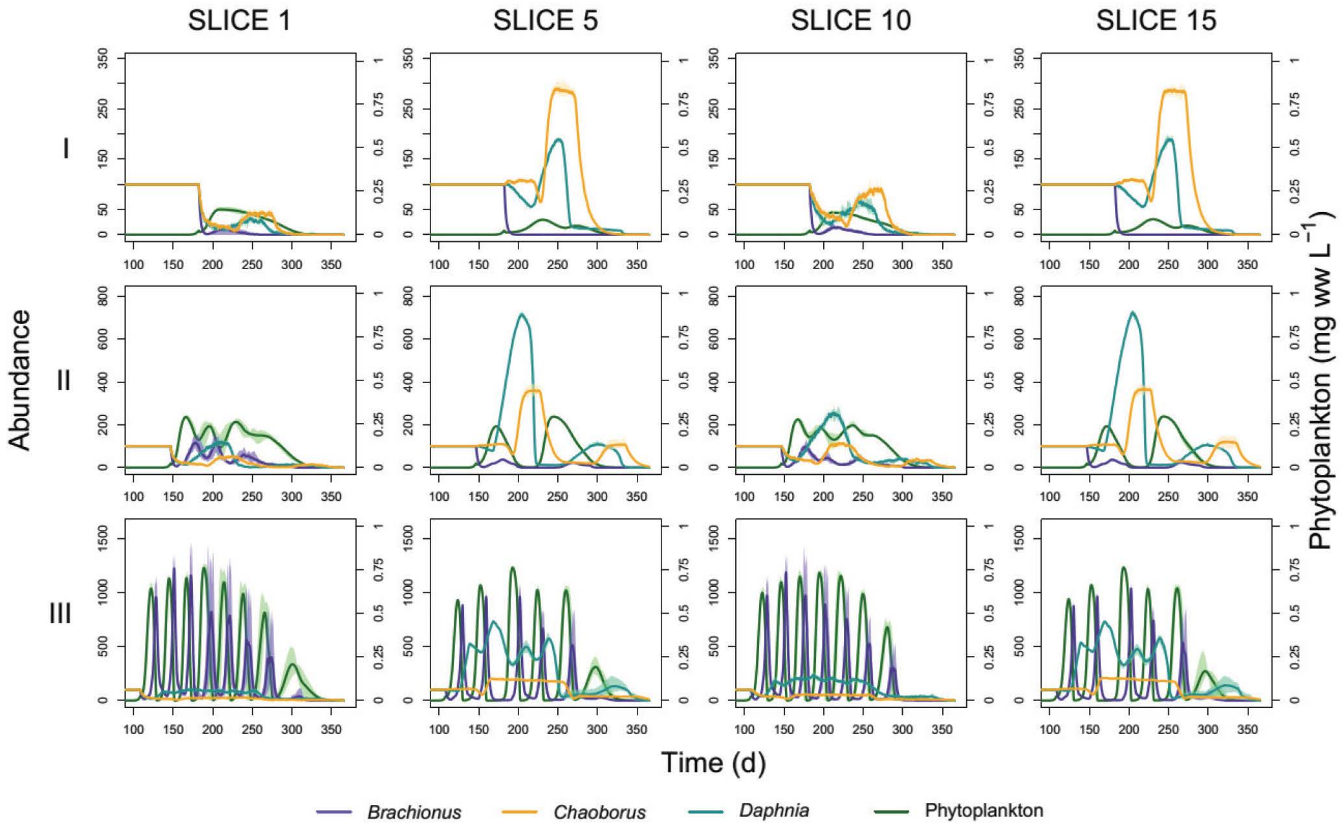


Figure 6.4: *Food web dynamics without chemical exposure as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the phytoplankton-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.*

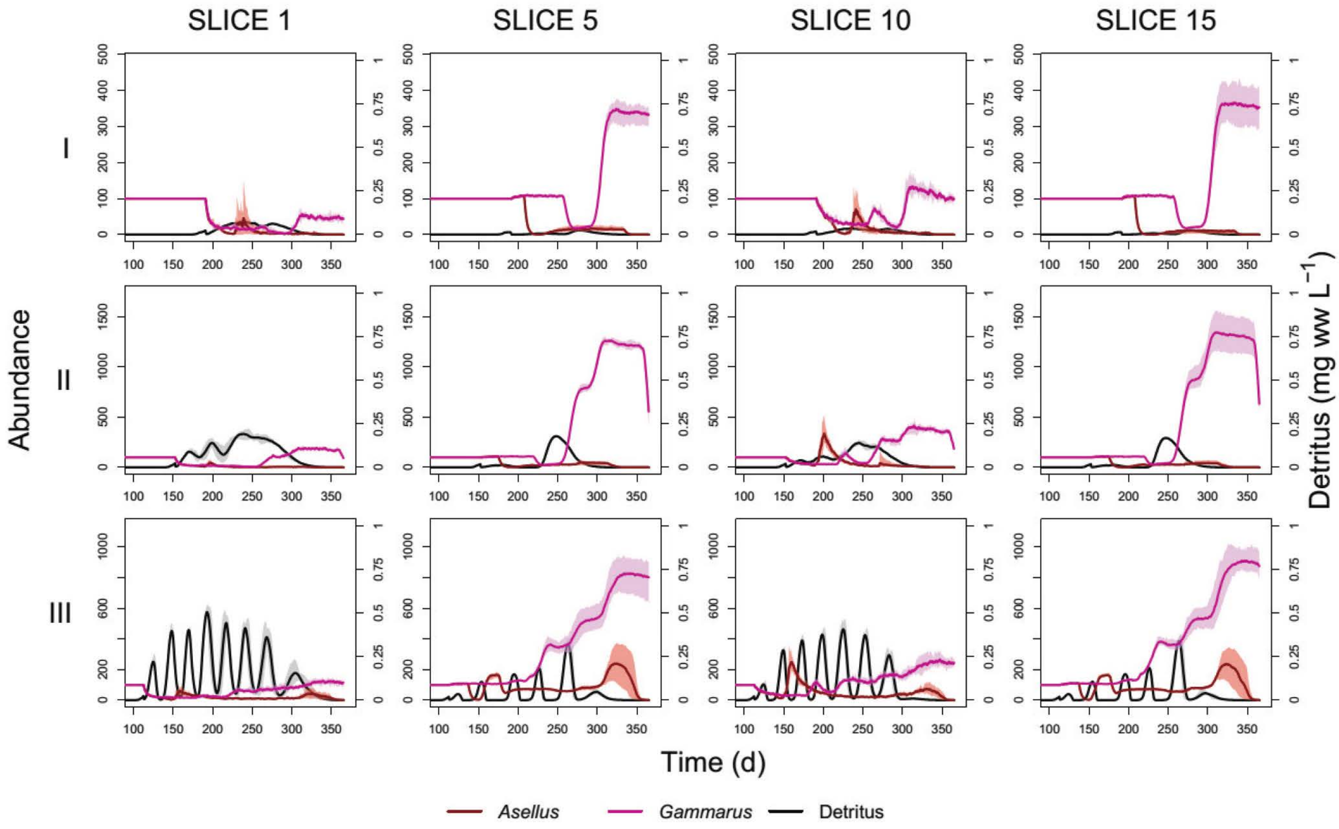


Figure 6.5: *Food web dynamics without chemical exposure as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the detritus-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario*

6.3.2.2. Carbendazim exposure

Direct effects

As a result of the low carbendazim concentrations, only minimal effects of carbendazim on the food web dynamics were observed in scenario I (Figure 6.6-6.7). In scenario II and III, the expected negative effects of carbendazim exposure to *Daphnia* and *Gammarus* densities were observed. High spatial differences between the direct effects of carbendazim on *Daphnia* densities were observed in scenario II and III: carbendazim effects were mainly observed in the first pond and were lower and occurred later in the year in the second pond (slice 15), reflecting the lower carbendazim concentrations downstream. As a result of the higher carbendazim concentrations, effects were more pronounced in scenario III than in scenario II: after the first carbendazim application, the *Daphnia* densities decreased by 56% in the first pond (slice 5) in scenario II while, in scenario III, *Daphnia* went extinct in the first pond (Figure 6.6). Recovery was only observed in the first pond in scenario II, where *Daphnia* densities increased again after the first exposure peak (around day 250). The reached densities after recovery were, however, only about 40% of the densities in the control scenario and the population peak occurred later (day 250 instead of day 200).

Carbendazim effects on *Gammarus* densities were observed in the whole system for both scenario II and III (Figure 6.7): the strong population growth phase at the end of the year, observed in the control scenarios (Figure 6.5), was no longer observed. Similar to the carbendazim effects on *Daphnia*, the effects of carbendazim were lower and occurred later in the second pond, especially in scenario III.

Indirect effects

Because direct effects of carbendazim were nearly absent in scenario I, indirect effects were mainly observed in scenario II and III on *Asellus*, *Brachionus* and *Chaoborus*. Indirect effects of carbendazim were especially visible for the detritus feeders (Figure 6.7): the tolerant *Asellus* was able to become the dominant species throughout in the system in both scenarios e.g. an increase in density by 329% in scenario II. Reflecting the direct effects of carbendazim on *Gammarus*, the indirect effects occurred later and the *Asellus* densities were lower in the second pond than in the first pond.

For the grazers, in scenario II, the densities of the more tolerant *Brachionus* were higher after carbendazim exposure, although no shift in dominance occurred (Figure 6.6). As was the case for the direct effects, spatial differences were observed: *Brachionus* initially increased in density in the first pond after the first carbendazim application (day 160) but decreased again once *Daphnia* densities recovered. In the second pond, carbendazim effects were nearly absent after the first carbendazim application but an increase in *Brachionus* density was observed after the second application (day 250). In scenario III, these spatial differences were even more pronounced. In the first pond, the carbendazim application further increased the dominance of *Brachionus* leading to an increase in the number of peaks in *Brachionus* density (from 5 to 7). In the second pond, however, indirect effects were absent.

Because *Chaoborus* can feed on both grazers, it was hard to formulate expectations for the indirect effects of carbendazim. Apparently, the predator *Chaoborus* was affected indirectly in scenarios II and III through the effects of carbendazim on the prey species *Daphnia* (Figure 6.6). As was the case for *Daphnia*, effects were most pronounced in the first pond. In scenario II, *Chaoborus* densities decreased by 32% in slice 5 but, following the recovery of *Daphnia*, stayed high for a longer period. In the second pond, indirect effects were only observed at the end of the year by the absence of a second population peak. In scenario III, *Chaoborus* followed the extinction of *Daphnia* in the first pond but no indirect effects were observed in the second pond.

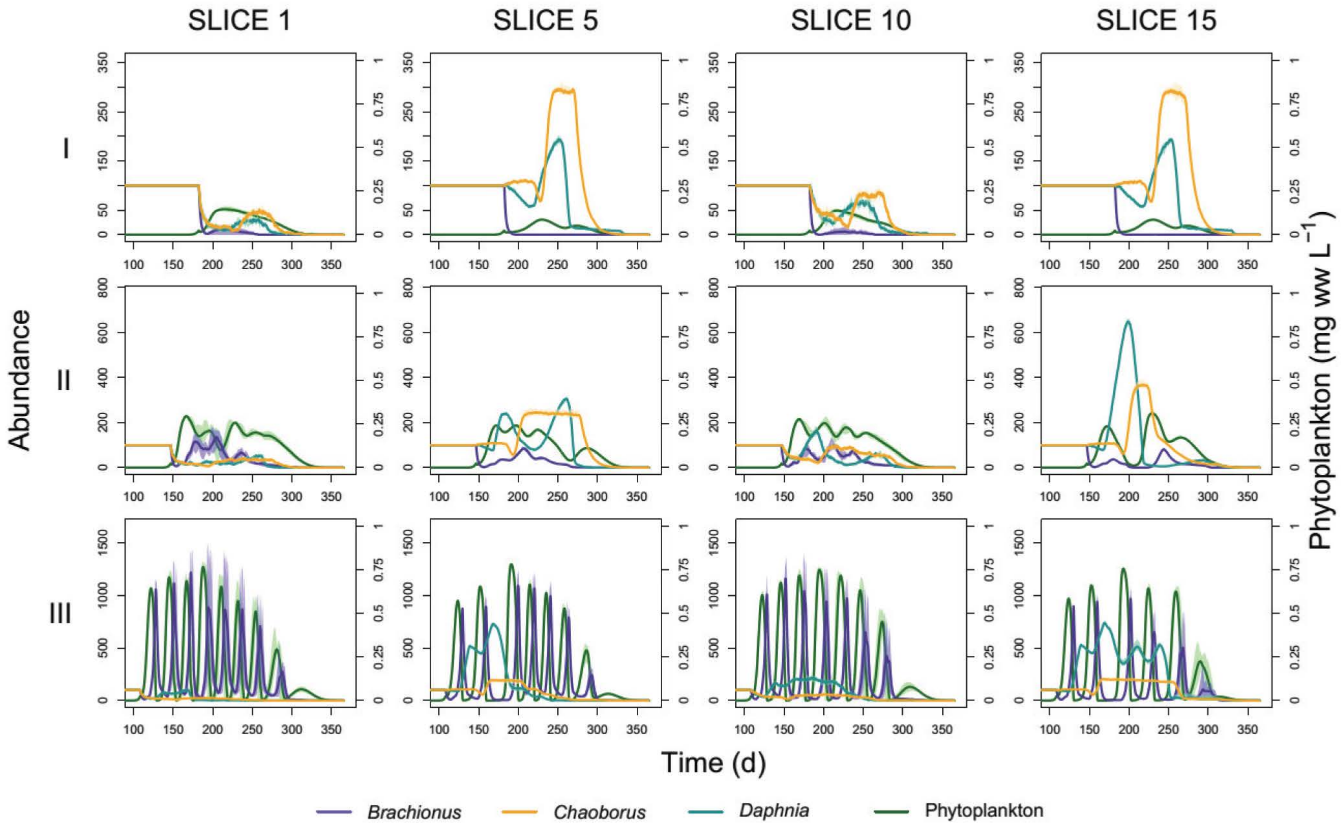


Figure 6.6: *Food web dynamics with carbendazim exposure as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the phytoplankton-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.*

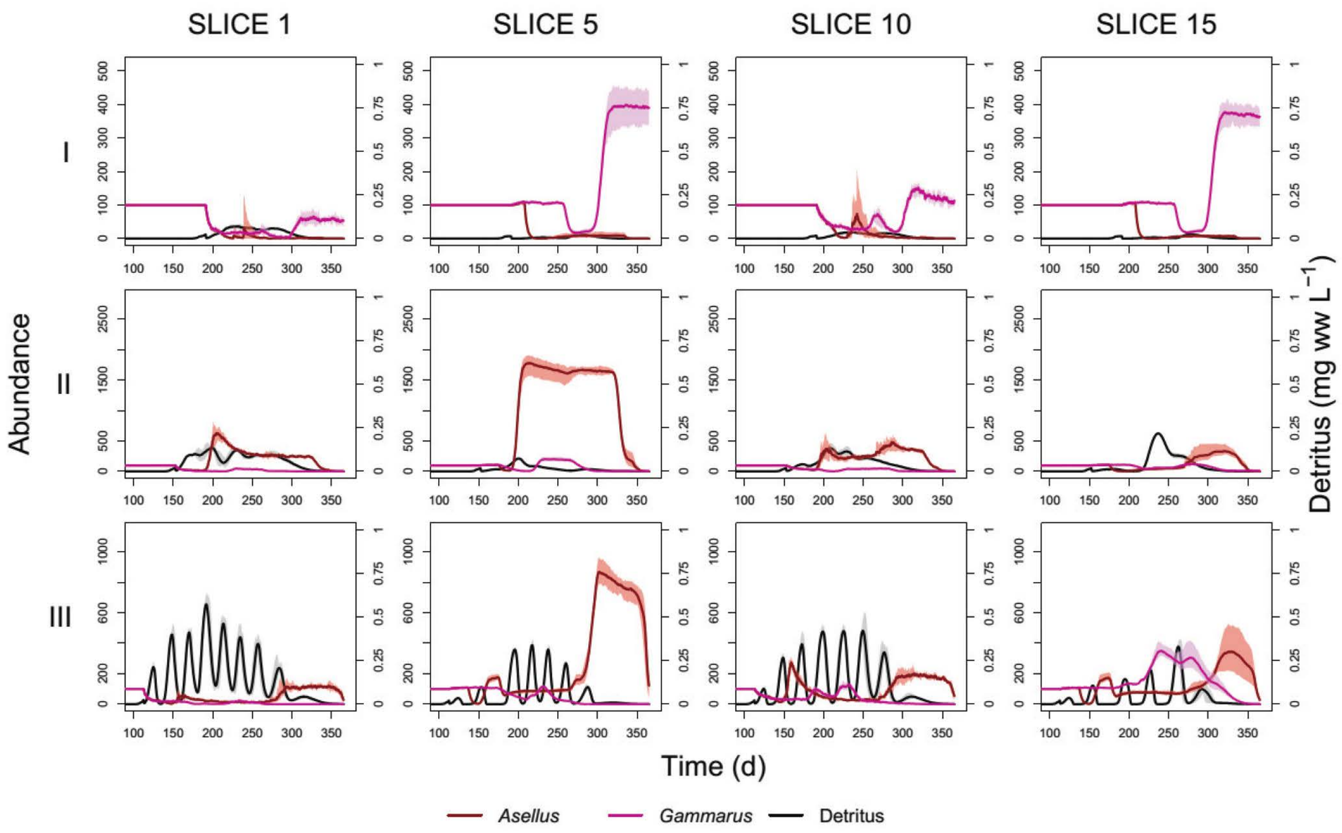


Figure 6.7: *Food web dynamics with carbendazim exposure as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the detritus-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.*

6.3.2.3. Chlorpyrifos exposure

Direct effects

As expected, direct effects of chlorpyrifos were observed for *Chaoborus*, *Daphnia* and *Gammarus* (Figures 6.8 and 6.9). The magnitude of the effects was directly linked to the exposure concentrations that were predicted by the ChimERA$_{\text{fate}}$ model (Figure 6.3): clear differences in effects could be observed between environmental scenarios (I, II and III) and between different locations in the system.

Effects in scenario I were limited for the phytoplankton-based part of the food web (Figure 6.8). Because of the higher and prolonged exposure to chlorpyrifos, effects were more pronounced in scenario II and III than in scenario I. The fast decrease in chlorpyrifos concentrations away from the emission point resulted in effects mainly occurring in the inflow stream (slice 1) and the first pond (slice 5). In scenario II, the chlorpyrifos effects on *Daphnia* were clear in the first pond but recovery occurred quickly: the population peak occurred later (day 250 instead of day 200) and was lower ($551 \cdot 10^2$ ind L^{-1} instead of $733 \cdot 10^2$ ind L^{-1}). A second population growth period at the end of the year (Figure 6.4) was no longer observed. For scenario II, chlorpyrifos effects on the predator *Chaoborus* were also mainly observed in the first pond e.g. the maximum density in slice 5 dropped from 397 ind L^{-1} to 114 ind L^{-1}. In scenario III, the direct effects of chlorpyrifos were lower and the same spatial trend was observed: clear effects in the first pond and no effects in the second pond.

For the detritus feeders, the densities of *Gammarus* were negatively affected in scenario I and effects were observed throughout the system (Figure 6.9). Gammarus completely disappeared in the first pond and the observed population growth in the second pond was only a fraction (<10%) of the control scenario. In scenario II, chlorpyrifos exposure led to the elimination of *Gammarus* in the first pond and severely affected the densities in the second pond, where maximum densities dropped from 1578 ind L^{-1} to 1066 ind L^{-1} for slice 15. In scenario III, although chlorpyrifos effects on *Gammarus* were lower than in scenario II, the differences between the first pond and the second pond were even greater than in scenario II: while chlorpyrifos effects were still observed in the first pond (52% reduction in density), these were negligible in the second pond.

Indirect effects

The expected indirect effects of chlorpyrifos were observed and reflected the spatial pattern of the direct effects (Figure 6.8-6.9). Indirect effects on *Brachionus* were absent in scenario I (Figure 6.8). In scenario II, the indirect effects on *Brachionus* only occurred in the inflow stream and the first pond and were limited because of the recovery of *Daphnia* after chlorpyrifos exposure. Chlorpyrifos effects on *Daphnia* also influenced the phytoplankton dynamics in scenario II: the first growth season of phytoplankton lasted longer in the first pond (from day 150 to day 230 versus from day 150 to day 190). In scenario

III, indirect effects on Brachionus were again limited to the inflow stream and the first pond and only resulted in a small increase in Brachionus density (<10%).

In scenario I, the densities of *Gammarus* were negatively affected but indirect effects on its competitor *Asellus* were not observed (Figure 6.9). In scenario II, however, *Asellus* densities increased strongly after chlorpyrifos emission in the inflow stream, first pond and connecting stream, leading to maximum densities of 5708 ind L^{-1} in slice 5 versus 116 ind L^{-1} in the control scenario. The sharp population decline in *Asellus* observed in the first pond around day 250 was a result of starvation. In scenario III, the spatial pattern of the direct effects on *Gammarus* was confirmed and (low) indirect effects of chlorpyrifos on *Asellus* were observed in slices 1 and 5 only.

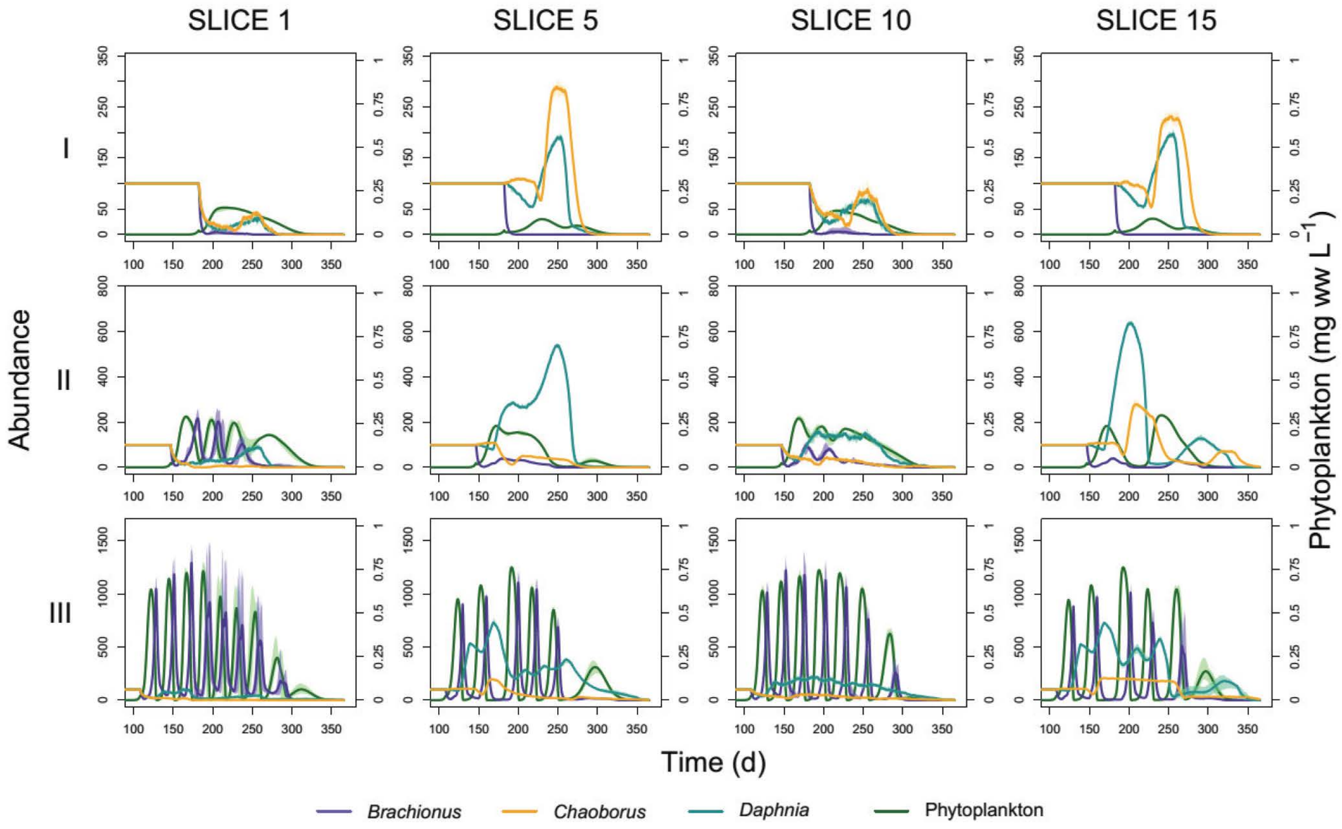


Figure 6.8: *Food web dynamics with chlorpyrifos exposure as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the phytoplankton-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.*

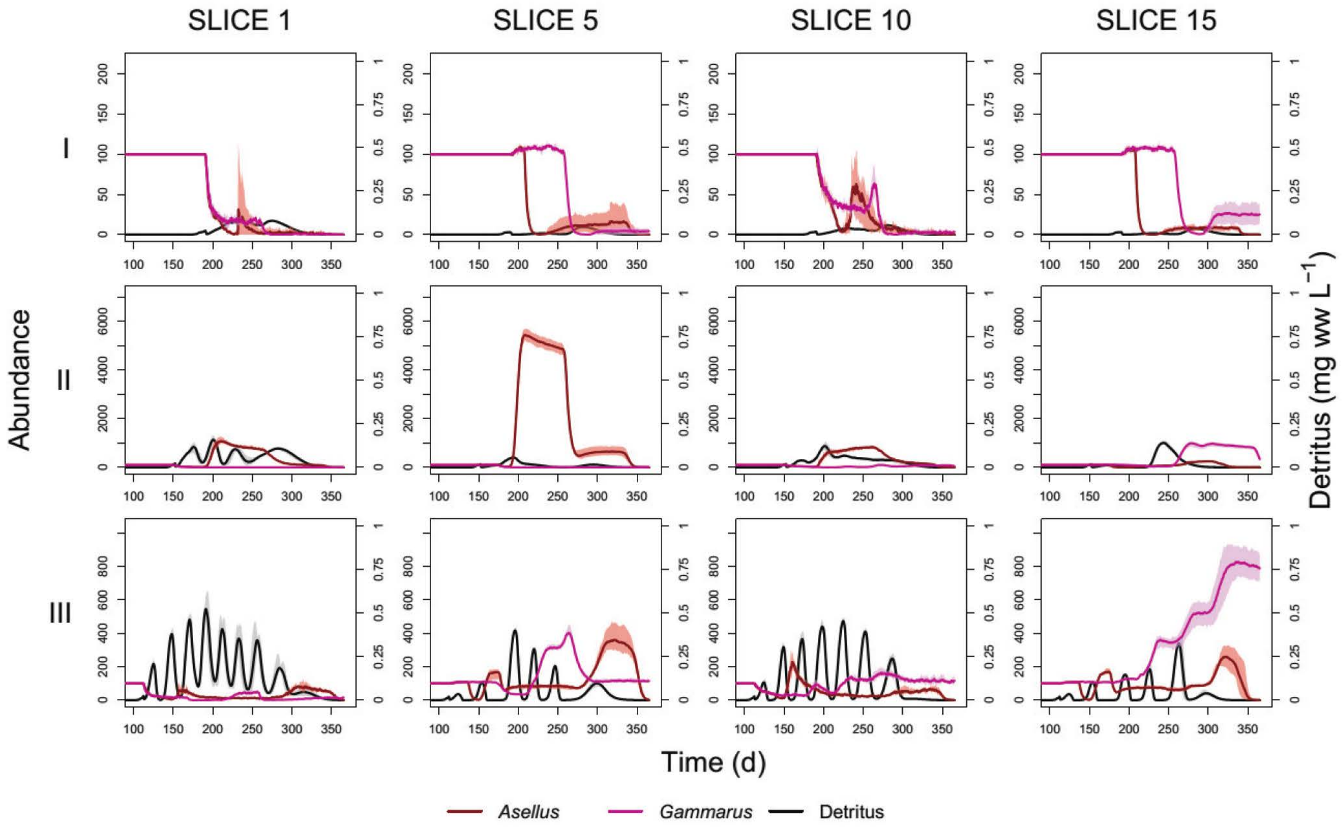


Figure 6.9: *Food web dynamics with chlorpyrifos exposure as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the detritus-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.*

6.3.2.4. Pyrene exposure

Direct effects

In scenario I, *Daphnia* population dynamics exposed to pyrene (Figure 6.10) followed the same pattern as in the control scenario (Figure 6.4). However, the maximum observed densities of *Daphnia* were lower than those in the control scenario ($131 \cdot 10^2$ ind L^{-1} versus $205 \cdot 10^2$ ind L^{-1}). In scenario II and especially scenario III, effects on *Daphnia* were higher than in scenario I and eventually daphnids were eliminated from the food web. Following the exposure pattern, effects occurred first close to the emission point (slice 1). Direct effects on *Gammarus* were absent (Figure 6.11): in none of the scenarios was there a decrease in *Gammarus* densities that could be directly related to the pyrene concentration and *Gammarus* concentrations were even higher in scenario III. The observed effects on *Gammarus* were mainly indirect effects (see below).

Indirect effects

In scenario I, although expected, the decreased *Daphnia* densities did not lead to higher *Brachionus* densities (Figure 6.10). In scenario II, the expected indirect effects of pyrene were observed: *Brachionus* was clearly more abundant than in the control scenario and three peaks in density were observed throughout the year. In scenario III, after the early disappearance of *Daphnia*, *Brachionus* was even more dominant than in the control scenario: peaks in population density were even higher and the number of peaks in density increased from 5 to 8. The predator *Chaoborus* was affected indirectly by pyrene in all three scenarios through the lower prey density. In scenario I, *Chaoborus* densities decreased from 314 ind L^{-1} in the control scenario to 158 ind L^{-1}. Both in scenario II and III, despite the increase in the other prey species *Brachionus*, *Chaoborus* disappeared once *Daphnia* was eliminated from the food web by pyrene.

The combination of direct effects on *Daphnia* and indirect effects on *Brachionus* altered the phytoplankton dynamics (Figure 6.10). This, in turn, affected the detritus dynamics, resulting in unexpected indirect effects on the detritus feeders (Figure 6.11). In scenario I, the altered detritus dynamics resulted in the absence of *Gammarus* population growth at the end of the year. In scenario II, the phytoplankton concentration was more constant in the ponds between day 175 and 275, resulting in more constant detritus concentrations and the absence of a peak in detritus concentrations compared to the control scenario (Figure 6.5). This resulted in lower *Gammarus* densities (only 464 ind L^{-1} compared to 1579 ind L^{-1} in the control scenario) but did not affect *Asellus* densities. In scenario III, the number of phytoplankton growth periods increased from 6 to 9, which was reflected in the detritus dynamics. As a result, both *Gammarus* and *Asellus* densities increased in comparison to the control scenario, from 1034 ind L^{-1} and 405 ind L^{-1} to 1401 ind L^{-1} and 545 ind L^{-1}, respectively.

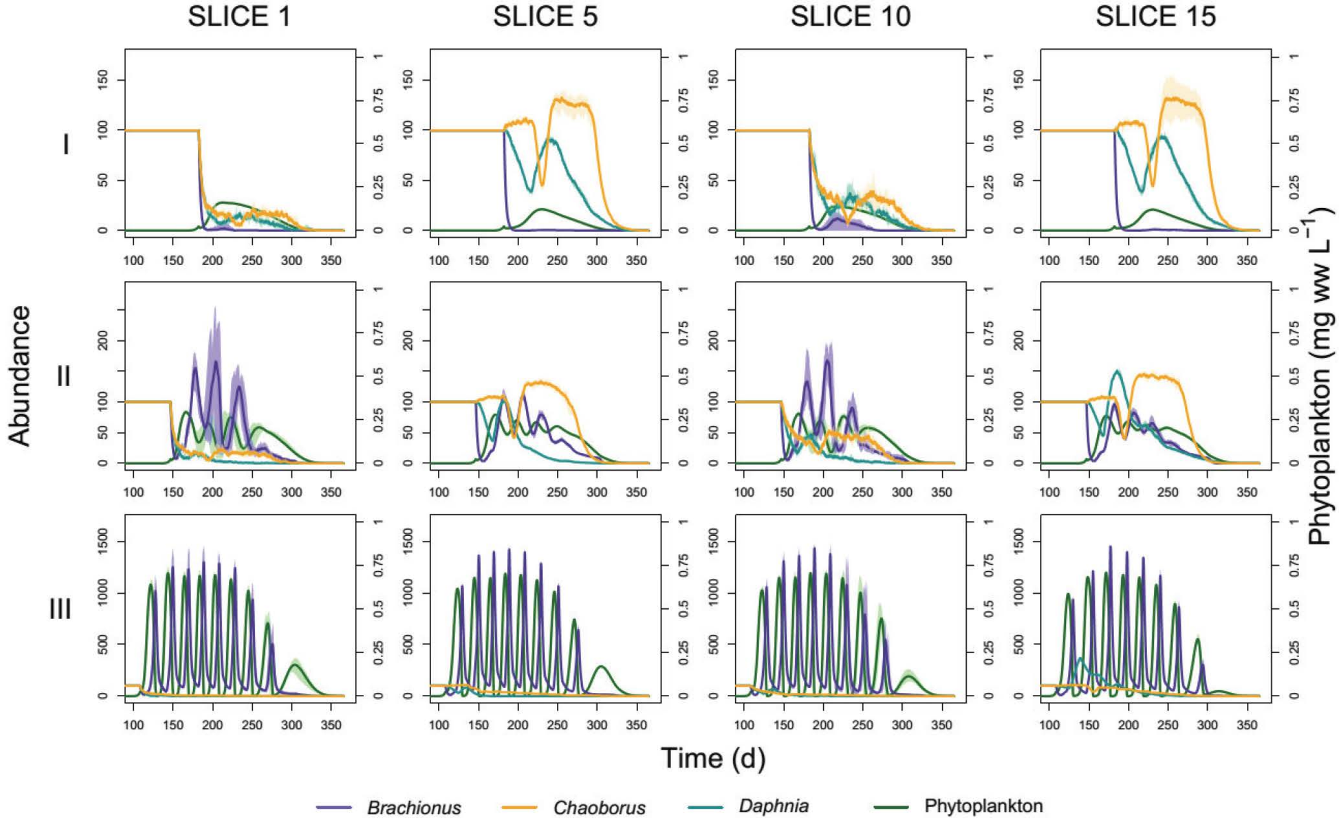


Figure 6.10: *Food web dynamics with pyrene exposure as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the phytoplankton-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.*

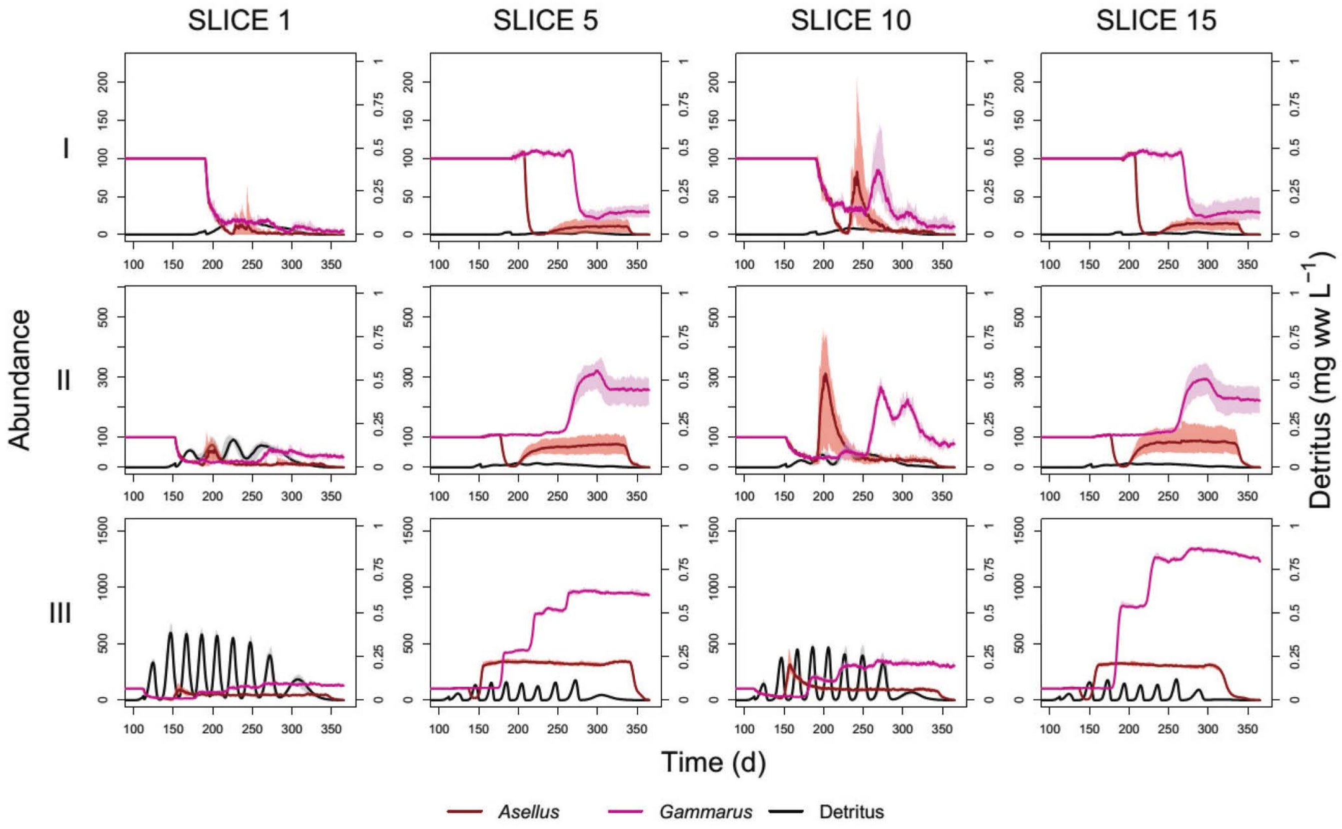


__Figure 6.11__: Food web dynamics with pyrene exposure as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the detritus-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.

6.3.2.5. Exposure to the mixture of all three chemicals

Direct effects

In scenario I, the pattern in *Daphnia* densities was similar to the control scenario (Figure 6.4) but the maximum densities were lower (Figure 6.12). In scenario II, *Daphnia* was eliminated quickly in the whole system with effects appearing earlier close to the emission source (slice 1). *Daphnia* disappeared from the first pond by day 200 and from the second pond by day 250. In scenario III, the effects of the mixture occurred even faster, with *Daphnia* disappearing from the first pond by day 150.

For *Gammarus* in scenario I, direct effects of the mixture on the densities were limited and only occurred after day 250 (Figure 6.13). In scenario II, the effects were more clear, although they only started occurring after carbendazim and chlorpyrifos were first applied (day 180): *Gammarus* disappeared from the system and this occurred first close to the emission point. Effects of the mixture on *Gammarus* were also clear in scenario III but *Gammarus* was able to persist longer in the second pond compared to scenario II.

Direct effects of the mixture for *Chaoborus* are expected to be similar to the direct effects of chlorpyrifos as this species is tolerant for the other two chemicals. Direct effects of the mixture on Chaoborus densities were not observed in scenario I (Figure 6.12). In scenario II, a sharp decrease in Chaoborus density is observed in the first pond (slice 5) after the application of chlorpyrifos and carbendazim (day 180). Direct effects in the second pond were limited. In scenario III, direct effects of the mixture were no longer observed and the population dynamics of *Chaoborus* were determined by the indirect effects of the mixture (see below).

Indirect effects

For *Brachionus* in scenario I, the indirect effects were negligible (Figure 6.12) because the densities were very similar to the control scenario (Figure 6.4). In scenario II, following the disappearance of *Daphnia* after chemical exposure, densities of the tolerant *Brachionus* increased in all slices and showed three distinct population peaks. In scenario III, indirect effects of exposure to the mixture of three chemicals resulted in an increase in *Brachionus* densities. Also, the frequency of the peaks in *Brachionus* density increased from 5 to 8 compared to the control scenario.

Through the strong direct and indirect effects of the mixture on the grazers, phytoplankton dynamics were altered. Because of the close link between phytoplankton and detritus, this resulted in indirect effects of the mixture on the detritus feeders. In scenario I, the altered detritus concentrations resulted in the absence of *Gammarus* growth at the end of the year (Figures 6.5 and 6.13). This did, however, not result in an increase in *Asellus* densities. In scenario II, the exposure to the mixture resulted indirectly in increased *Asellus* densities in the whole system and this increase was highest in the first pond (slice

5). In the case of scenario III, the increase of *Asellus* was only observed for the first pond. Also in scenario III, because of the altered detritus dynamics, *Gammarus* was able to reach higher densities in the second pond (1314 ind L^{-1}) compared to the control scenario (1034 ind L^{-1}).

In scenario I, *Chaoborus* densities were lower (Figure 6.12) than the control scenario (Figure 6.5) because of the decreased densities of *Daphnia*. In scenario II, in addition to the direct effects of the mixture, *Chaoborus* was indirectly affected through the effects on *Daphnia* and population densities were never higher than 100 ind L^{-1}. In scenario III, the effects of the mixture on *Daphnia* resulted in the elimination of *Chaoborus* even before carbendazim and chlorpyrifos were applied (day 180).

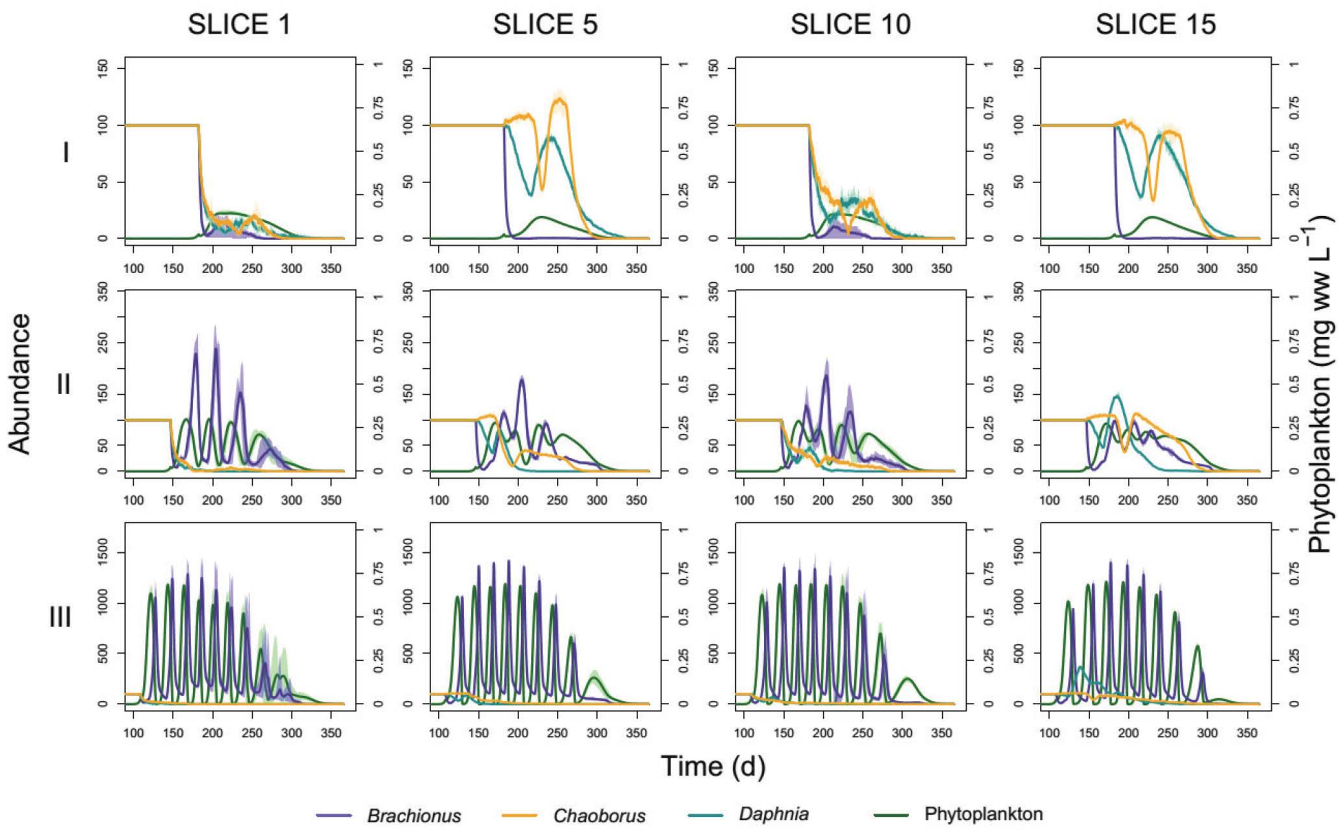


Figure 6.12: *Food web dynamics exposed to three chemicals as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the phytoplankton-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.*

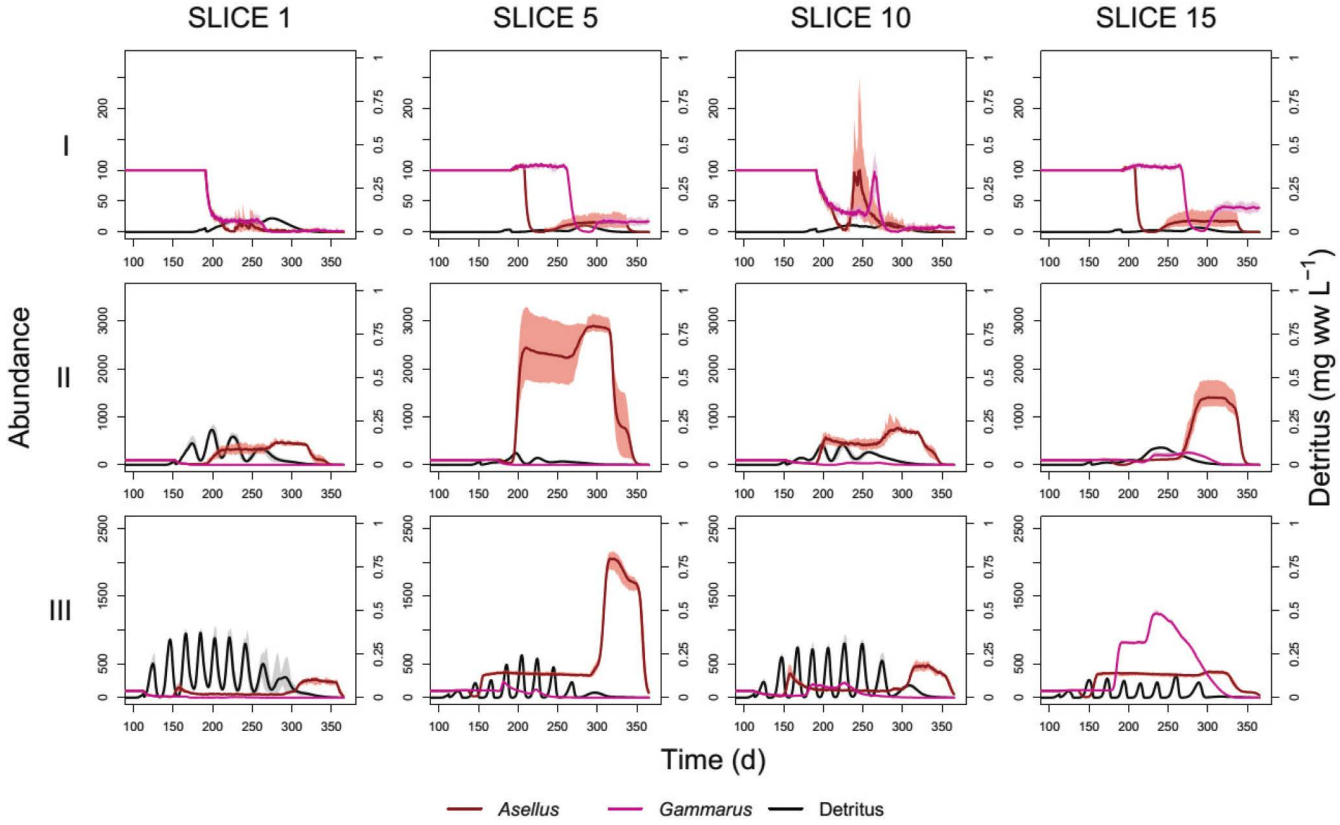


Figure 6.13: *Food web dynamics exposed to three chemicals as predicted by the ChimERA model for three environmental scenarios (scenarios I, II and III) and four selected locations in the two-pond system: at the inflow of the first pond (Slice 1), in the middle of the first pond (Slice 5), between the two ponds (Slice 10) and in the middle of the second pond (Slice 15). Only the detritus-based part of the food web is shown. Lines and shaded areas indicate the average and minimum and maximum densities, respectively, of five iterations per scenario.*

6.4. Discussion

6.4.1. ChimERA$_{fate}$ simulations

When comparing the fate model predictions among the different scenarios, the residence time –reflecting the removal of the chemical from the ponds via with the water (out)flow – was the most important factor. Higher residence times resulted in less dilution of the chemical i.e. more chemical was emitted in the same volume of water. This explains why higher water concentrations were observed (for all chemicals) in scenario III (residence time of 100 days) compared to the concentrations in scenario II and I (residence time of 25 and 5 days, respectively). As discussed in the expectations section (6.2.2.1), sediment accumulation of the chemical also increased with water residence time, decreasing the concentration in the water. Apparently, sediment accumulation was not strong enough to compensate for the positive effect of retention time on the water concentration. However, the effects of sediment accumulation were visible in the spatial patterns within the system: because most of the chemical accumulated in the sediment, the water concentrations of the hydrophobic chemical chlorpyrifos were much lower in the second pond than in the first pond and this was more expressed in scenarios with long residence times. The expected negative effect of a higher temperature – and the resulting increased phytoplankton and detritus biomass – and a higher organic matter concentration on the water concentration were not observed. These two processes were apparently not strong enough to compensate for the positive effect of a higher residence time.

The fate simulations for carbendazim differed substantially from the expectations (6.2.2.1). Despite the short peaks in emission, concentrations remained high for much longer than expected further away from the emission point in scenarios with higher residence times. Although no accumulation in sediment occurred for this hydrophilic chemical, diffusion into the sediment pore water and later again into the water column was an important fate process for carbendazim. This allowed for more constant dissolved water concentrations. More time for equilibrium between the sediment pore water and the water column was available in systems with high water residence times. This resulted in more diffusion into the sediment, explaining the stronger decrease in carbendazim water concentration further downstream in scenarios III than in scenario II and I.

As expected, because of the hydrophobic nature of chlorpyrifos, accumulation in the sediment occurred and this led to constant water concentrations even when emission had stopped. This was best visible in slice 1, where the emission occurred: once emission stopped, the water concentration quickly decreased to a low but relatively constant ambient concentration. Because most of the chlorpyrifos accumulated in the sediment, maximum chlorpyrifos concentrations were lower and reached later further downstream in the system, especially in scenarios with high water residence time. Simulated pyrene concentrations became stable over time, as expected, because of the constant emission strength. Water residence time and sediment accumulation determined how fast water concentrations stabilized: the emitted pyrene flowed downstream slower with increased water residence time and accumulation in the sediment further prolonged the stabilization of the water concentrations.

With the ChimERA$_{\text{fate}}$ model, we clearly showed that spatiotemporal differences in exposure were determined by the physico-chemical properties of the chemical and the environmental variables considered here (hydrodynamics, trophic state and temperature). Although environmental fate processes are well studied and can be described accurately with equations, the predicted patterns in exposure sometimes differed from our expectations e.g. for carbendazim. The relative importance of each environmental process is not always clear *a priori* and can change with the environmental conditions. Fate models are thus ideal tools to identify which chemicals will show a strong spatial pattern and in which environmental conditions spatial patterns will be most prominent. Identifying whether chemicals will only be present in high concentrations close to the emission source or also further downstream is an invaluable tool to predict the risk of the chemical.

6.4.2. ChimERA$_{\text{foodweb}}$ without chemical exposure

Both phytoplankton growth and maximum phytoplankton concentration increased with the increase in trophic state and temperature. As a result, grazer densities increased rapidly in eutrophic, high temperature environments such as scenario III. The higher trophic state and temperature in scenario III compared to the other two scenarios also led to a more dynamic system, with a typical cyclic pattern of phytoplankton growth, increase in grazer densities, phytoplankton decline and decrease in grazer densities. These faster food web dynamics in mesotrophic systems compared to those in oligotrophic systems were predicted before for a similar food web using ordinary differential equations to describe the biomass of the species in the food web (De Laender et al. 2015). Because *Brachionus* has a much shorter life cycle than *Daphnia*, the rotifers were able to increase in density more rapidly during phytoplankton growth periods, leading to the observed shift in dominance in scenario III compared to scenario I and II. However, this does not seem to reflect observations in real ecosystems, where daphnids are known to outcompete rotifers (Gilbert 1985; MacIsaac and Gilbert 1989). Other competitive mechanisms between large daphnids and rotifers have been suggested in literature e.g. through mechanical interference (MacIsaac and Gilbert 1989). As shown in Chapter 4, accounting for competitive exclusion was sufficient to simulate the outcome of competition between *Daphnia magna* and *Brachionus calyciflorus* for the experimental conditions used in Chapter 2. However, alternative mechanisms of competition could be more important when environmental conditions are more realistic than the controlled conditions used in Chapter 2. Because the relative importance of such alternative modes of competition was not known, they were therefore not implemented in the DEBkiss IBMs.

The current food web dynamics are clearly bottom-up regulated: grazer dynamics were limited by the phytoplankton, detritus feeders followed the detritus pattern and prey dynamics. Although they are able to prey on both *Brachionus* and *Daphnia*, *Chaoborus* was most abundant when *Daphnia* was dominant i.e. in scenario II. Seemingly, *Chaoborus* was thus not able to sustain high population densities in scenario III when maximum *Brachionus* densities were high. However, *Brachionus* densities also fluctuated strongly in scenario III. The lower *Chaoborus* densities in scenario III were probably related to the absence of a reserve compartment in the DEBkiss theory. When prey populations were high, energy uptake by *Chaoborus* was also high and this energy was immediately spent. When prey densities were low, the energy uptake by *Chaoborus* was insufficient. In reality, *Chaoborus* would first utilize energy from the reserve buffer in that situation (Kooijman 2010). However, since a reserve compartment is lacking in DEBkiss, energy is instead taken from the reproduction buffer or, when all energy in the reproduction buffer is spent, from structural biomass. This led to a much faster response to food shortage compared to reality, as discussed in Chapter 4. Daphnids show much less extreme population dynamics, serving as better prey species for the *Chaoborus* DEBkiss IBM.

Even without chemical exposure, there were notable spatial differences in food web dynamics: except for *Brachionus*, population densities were lower in the streams than in the ponds. This is a direct result of the way movement was implemented. Movement was implemented as a species-specific chance to move to a neighbouring water patch. Patches located in a pond were surrounded by more water patches than patches in a stream. Patches in a pond thus had a higher chance of receiving new individuals than patches in a stream. This results in highly different population dynamics between ponds and streams for mobile species. For example, the probability for *Daphnia* [1] to move to a neighbour water patch was 1 d^{-1}. As a result, daphnid density was much higher (e.g. up to five times for scenario II) in ponds than in streams. The lower *Daphnia* and *Chaoborus* densities in the streams meant less competition and predation pressure, allowing *Brachionus*, which had a lower movement chance (0.1 d^{-1}), to reach higher densities in the streams than in the ponds e.g. up to two times higher for scenario II.

6.4.3. ChimERA$_{\text{foodweb}}$ with chemical exposure

The magnitude of effects was directly linked to the concentrations that were predicted by the ChimERA$_{\text{fate}}$ model: clear differences in effects could be observed between the different environmental scenarios (I, II and III) and between the different locations in the system. Recovery of the phytoplankton-based part of the food web was observed for chlorpyrifos and carbendazim after the first emission peak (around day 200). The second emission peak, combined with the decreased food availability and the increased competition with the tolerant species (*Brachionus*), prevented recovery during the simulated period. Unsurprisingly, recovery was not observed for pyrene exposure because the concentration was relatively constant throughout the year. Recovery of the detritus-based part of the food web was observed to be much more difficult. This was mainly due to the longer reproduction times of the detritus feeders: i.e. these were at least 40 and 80 days between reproduction events for *Gammarus* and *Asellus*, respectively. Slower recovery of species with long life cycles or generation times has been raised as a concern for ERA before (De Lange et al. 2010; Rubach et al. 2010). By including species with different life cycle times or by adjusting the currently implemented generation times, the ChimERA model can be a tool to assess how life cycle duration affects food web recovery.

Simulations performed using the ChimERA model showed a strong spatial pattern, with effects of chemicals being absent, smaller or delayed in the second pond compared to those observed in the first pond. A good example of the absence of effects in the second pond is the exposure to chlorpyrifos in scenario II: the water concentrations in the first pond clearly caused a delay in daphnid population growth while the food web dynamics in the second pond were completely unaffected. Smaller effects in the second pond than in the first pond were, for example, observed for carbendazim in scenario III: *Gammarus* densities were less affected in the second pond compared to the first pond. Delayed effects were clearly visible for pyrene in scenario III: *Daphnia* populations in the second pond also became extinct but this occurred much later than in the first pond. Also, because *Daphnia* and *Chaoborus* tended to remain in the ponds and were much less abundant in the streams, the food web dynamics in the streams were already more determined by the tolerant *Brachionus* and effects of the chemical were generally less severe there. This highlights the need in a spatial landscape to account for the local conditions. Environmental variables such as temperature, light conditions and water flow strength are distributed heterogeneously in a landscape. Environmental conditions determine which species are successful and local food webs can thus differ greatly in their structure and composition. Chemical risk for a landscape is not homogeneously distributed but local differences will exist because exposure and food web vulnerability differ locally. The ChimERA model, although only applied here for a two-pond system, is a good example of how an integrated fate and effect model can be used to differentiate the local risks within a spatial system.

Indirect effects were prevalent in the ChimERA simulations and included indirect effects on competitors, on predators and on a food source (i.e. phytoplankton and detritus). Chemical effects on the sensitive species often led to increased densities of the tolerant species which became dominant in some cases. Especially the indirect effect of chlorpyrifos on *Asellus* densities in scenario II was striking. *Brachionus* typically also increased in density after chemical effects on *Daphnia* had occurred. This effect was, however, less drastic than those observed on *Asellus* and mainly occurred in scenario II. In scenario I, rotifers were quickly outcompeted by the daphnids, even before chemical effects became apparent, preventing possible indirect effects on *Brachionus*. In scenario III, rotifers were already quite high in density and indirect effects only resulted in small increases in density. A shift in dominance between competitors is often observed after chemical exposure. For example, increases in rotifer abundance after chemical effects on cladocerans were observed after the application of fenvalerate in lake enclosures (Day et al. 1987). However, rotifers can also be outcompeted by cladocerans after chemical exposure e.g. the cladoceran *Moina macrocopa* outcompeted the sensitive rotifer *Brachionus calyciflorus* after exposure to cadmium (Gama-Flores et al. 2006). Other examples of shifts in dominance after chemical exposure include the replacement of green algae by cyanobacteria and diatoms after fomesafen application (Caquet et al. 2005) or the increase in tolerant rotifers species after carbendazim effects on cladocerans and copepods (Van den Brink et al. 2000). Theoretically, it makes sense that an inferior competitor can be successful when the superior competitor is affected by chemical stress because less resources are consumed by the superior competitor. However, this is not always observed in experiments (Chapter 2). In the ChimERA simulations, even when the superior competitor was affected, other conditions were also important. For example, in the simulations performed for pyrene exposure in scenario I, effects of pyrene on *Daphnia* densities were not sufficiently high to prevent the elimination of *Brachionus*. Also, the food concentrations in scenario I were too low to sustain high densities of *Brachionus*, which was demonstrated in the streams where competition with *Daphnia* was almost absent but where *Brachionus* densities were still close to zero. Models like the ChimERA model can help to understand and predict when and why indirect effects occur.

Indirect effects of carbendazim and pyrene were observed on the predator *Chaoborus*. Although the maximum densities of *Brachionus* increased after chemical exposure, these rotifer densities fluctuated much more than *Daphnia* densities. With the current *Chaoborus* DEBkiss IBM implementation, it was difficult to simulate stable population densities when food availability is highly dynamic, as discussed before (see 6.4.2). Because of the high fluctuations in *Brachionus* densities, *Brachionus* was thus a less suitable prey for *Chaoborus*, leading to the observed indirect effect on *Chaoborus* densities: reduced population densities when *Daphnia* density was negatively affected by chemical stress and even extinction e.g in the case of pyrene exposure in scenario III. Altered *Daphnia* densities due to chemical stress lowered the *Chaoborus* densities but could also lead to a longer period of stable predator densities when daphnid densities recovered from chemical exposure e.g. after carbendazim exposure in scenario II.

Phytoplankton is the only source of detritus in the fate model. Therefore, it is expected that a chemical exposure affecting grazer dynamics will lead to changes in phytoplankton dynamics and as a result, in different detritus dynamics. For example, due to the pyrene exposure in scenario II, the phytoplankton dynamics shifted from two distinct peaks at day 175 and 250 in the ponds to a more constant phytoplankton concentration with four smaller peaks between day 150 and 250. This resulted in different detritus dynamics, ultimately also affecting the detritus feeders, in this case by preventing the strong increase in *Gammarus* density at the end of the year. Interestingly, shifts in grazer densities did not necessarily affect the detritus feeders negatively. For pyrene in scenario III for example, the dominance shift to *Brachionus* increased the amount of detritus available, resulting in higher *Asellus* and *Gammarus* densities. In reality, however, the main food sources of *Asellus* and *Gammarus* are not from phytoplankton origin. The diet of *Asellus* and *Gammarus* is much more diverse with leaf litter from external sources (terrestrial plants) often cited as the most important food source (Moore 1975). This was not considered in the ChimERA model and *Asellus* and *Gammarus* were thus much more dependent on phytoplankton dynamics than in reality. However, this did allow us to study the interactions between phytoplankton, detritus and associated consumers.

For the grazers and predator in the food web, the overall effect(s) of the mixture seemed to be dominated by the effects of pyrene: effects of the mixture on the grazers corresponded mostly with the effects of pyrene alone, with effects occurring almost immediately after the start of the simulated exposure. For the detritus feeders, however, this was not the case: direct effects of pyrene on *Gammarus* were limited in the pyrene only exposure, but effects of the mixture were clearly visible leading to a dominance of *Asellus*. Identification of the dominant chemical in the mixture was done by comparing which effects of the single exposure corresponded most to the effects of the mixture. For more complex food web models or mixtures, where more effects can occur, this approach is insufficient and alternative approaches need to be considered e.g. similarity indices such as the Bray-Curtis index (Bray and Curtis 1957). Similarity indices describe the composition of the community/food web with a single number. For example, the Bray-Curtis indices of the communities exposed to the mixture and of those exposed to the chemicals in the mixture individually could be compared. The Bray-Curtis index of the individual chemical closest to the index of the mixture then indicates in which community the effects of the individual chemical are most similar to the effects of the mixture i.e. which effect dominates.

Mixture toxicity at the community level can be very different from mixture toxicity at the level of individuals and populations. For example, the effects of a herbicide and an insecticide, affecting phytoplankton and grazers, respectively, are not necessarily additive (i.e. the sum of the individual effects) at the food web level. Synergistic effects at the food web level (i.e. higher effects than what is expected from the individual effects) could occur when the grazers are affected both by direct effects of the insecticide and by indirect effects due to herbicide effects on the phytoplankton. Antagonistic effects (i.e. smaller effects than what is expected from the individual effects) could occur when the phytoplankton is affected by the herbicide but is less grazed upon because of the adverse insecticide effects on the grazers. Models like the ChimERA model offer a unique way to account for this.

6.4.4. The ChimERA model as a risk assessment tool

The ChimERA model demonstrates how integrating a fate and food web model offers insights that cannot be obtained when running both models separately. Experimental/field data to compare our model simulations with were not available. However, the underlying models – ChimERA$_{\text{fate}}$ and ChimERA$_{\text{foodweb}}$ – have been tested: the ChimERA$_{\text{fate}}$ model was successfully applied in three case studies for a pond system (Morselli et al. 2015) and the application of the DEBkiss IBMs for competing species – used in the ChimERA$_{\text{foodweb}}$ model –was evaluated in Chapter 4. The simulations of the

ChimERA model were therefore considered realistic although it cannot be excluded that certain processes or mechanisms important for the integrated model were not incorporated.

The ChimERA model can be seen as a proof of principle study of how a coupled fate and effect model can be used as a realistic risk assessment tool incorporating more ecological and fate processes. The uniqueness of the ChimERA model is that it used the input of various abiotic and biotic variables and translated these into food web dynamics that varied greatly between the selected scenarios. This makes models such as the ChimERA model ideally suited to address the question of multiple stressors (SCHER et al. 2013; Gunderson et al. 2016): the ChimERA model provides an integrated prediction of all the stressors present in the environment. Although the ChimERA model should be further improved with e.g. additional environmental stressors (see 7.6), the integrated model (1) was able to provide realistic effects of chemicals on food web dynamics based on the modelling of individual species, (2) included spatiotemporal differences in exposure and effects on food web dynamics, (3) showed how the effects of a chemical depend on the selected scenario, (4) included a feedback mechanisms between the abiotic and biotic part of the system, (5) implemented an effect model capable of taking exposure history into account and (6) considered the simultaneous effect of multiple chemicals.

(1) Realistic effects of chemicals on food web dynamics

Starting from the effects of a chemical assessed under laboratory conditions, the ChimERA model was able to suggest how the chemical affects a hypothetical food web, taking into account both direct and indirect effects. Indirect effects, occurring through competition and predation, need to be accounted for in realistic ecological risk assessments (Fleeger et al. 2003). Indirect effects are very difficult to predict based on single species toxicity tests alone and are difficult to account for in mesocosm studies (Van den Brink and Ter Braak 1998). Ecological models have been suggested as good alternatives to account for these indirect effects (Galic et al. 2010) and the ChimERA model demonstrated here how this can be achieved: *Asellus* and *Brachionus* became dominant after chemical effects on *Gammarus* and *Daphnia*, respectively, and the predator *Chaoborus* was negatively affected by chemical effects on its prey *Daphnia*.

(2) Spatiotemporal differences in exposure and effects on food web dynamics

Although typically ignored in ecological risk assessments, the spatial structure of the system and the location of emission can influence the effects of a chemical, e.g. as observed in mesocosms (Brock et al. 2009) and predicted by models (Galic et al. 2012). Spatial structure will determine where and when exposure is occurring (Wickwire et al. 2011), what fraction of the system is affected by the chemical (Brock et al. 2009) and how fast the system can recover e.g. through immigration of biota from unaffected locations (Dohmen et al. 2015). Because the spatial structure is explicitly taken into account in the ChimERA model, the effects of the tested chemicals clearly differed between different locations in the system. The spatial dimension was not only important for differences in exposure. Differences in food web structure in different parts of the system were also observed.. For example, as a result of differences in movement potential of the different organisms, rotifers were more abundant and daphnids less abundant in the streams than in the ponds.

(3) The outcome of chemical exposure depends on the selected scenario

Although the amount of chemical emitted to the system was identical in all scenarios, exposure concentration and thus effects on the food web dynamics were highest in scenario III. The ChimERA model was able to translate differences in environmental variables to differences in (1) food web dynamics and (2) effects of chemical exposure. The three environmental scenarios studied here were only a limited selection of the many possible combinations of temperature, trophic state and hydrodynamics. Because of its flexibility, the ChimERA model structure can be tailored to specific real-world situations by adjusting these environmental inputs. Similarly, the structure of the food web and the species involved are exchangeable, thus allowing for adjustments to site-specific communities. The scenarios studied here were limited to one spatial pattern i.e. two connected ponds. However, the ChimERA model can be used to simulate scenarios for any kind of spatial structure and can thus be applied to answer specific questions. For example, it could be used to assess how the presence of a non-exposed part of the system affects the recovery of the food web.

The large number of possible scenarios can be confusing for risk assessors who need to decide e.g. if the selected scenarios are applicable and/or if the food web and species are realistic. During the development of Chimera, a similar problem was encountered for exposure assessment where there was also a multitude of environmental fate models and scenarios available. To solve this, environmental modelling was standardized and the FOCUS scenarios were developed (FOCUS 2001). A similar standardization of ecological and effect models will help facilitate the acceptance of ecological models as tools for effect assessment.

(4) Feedback mechanisms between the abiotic and biotic component of the system

The typical separate assessment of the fate and effects of chemicals ignores the fact that the environmental fate is also influenced by the biological/ecological component of the system, and *vice versa.* For example, the biomass of phytoplankton is an important factor for determining the bioavailable concentration of a hydrophobic chemical (Morselli et al. 2015). However, phytoplankton is also a component of the food web as it is consumed by grazers. Strong grazing pressure will limit the amount of phytoplankton biomass in the system thus reducing the accumulation of the chemical in the phytoplankton. The impact of this feedback mechanism was limited for the scenarios chosen here because the effect of phytoplankton biomass on the chemical concentrations was limited. Other biological compartments like macrophytes (Morselli et al. 2015) and zooplankton (Turner 2002) have been shown to influence the environmental fate of a chemical significantly and can be included in future scenarios.

(5) Effects of chemicals take earlier exposure into account

A TKTD model was used to predict the effects of chemicals on the survival of individuals. The nature of this type of model allows effects to occur even when the environmental concentration of the chemical have already decreased. This was visible e.g. for *Daphnia* exposed to chlorpyrifos in scenario II (Figure 6.8): at day 200, chlorpyrifos concentrations in the system had dropped to almost background concentrations but adverse effects persisted and a population increase was only observed after day 210. Although not tested here, TKTD models are especially useful when the emission is highly dynamic (Jager et al. 2011). Even for chemicals that are emitted very irregularly, the TKTD approach is able to predict the effects when only information of a typical toxicity test is available i.e. short term exposure to a constant concentration.

(6) Simultaneous effects of multiple chemicals

The current implementation of the ChimERA model is able to predict the effects of a mixture of chemicals, although the validity of the used mixture model is uncertain for the chemicals used in this study. Mixture toxicity is complex and the selection of the appropriate mixture toxicity model depends on the chemicals involved, although concentration addition was the preferred model in over 90% of pesticide mixtures (Deneer 2000). The mechanisms of mixture toxicity between carbendazim, chlorpyrifos and pyrene are unknown and an appropriate mixture toxicity model could therefore not be chosen. The mixture toxicity model used here, although possibly invalid, did allow us to explore how mixture toxicity effects may affect food web dynamics in comparison with single substance exposures. Interestingly, different chemicals impacted different species: *Daphnia* was mainly affected by pyrene while the effects of carbendazim and chlorpyrifos mainly affected *Gammarus*. Assessing the effects of multiple chemicals on the food web is an important feature of the ChimERA model. Traditionally, mixture toxicity studies focus on effects on the same organism. With the ChimERA model, mixture toxicity effects can be evaluated for the whole food web. For example, how will the food web be affected by the simultaneous exposure to a chemical affecting the grazers and one affecting the predators? The ChimERA model can provide an answer to such a question by e.g. predicting that the negative effects on the grazers are compensated by the reduced predation pressure, resulting in negligible effects of the mixture on the grazer density.

Ecological risk assessment needs new methods to deal with the large amount of chemicals to which ecosystems are possibly exposed and the increasing number of stressors that can interact with the effects of a chemical (SCHER et al. 2013; Gunderson et al. 2016). Modelling has been named as one of the most promising tools to face these challenges (SCHER et al. 2013). The simulations performed here with the ChimERA model are an innovative approach to risk assessment: environmental fate predictions of the chemical are immediately coupled to its ecological effects and the effects could be differentiated in space and time. However, models like the ChimERA model are so complex and offer so many options that it can be difficult for risk assessors to correctly use them. How can such models be used as regulatory instruments? Lessons can be learned here from how environmental fate models were accepted in Europe i.e. the FOCUS scenarios and models (FOCUS 2001). Before FOCUS was defined, a multitude of environmental fate models had been developed by scientists but risk assessors had no foundation to evaluate these models and were reluctant to use them. Intensive collaboration between the scientific community, industry and risk assessors led to the description of the FOCUS scenarios and a selection of accepted FOCUS models for these scenarios. The FOCUS scenarios were selected to represent a limited number of typical European situations such as a ditch system in The Netherlands or a typical Scandinavian pond. The FOCUS models are environmental fate models that have been extensively validated and are now accepted as 'approved' models to predict the environmental fate of a chemical in the FOCUS scenarios. When a new chemical is developed, these FOCUS models can be used to predict the environmental fate of the chemical in the FOCUS scenarios and thus be used in the exposure assessment of the chemical. Because of the standardization, risk assessors can evaluate the application of the FOCUS models, judge whether the environmental fate has been correctly assessed and ultimately if the chemical poses a risk to the environment. Also, when new fate models are developed, these can be tested with the FOCUS scenarios and compared with the validated FOCUS models. This allows new models to be more easily accepted – and thus used – by regulators.

A similar standardization is required for ecological and toxicity models before these models will be accepted by regulators and risk assessors. However, such standardization is hampered by the apparent lack of universal ecological rules and the lack of clear protection goals (Van den Brink et al. 2006). Question like what are acceptable effects in an ecosystem, should we focus on protecting biodiversity or ecosystem functioning (or both?) and do we allow effects if recovery occurs (but within what time frame?) need to be answered before standardized scenarios and models can be approved. This will require a large effort from both the scientific community, industry and regulators but is, I believe, absolutely necessary if we want ecological models and integrated models like the ChimERA model to be accepted as risk assessment tools.

7

GENERAL CONCLUSIONS AND FUTURE RESEARCH SUGGESTIONS

7.1. Introduction

To perform adequate ecological risk assessment for the large amount of chemicals produced each year, appropriate methods and procedures reflecting the environmental diversity and reality are needed. Overall, current methods are, however, inadequate and lack ecological realism. New methods are therefore needed and the use of ecological models has been suggested as a powerful tool to improve ERA. In this PhD thesis the potential role of ecological interactions, one of the key characteristics lacking from current ERA methods, was first studied experimentally. Individual based models (IBMs) were then developed to simulate and evaluate the patterns observed in these experiments. Next, the developed IBMs were integrated with an environmental fate model into the ChimERA model, capable of simulating spatially-explicit effects of chemicals on a food web in realistic conditions. Finally, the ChimERA model was tested as a scenario analysis tool.

The results of this PhD thesis are summarized in this chapter and suggestions for future research are given. Each paragraph starts with the research question addressed in each chapter, then presents a summary of the conclusions and finally suggests possible future research directions.

7.2. Species interactions and chemical stress: combined effects of intra- and interspecific interactions and pyrene on *Daphnia magna* population dynamics

The main research question addressed in Chapter 2 was how interactions within and between species – intra- and interspecific competition with Brachionus calyciflorus and predation by Chaoborus sp. larvae – influenced the effects of pyrene on populations of Daphnia magna.

Exposure to pyrene led to decreased *D. magna* densities and pyrene predominately affected smaller individuals. Predation pressure by *Chaoborus* sp. larvae and intraspecific competition limited the *D. magna* population densities but competition with *B. calyciflorus* had no significant effects on *D. magna*. Predation pressure and intraspecific competition altered the size structure of the *D. magna* population, reducing the fraction of small, more sensitive individuals in the population. As a result, predation and intraspecific competition both interacted antagonistically with pyrene exposure i.e. reduced the effects of pyrene. It can be concluded that, overall, interactions within and between species altered the effects of pyrene, highlighting the need to account for such interactions in ecological risk assessments.

Future research suggestions: *D. magna* is a much stronger competitor and quickly outcompetes *B. calyciflorus* in the used system. Probably, the influence of interspecific competition on the effects of a chemical depends on the strength of the competition. How the strength of competition alters the effect of the chemical can be studied by e.g. using a smaller daphnid species. Similarly, the strength of the predation pressure is expected to alter the effects of chemical exposure. This interaction can be studied by using different predator species or densities. The effects of pyrene only became apparent after at least one week of exposure. It would be interesting to see if more acute chemical effects (i.e. occurring sooner) interact with species interactions in a different way. In the experiment, pyrene mainly affected the prey (*D. magna*) and not the predator (*Chaoborus* sp. larvae). Applying a chemical that affects the predator and not the prey can result in very different results.

The effects of a chemical are not only dependent on the ecological interactions studied in this chapter i.e. competition and predation, but also on abiotic conditions. Multiple abiotic stressors can be present in the environment and influence the toxicity of a chemical (SCHER et al. 2013; Gunderson et al. 2016). Mixture toxicity of chemicals, for example, has received a lot of attention e.g. for metals (Jonker et al. 2005) and pesticides (Deneer 2000). Other, non-chemical stressors such as temperature (Scherer et al. 2013) can also greatly alter the effects of a chemical. Moreover, when and how often organisms are exposed to these stressors is an important determining factor of their effects (Gunderson et al. 2016). Chemical effects occurring in realistic conditions are thus much more complex than the controlled environment studied in the laboratory. Ideally, future research should focus on studying how this complex reality – including ecological interactions, multiple stressors and differences in timing – influences the toxic effects of a chemical and how these can be accounted for in ecological risk assessment. Alternative approaches to experimental work such as the models presented in this PhD thesis are useful to address these challenges.

7.3. Development of the DEBkiss IBM

In Chapter 2, I have shown that interactions between species are essential to understand how chemicals affect populations. The objective of Chapter 3 was to identify and develop a modelling approach that is able to account for species interactions when assessing effects of chemicals.

Individual-based models (IBMs) are ideal tools to simulate effects of chemicals on populations. These models, however, need to be based on a sound theoretical basis. Dynamic energy budget theory based on the keep it simple, stupid principle (DEBkiss) offers a good compromise between complexity and the amount of data required to parameterize such a model. DEBkiss IBMs were developed to account for species interactions when species are exposed to chemicals. Two possible methods to calculate the effects of a chemical were included: dose-response (DR) curves and toxicokinetic-toxicodynamic models. The applicability of the DEBkiss IBMs and two toxicity model was evaluated in Chapter 4.

7.4. Application of the DEBkiss IBM framework to assess the effects of competition and chemical stress on the population dynamics of *Brachionus calyciflorus* and *Daphnia magna*

In Chapter 4, I explored how the DEBkiss IBMs developed in Chapter 3 can be used to predict how species interactions influence population responses to chemical exposure. Therefore, I applied the DEBkiss IBMs developed in Chapter 3 to the experiments performed in Chapter 2.

DEBkiss IBMs were parameterized for *D. magna* and *B. calyciflorus* and compared to the experimental results of Chapter 2. Population dynamics of isolated *D. magna* populations were reasonably well predicted but the size structure of the population observed in Chapter 2 was not accurately predicted. This was attributed to the absence of an energy reserve compartment in the models, leading to faster starvation of the individuals when food is limited. The population dynamics of isolated *B. calyciflorus* were accurately predicted using the DEBkiss IBM. Pyrene effects were predicted to occur too early with both toxicity models but the TKTD model approached the observed effects best. The result of competition was accurately predicted when both DEBkiss IBMs were coupled to a shared food source: *D. magna* quickly outcompeted *B. calyciflorus*. Using the models to simulate competition when exposed to pyrene resulted in an increase of *B. calyciflorus* when *D. magna* densities decreased. This was not observed in the experiments and this can be explained by the fact that the predicted effects of pyrene occurred earlier in the simulations than in the experiments.

Future research suggestions: the absence of a reserve compartment is proposed as the most likely reason for the inability of the developed DEBkiss IBMs to predict the size structure of the *D. magna* population. Application of an IBM based on the full DEB theory, where a reserve compartment is included, would allow to evaluate this hypothesis. An IBM based on the full DEB theory is available for *D. magna* (Martin et al. 2013a) and could be applied to these experiments to test whether this would alleviate the problems observed with the DEBkiss IBM implementation. This full DEB IBM was successfully applied to similar experiments with *D. magna*, although an additional starvation rule was also required (Martin et al. 2013a). However, different *D. magna* clones can still differ significantly in their parameters (Baird et al. 1991) and the experimental conditions were not identical e.g. different algal food source and feeding regime. It is therefore unsure whether this DEB IBM could be applied without adjustments to the experiments of Chapter 2.

Ideally, the DEBkiss IBM implementation needs to be validated further. The models need to be compared with experiments where the same species were used in other conditions e.g. different food concentrations, different starting densities or exposure to different chemicals. This would greatly increase the acceptance of these models. Also, application of DEBkiss IBMs to other combinations of species where e.g. the strength of competition is different, would show the general applicability of DEBkiss IBMs to other species. The applicability for different types of species interactions (e.g. predation) could also be explored further.

7.5. Development of the integrated ChimERA model

The objective of Chapter 5 was to develop an integrated ecological risk assessment model. As a first step, a food web model was described based on the DEBkiss IBMs from Chapter 3. To provide realistic simulations of the effects of a chemical on food web dynamics, the food web model was integrated with an environmental fate model.

The ChimERA model was developed as a tool to perform more realistic ecological risk assessments. The ChimERA model integrates an environmental fate model ($\text{ChimERA}_{\text{fate}}$) and a food web model based on DEBkiss IBMs ($\text{ChimERA}_{\text{foodweb}}$). $\text{ChimERA}_{\text{fate}}$ is a dynamic and spatially explicit fate model that predicts environmental concentrations based on environmental variables (hydrodynamics, temperature and trophic state). $\text{ChimERA}_{\text{foodweb}}$ was developed based on the earlier implementation of competition between DEBkiss IBMs and a newly implemented predation interaction. The food web model included *Brachionus* and *Daphnia* as grazers, *Chaoborus* as a predator and *Asellus* and *Gammarus* as detritus feeders. The ChimERA model was used in Chapter 6 to evaluate the effects of chemicals on food web dynamics for different hypothetical scenarios.

7.6. The ChimERA model as a scenario analysis tool

Models have been suggested as good alternatives for current risk assessment methods. In Chapter 6, I explored how the developed ChimERA model could be used to predict the risk of chemicals for a range of different environmental conditions.

The ChimERA model was applied to a two-pond system in 15 hypothetical scenarios, differing in water residence time, temperature, trophic state and the applied chemical. In general, the ChimERA model predicted food web dynamics and effects of chemicals that varied greatly between the 15 scenarios considered. Densities of the species were highest in scenarios with a high trophic state, high temperature and high water residence time. Daphnids were the dominant grazers in the ponds while rotifers dominated in the streams. *Gammarus* was the dominant detritus feeder. Concentrations of applied chemicals (carbendazim, chlorpyrifos and pyrene) were highest in scenarios with a high water residence time. The physico-chemical properties of the chemical determined the spatio-temporal pattern of the exposure, i.e. where the concentrations were highest and how fast the chemical disappeared. Direct effects of the chemicals on *Chaoborus, Daphnia* and *Gammarus* densities were predicted, as expected from their sensitivities to these chemicals. These effects were, however, heterogeneously distributed in space and time, following the differences in exposure. The most notable indirect effects were a shift in dominance from *Daphnia* and *Gammarus* to *Brachionus* and *Asellus*, respectively. Also, the predator *Chaoborus* was affected indirectly through effects on its prey species *Daphnia*. The effects of the mixture of three chemicals differed between species, where the effects of pyrene dominated for *Daphnia* and the effects of carbendazim and chlorpyrifos for *Gammarus*.

Future research suggestions: although the current work gives a good overview of the potential use of an integrated fate and effect model for risk assessment, further steps are needed before the model can be applied as a risk assessment tool. The suggested steps can be divided in three categories: more complexity, more testing and guidance on the model output.

(1) More complexity

Multiple stressors and the timing of exposure to these stressors have been raised as a critical points missing in current ecological risk assessment (SCHER et al. 2013; Gunderson et al. 2016). The ChimERA model presented in this thesis offers a good first step on how these can be accounted for. However, although the ChimERA model is already able to account for realistic exposure scenarios, the complexity of the submodels is limited and probably needs to be increased further to better simulate reality. Some key areas where complexity can be improved are discussed here.

The DEBkiss IBMs currently used in the food web model are most likely insufficient to accurately simulate the full life cycles of *Asellus*, *Chaoborus*, *Gammarus* and *possibly* also *Daphnia*. DEBkiss IBMs were used here because of their transparency and simplicity. This allowed for an easy interpretation of the food web dynamics and resulted in reasonable the computation times. Although their parametrization was based on a verified database and literature sources, the DEBkiss implementation has not been tested extensively against actual observational data. However, given the problems with the DEBkiss implementation for *Daphnia* (Chapter 4), it is likely that similar problems with e.g. the absence of a reserve compartment would arise for other species. The ChimERA framework was developed as a very flexible tool and, as long as the same information is exchanged with the server, other IBMs can be implemented in the food web. However, a tested implementation of the full DEB theory into an IBM is only available for *Daphnia* (Martin et al. 2012). Adaptation and validation of this implementation for the other species was not in the scope of this research. Validated IBMs not based on DEB theory are available for *Asellus* (Van den Brink et al. 2007), *Chaoborus* (Strauss et al. 2016) and *Gammarus* (Galic et al. 2014). However, these implementation do not always explicitly consider food and the use of different underlying theories could hinder interpretation.

The complexity of several ecological processes included in the ChimERA model can be improved, of which a few will be discussed here in detail. One example of such an ecological process deals with the movement of individuals within the spatial structure. In the current simulations, random movement was assumed and this was shown to cause mobile individuals to accumulate in the ponds. However, movement of individuals is not random and is dependent on the prevalent conditions. For example, the spatial distribution of *Gammarus* is highly dependent on the stream conditions (Adams et al. 1987), *Chaoborus* larvae move to other regions when prey become limited (Liljendahl-Nurminen et al. 2002) and *Daphnia longispina* are known to actively avoid contaminant exposure (Lopes et al. 2004). Movement within the system is thus dependent on many species-specific, often unknown, factors , making the modelling of realistic movement very complex and not within the scope of this PhD thesis. However, it could be worthwhile to explore this for species for which there is a known relationship between movement and an environmental factor.

The selection and quality of food is another example of complexity that could be added. Food in the current implementation of the ChimERA model is limited to a very general description of phytoplankton (or detritus). However, in reality, phytoplankton consists of many species belonging to different algal classes. Grazers prefer to eat certain phytoplankton species and phytoplankton species differ in their nutritional value for the grazer. It is likely that differences in food selectivity and food quality (nutritional value) will impact the energy budgets of the individual species. Modelling multi-species grazing e.g. similar to how multi-species predation was added to the ChimERA model would allow to explore the implications of increasing the complexity of the phytoplankton compartment. Similarly, the detritus consists of many fractions, also from non-algal origin, and accounting for this will increase the realism of the models.

Similarly, increasing the complexity of the fate model can be considered by including additional parameters and processes of importance. The fate model at the moment does not consider high frequency fluctuations in water chemistry parameters that can be important for the environmental fate of the chemical. For example, pH influences several environmental fate processes such as adsorption to soil and degradation (Vala Ragnarsdottir 2000) but high frequency (e.g. day-night) fluctuations are not included in $\text{ChimERA}_{\text{fate}}$. Inclusion of such processes will further increase the accuracy of the fate model. Another important improvement to the chemical fate model would be the addition of chemical effects on the phytoplankton dynamics. For the three chemicals studied, phytoplankton effects were considered negligible. However, for other chemicals such as herbicides, effects on phytoplankton are likely. Because phytoplankton dynamics in the fate model are modelled using an ordinary differential equation (Equation 5.1), the effects of chemicals could be included easily by multiplying the phytoplankton growth rate with a concentration-response term (De Laender et al. 2015).

Further development of the ChimERA model, or similar models, should strive to increase the spatial scale and the simulated period. Current application was limited to two connected ponds simulated for one year. Increasing the spatial scale and the simulated period will provide more in-depth, realistic ecological risk assessments. Questions such as what are the multi-annual effects of a chemical? Or how will a different spatial scale and structure affect the effects of a chemical e.g. when multiple isolated ponds are located within a larger area? could be addressed. Also, new methods in ecology could be used to further increase the relevance of the risk assessment. For example, geographic information system (GIS) approaches have become popular in (landscape) ecology e.g. for fire risk assessment (Chuvieco et al. 2010). Combining a GIS approach with the ChimERA model could lead to ecological risk assessments for real-world situations.

Finally, it would be interesting to explore the effects of chemicals for other food web types. The current ChimERA$_{\text{foodweb}}$ model only considers five species and three trophic levels (phytoplankton/detritus, grazer/detritivore and a predator). Both direct and indirect effects of a chemical could be totally different when e.g. there are more trophic levels and more trophic links in the food web. Comparing effects between these food webs will allow more insight into how chemicals can affect food web dynamics.

(2) More testing

Parts of the ChimERA model were tested: e.g. DEBkiss IBMs for two competing grazers (see Chapter 4) and the ChimERA$_{\text{fate}}$ model (Morselli et al. 2015). However, the applicability of ChimERA for more complex cases needs to be tested further by comparing the simulations with observational data. The DEBkiss IBMs have only been applied for species with a short life cycle. The applicability for species that live multiple months and even years needs to be further tested. Also, the DEBkiss IBMs were only compared with data for two interacting species (Chapter 4). In real food webs, interactions occur between more than two species. To test how well more complex species interactions are modelled, DEBkiss IBMs could be compared to the results of experiments in the lab with simplified food webs, e.g. including multiple grazers and predators.

(3) Guidance on the model output

The interpretation of the output of complex models such as the ChimERA model is a challenge. For non-experts, the model output can be intimidating and confusing. To increase the acceptance of the ChimERA model, clear guidance needs to be provided on how the model works and how the output should be interpreted. For the ChimERA model to be used as a risk assessment tool, further definition is required of which endpoints should be used and what effects are considered acceptable. This is of course strongly dependent on what regulators require and input from them is therefore pivotal at this point. Additionally, good modelling practice and standardized model documentation protocols such as TRACE (Schmolke et al. 2010) are essential in this regard to increase the transparency and general acceptance of models in general and the ChimERA model specifically.

7.7. Overall contribution of this PhD thesis to the future of ecological risk assessment

This work has shown the importance of approaching chemical risk assessments from an ecological perspective. Species are not isolated entities but interact and influence the outcome of chemical exposure. The ChimERA model developed and applied here is an important proof of principle on how these interactions can be accounted for. In addition, the model also accounted for spatiotemporal differences in exposure and effects of chemicals. The ChimERA model, when further tested, validated and extended with more mechanisms, can serve as a blueprint for future efforts to model how chemicals affect ecosystems. The integration of a fate and effect model demonstrated the strength of simultaneously accounting for both the exposure and effects of chemicals when assessing potential ecological risks.

A

SUPPORTIVE INFORMATION CHAPTER 2

Table A1: *Percentage of the total variance explained by pyrene exposure and intraspecific competition. Percentages shown are calculated for the optimal GLM for log10-transformed total D. magna abundance after backwards model selection on the intraspecific dataset. Non-significant predictor variables are indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
Pyrene	/	13.7	10.4	2.1	16.6	12.3	75.4	32.1
Intraspecific	71.0	46.7	21.9	20.4	40.3	38.5	0.9	21.6
Pyrene X Intraspecific	/	/	/	23.2	13.9	/	8.4	/

Table A2: *Percentage of the total variance explained by of pyrene exposure and interspecific competition. Percentages shown are calculated for the optimal GLM for log10-transformed total D. magna abundance after backwards model selection on the interspecific dataset. Non-significant predictor variables are indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
Pyrene	/	19.5	/	/	5.1	23.1	81.3	24.0
Interspecific	/	12.0	/	51.8	55.8	32.1	4.0	14.0
Pyrene X Interspecific	/	/	/	/	9.6	/	/	18.2

Table A3: *Percentage of the total variance explained by of pyrene exposure and predation. Percentages shown are calculated for the optimal GLM for log10-transformed total D. magna abundance after backwards model selection on the predation dataset. Non-significant predictor variables are indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
Pyrene	/	/	/	/	/	/	45.1	13.3
Predation	/	11.4	51.8	42.4	46.8	54.6	19.5	55.0
Pyrene X Predation	/	/	/	/	/	/	16.8	/

Table A4: *GLM estimates of pyrene exposure and intraspecific competition for log10-transformed adult D. magna abundance after backwards model selection. For each time point, the significant estimates (p < 0.05) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	0.57	1.02	1.11	1.22	1.40	1.48	1.69	1.73
Medium pyrene	/	/	/	/	/	0.10	/	/
High pyrene	0.12	/	/	/	-0.12	/	-0.37	-0.18
Low intraspecific	0.30	0.24	0.19	0.16	0.08	/	/	/
High intraspecific	0.46	0.41	0.37	0.32	0.25	0.08	/	/

Table A5: *GLM estimates of pyrene exposure and intraspecific competition for log10-transformed juvenile D. magna abundance after backwards model selection. For each time point, the significant estimates ($p < 0.05$) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	0.74	0.56	1.15	1.57	1.89	1.79	1.56	1.08
Low pyrene	/	/	/	/	-0.17	/	/	0.44
High pyrene	/	/	/	/	/	/	-0.70	/
Low intraspecific	0.22	/	/	/	-0.24	-0.14	/	/
High intraspecific	0.49	0.57	0.27	-0.21	-0.33	-0.28	/	/
Low pyrene X Low intraspecific	/	/	/	/	0.32	/	/	/
Medium pyrene X High intraspecific	/	/	/	0.28	/	/	/	-0.63

Table A6: *GLM estimates of pyrene exposure and intraspecific competition for log10-transformed neonate D. magna abundance after backwards model selection. For each time point, the significant estimates ($p < 0.05$) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	/	/	1.25	1.47	1.47	0.69	/	-0.52
Medium pyrene	/	0.79	/	/	/	0.32	/	/
High pyrene	/	0.87	/	/	/	0.33	/	1.03
Low intraspecific	0.51	/	/	-0.39	-0.34	/	/	/
High intraspecific	0.75	/	-0.71	-0.75	-0.61	-0.55	/	/
High pyrene X Low intraspecific	/	-1.37	/	/	/	/	/	/
High pyrene X High intraspecific	/	-1.02	/	/	/	/	/	/

Table A7: *GLM estimates of pyrene exposure and interspecific competition for log10-transformed adult D. magna abundance after backwards model selection. For each time point, the significant estimates ($p < 0.05$) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	0.61	1.03	1.14	1.20	1.42	1.49	1.72	1.77
Medium pyrene	/	/	/	/	/	0.10	/	/
High pyrene	/	/	/	/	-0.09	-0.10	-0.57	-0.22
Low interspecific	/	/	/	0.13	/	/	/	/
High pyrene X High interspecific	/	/	/	/	/	/	/	-0.29

Table A8: *GLM estimates of pyrene exposure and interspecific competition for log10-transformed juvenile D. magna abundance after backwards model selection. For each time point, the significant estimates (p < 0.05) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	0.79	0.56	1.21	1.56	1.89	1.83	1.59	1.06
Low pyrene	/	/	/	/	-0.17	/	/	/
High pyrene	/	/	/	/	/	-0.14	-0.81	0.42
Low interspecific	/	0.35	/	/	/	-0.15	-0.32	-0.42
High interspecific	-0.35	0.32	/	-0.14	-0.27	-0.16	-0.23	/
Low pyrene X Low interspecific	/	/	/	/	0.22	/	/	/
Low pyrene X High interspecific	/	/	/	/	0.23	/	/	/
High pyrene X High interspecific	0.59	/	/	/	/	/	/	/

Table A9: *GLM estimates of pyrene exposure and interspecific competition for log10-transformed neonate D. magna abundance after backwards model selection. For each time point, the significant estimates (p < 0.05) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	-0.48	/	1.15	1.44	1.52	0.57	/	-0.81
Medium pyrene	0.56	/	/	/	/	0.56	/	/
High pyrene	/	/	/	/	/	/	/	1.79
Low interspecific	-0.59	/	/	-0.38	-0.49	/	/	/
High interspecific	/	/	/	-0.41	-0.57	-1.22	/	/
High pyrene X Low interspecific	/	-1.58	/	/	/	/	/	/
Low pyrene X High interspecific	/	/	/	/	/	1.21	/	/
High pyrene X High interspecific	/	/	/	-0.44	/	1.00	/	/

Table A10: *GLM estimates of pyrene exposure and predation for log10-transformed adult D. magna abundance after backwards model selection. For each time point, the significant estimates (p < 0.05) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	0.67	1.02	1.11	1.22	1.36	1.49	1.67	1.76
High pyrene	/	/	/	/	/	/	-0.36	-0.16
Predation	/	-0.20	-0.22	-0.22	-0.18	/	/	-0.16

Table A11: *GLM estimates of pyrene exposure and predation for log10-transformed juvenile D. magna abundance after backwards model selection. For each time point, the significant estimates ($p < 0.05$) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	0.67	0.55	1.01	1.56	1.89	1.79	1.56	1.27
Medium pyrene	/	/	/	/	-0.28	/	/	/
High pyrene	/	/	/	/	/	/	-0.70	/
Predation	/	/	/	-0.34	-0.36	-0.37	-0.50	-0.33

Table A12: *GLM estimates of pyrene exposure and predation for log10-transformed neonate D. magna abundance after backwards model selection. For each time point, the significant estimates ($p < 0.05$) of explanatory variables and their interactions are shown. Non-significant predictor variables are not shown (if never significant) or indicated with "/".*

Time (days)	**-4**	**0**	**2**	**4**	**7**	**10**	**15**	**22**
(Intercept)	/	/	1.25	1.51	1.47	0.57	-0.42	-0.57
High pyrene	/	0.79	/	/	/	/	/	0.96
Predation	/	/	-0.67	-1.03	-0.80	/	/	/
Low pyrene X Predation	/	/	/	/	/	-1.07	/	/

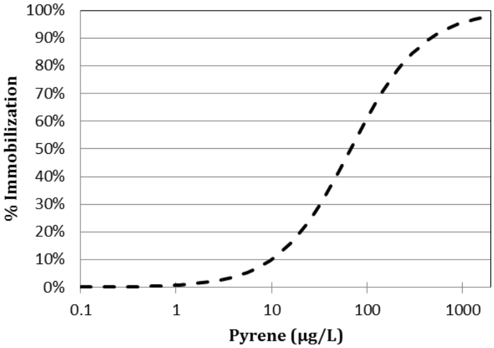


Figure A1. *Optimal concentration response curve for pyrene fitted to the results of a 48 hours test with neonate D. magna.*

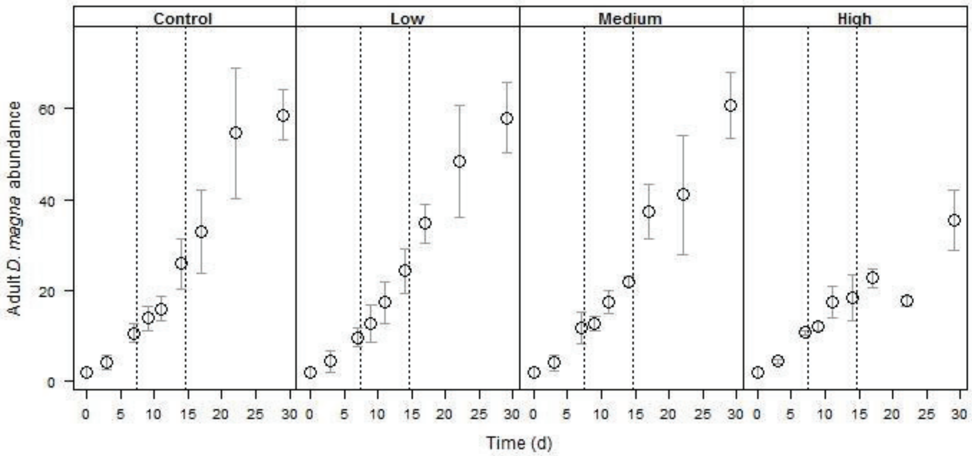


Figure A2. *Adult D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the adult D. magna abundances with no additional species interactions. Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

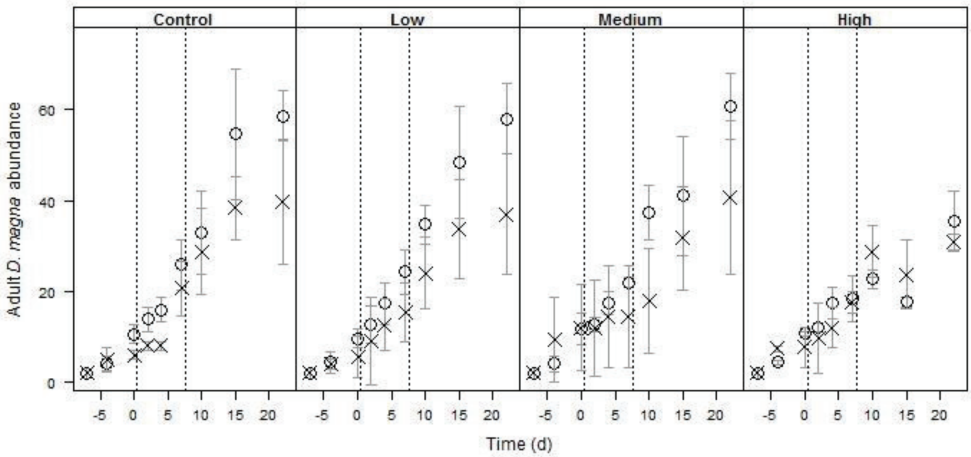


Figure A3. *Adult D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments without (points) and with predation (crosses). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

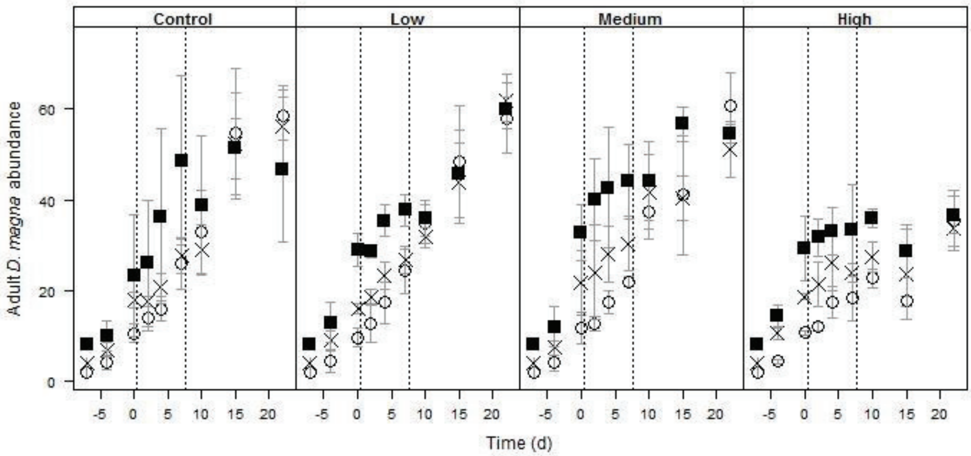


Figure A4. *Adult D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments with no additional species interactions (points), low intraspecific competition (crosses) and high intraspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

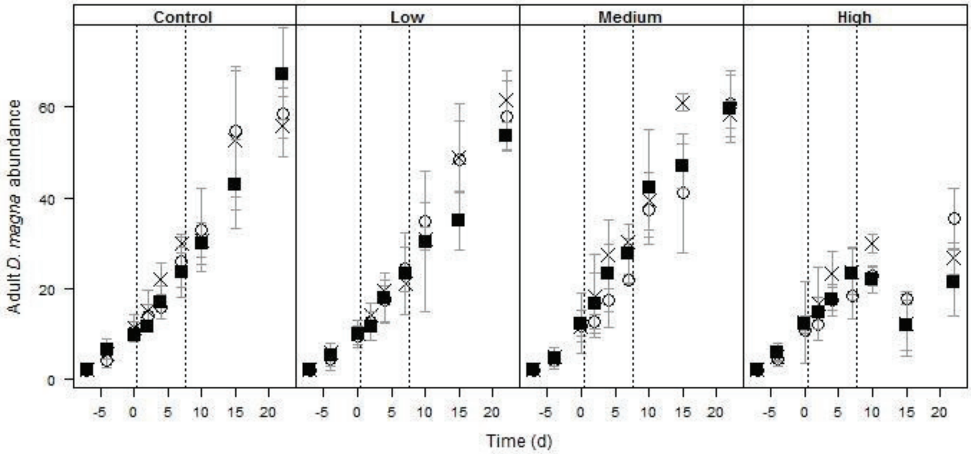


Figure A5. *Adult D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments with no additional species interactions (points), low interspecific competition (crosses) and high interspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

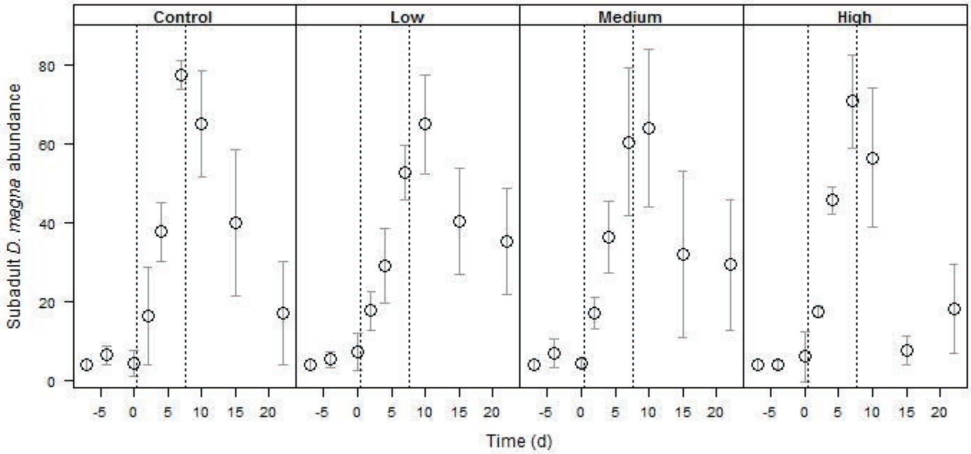


Figure A6. *Juvenile D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the juvenile D. magna abundances with no additional species interactions. Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

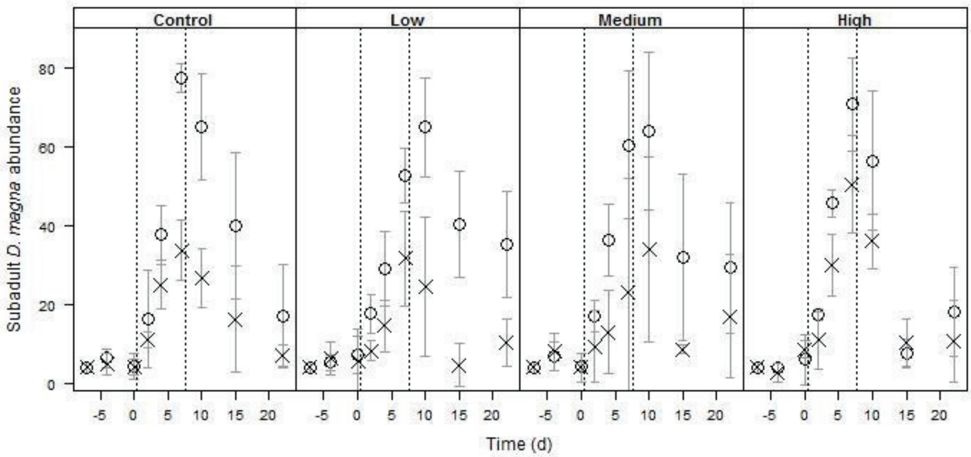


Figure A7. *Juvenile D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments without (points) and with predation (crosses). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

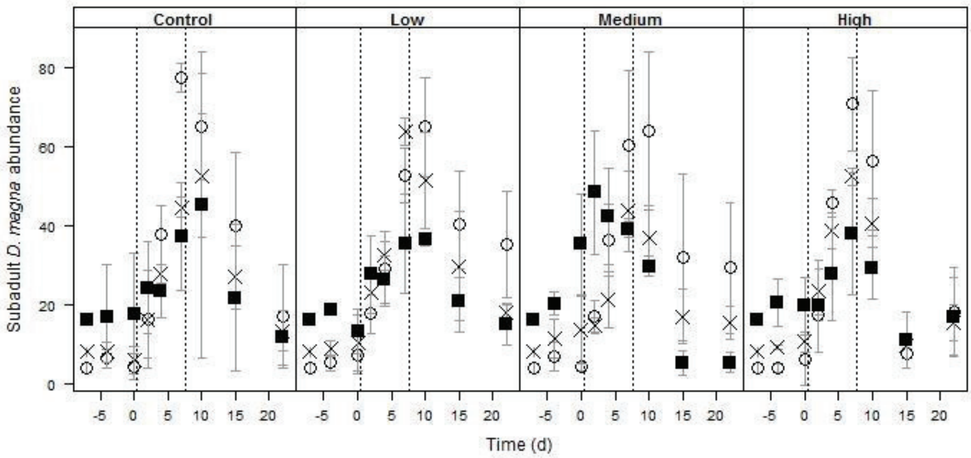


Figure A8. *Juvenile D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments with no additional species interactions (points), low intraspecific competition (crosses) and high intraspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

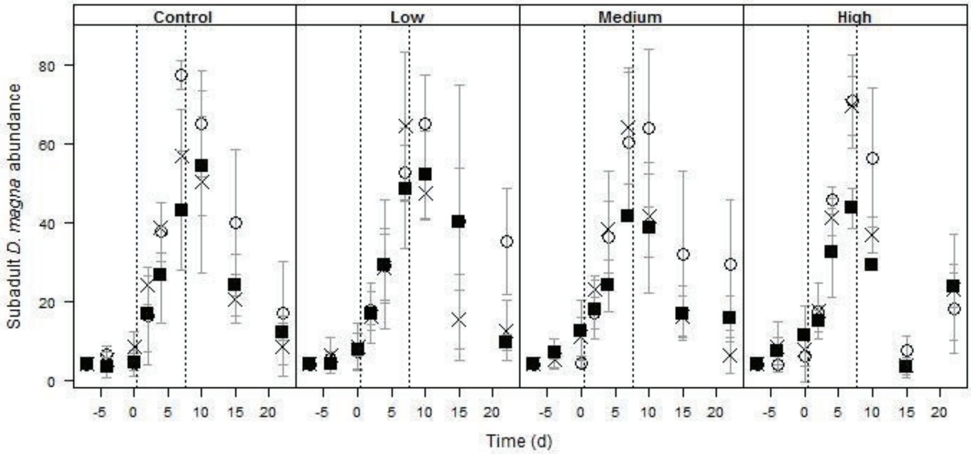


Figure A9. Juvenile D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments with no additional species interactions (points), low interspecific competition (crosses) and high interspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.

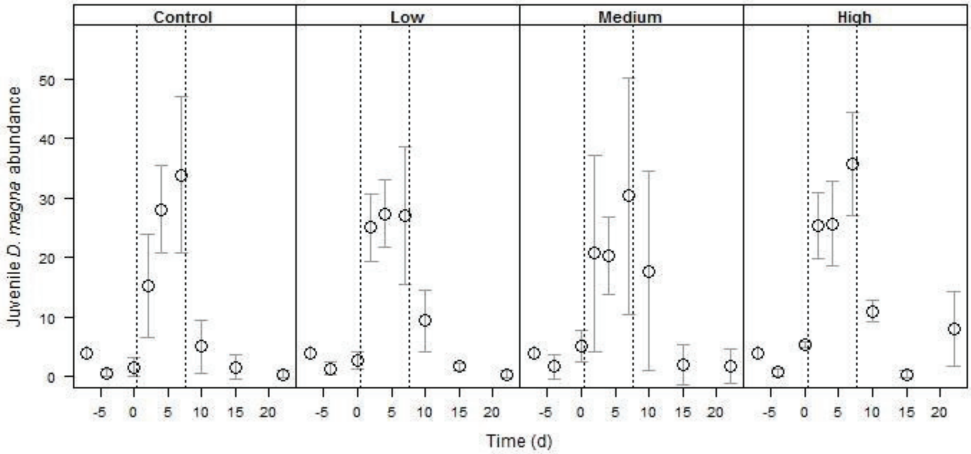


Figure A10. Neonate D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the neonate D. magna abundances with no additional species interactions. Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.

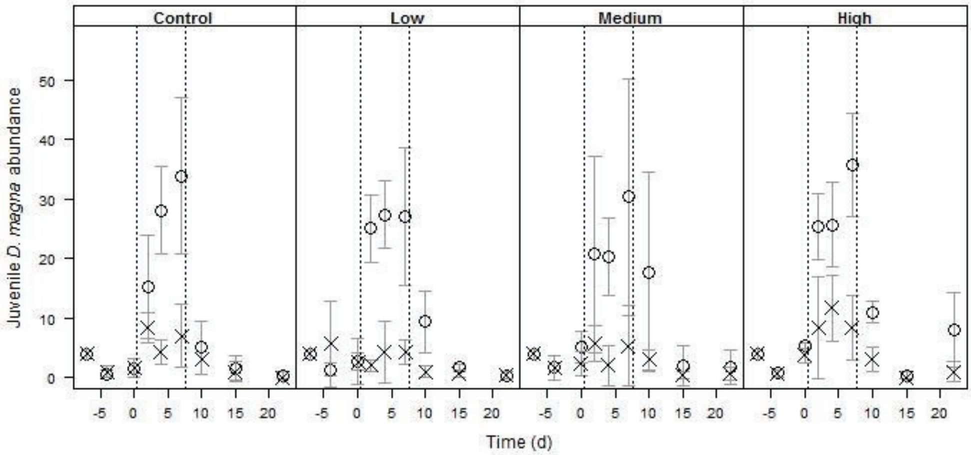


Figure A11. *Neonate D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments without (points) and with predation (crosses). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

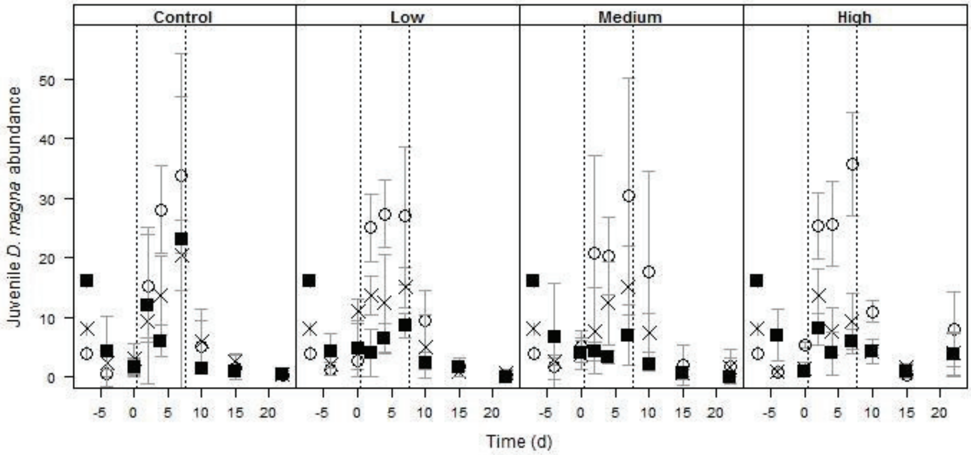


Figure A12. *Neonate D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments with no additional species interactions (points), low intraspecific competition (crosses) and high intraspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

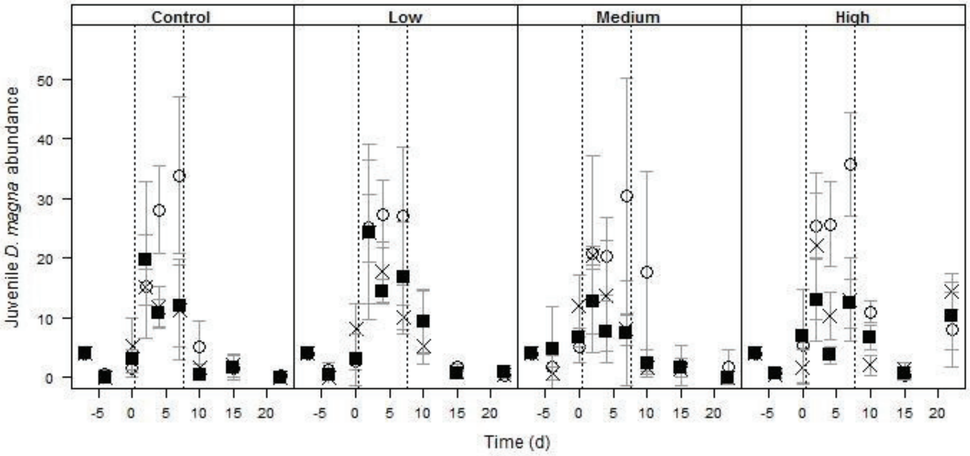


Figure A13. *Neonate D. magna abundance over time for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Data shown are the treatments with no additional species interactions (points), low interspecific competition (crosses) and high interspecific competition (black squares). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the first and the second pyrene application.*

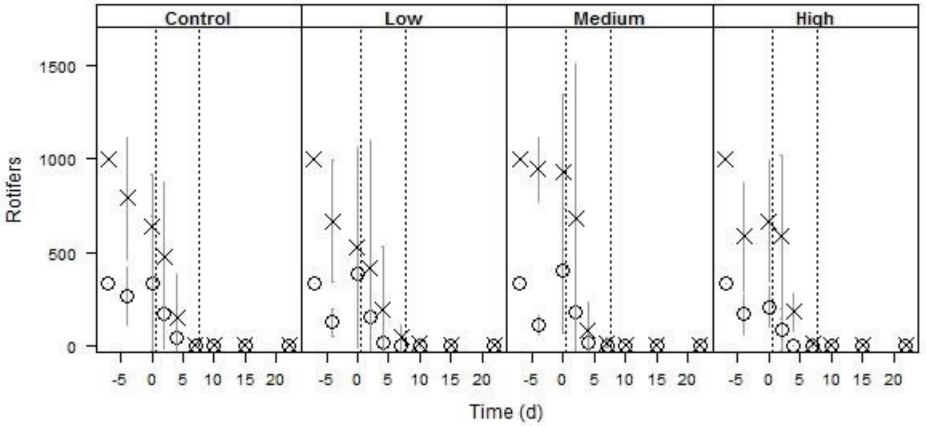


Figure A14. *B. calyciflorus population sizes over time with D. magna present for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Populations were started with either 333 rotifers · vessel-1 (points) or 999 rotifers · vessel-1 (crosses). Average values with standard deviations (error bars) are depicted. Dashed lines indicate first and the second pyrene application.*

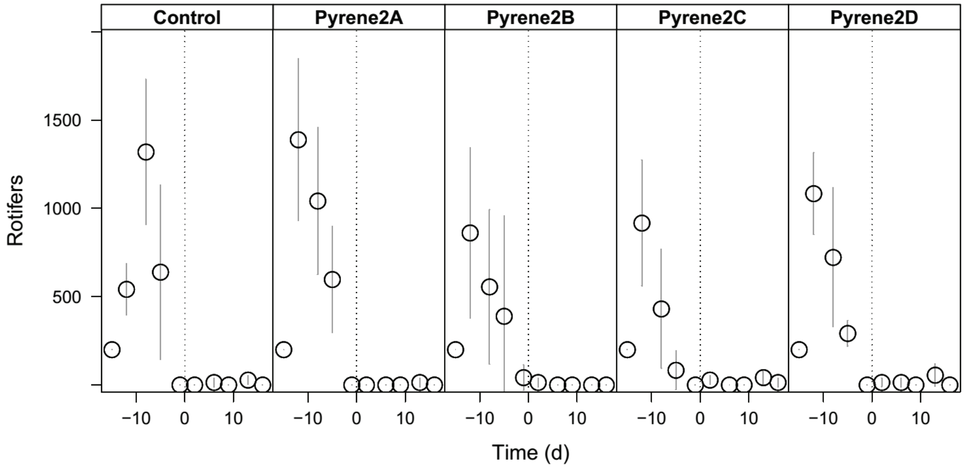


Figure A15. *B. calyciflorus population sizes over time with D. magna present for four pyrene exposure profiles: control, low, medium and high pyrene exposure. Populations were started with either 333 rotifers · vessel-1 (points) or 999 rotifers · vessel-1 (crosses). Average values with standard deviations (error bars) are depicted. Dashed lines indicate first and the second pyrene application.*

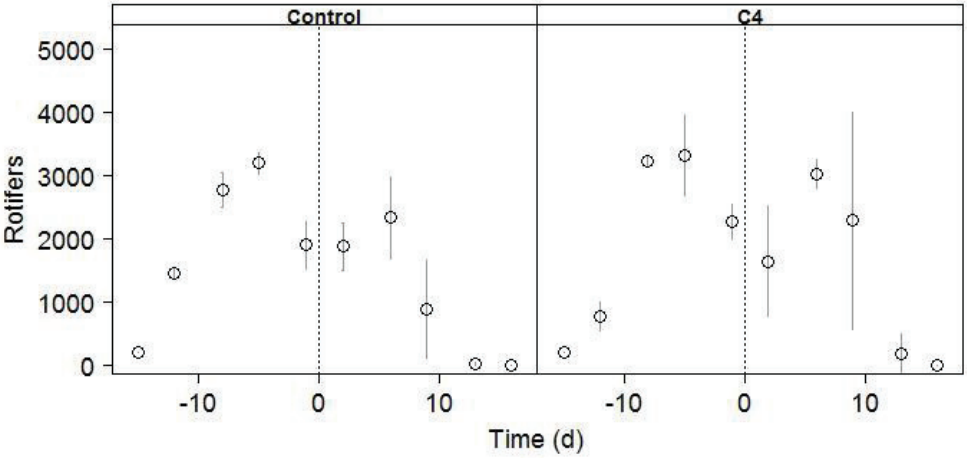


Figure A16. *B. calyciflorus population sizes over time without competition with D. magna and for two pyrene exposure profiles: no exposure ("Control") and high exposure (110 µg/L, "C4"). Average values with standard deviations (error bars) are depicted. Dashed lines indicate the time of pyrene application.*

B

SUPPORTIVE INFORMATION CHAPTER 4

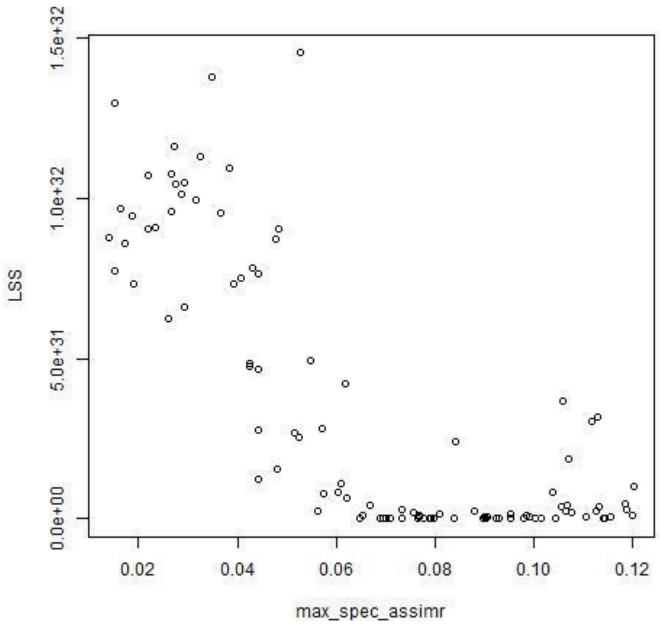


Figure B1: *Likelihood (LSS) of the DEBkiss models with values for the maximum area-specific assimilation rate (max_spec_assimr) between 0.006 and 0.121 mg mm-2 d-1.*

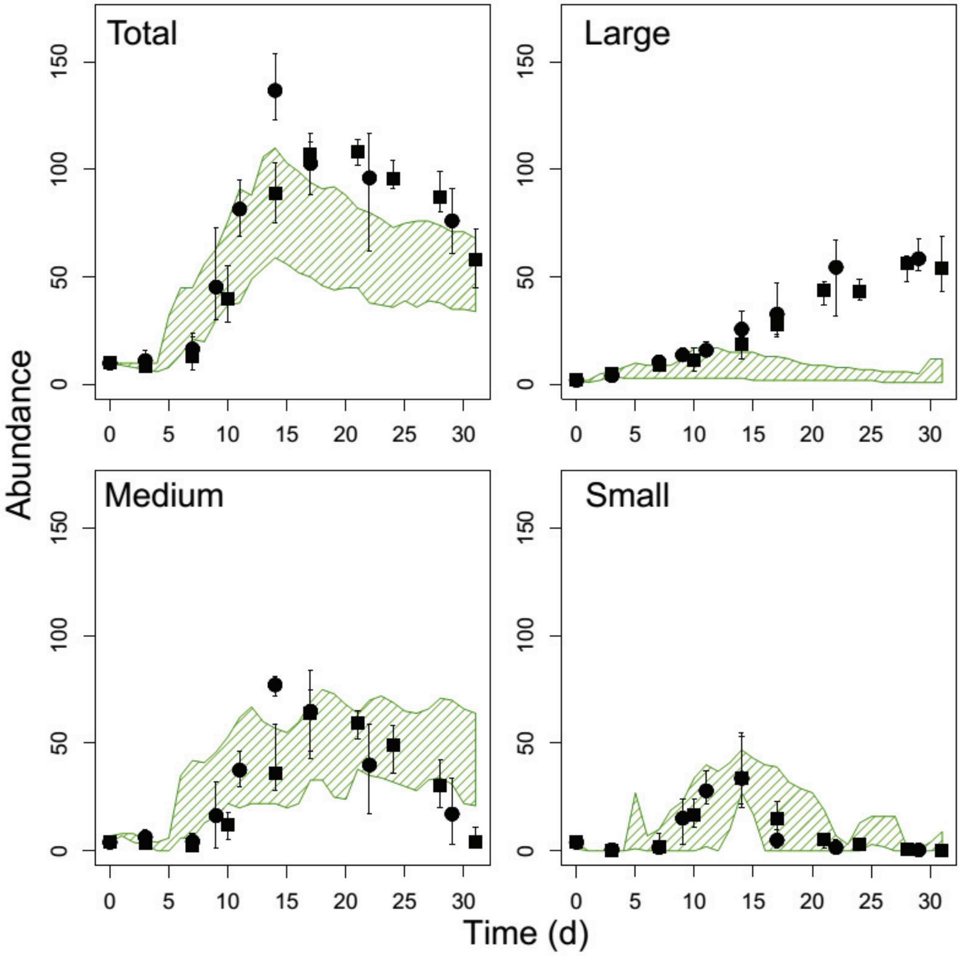


Figure B2: *Total population density (A) and densities of large (B), medium (C) and small (D) individuals for D. magna using a DEBkiss IBM without additional food starvation mortality. Black bullets and squares show the observed dynamics for populations without additional stress in two experiments. Green areas show the best predictions.*

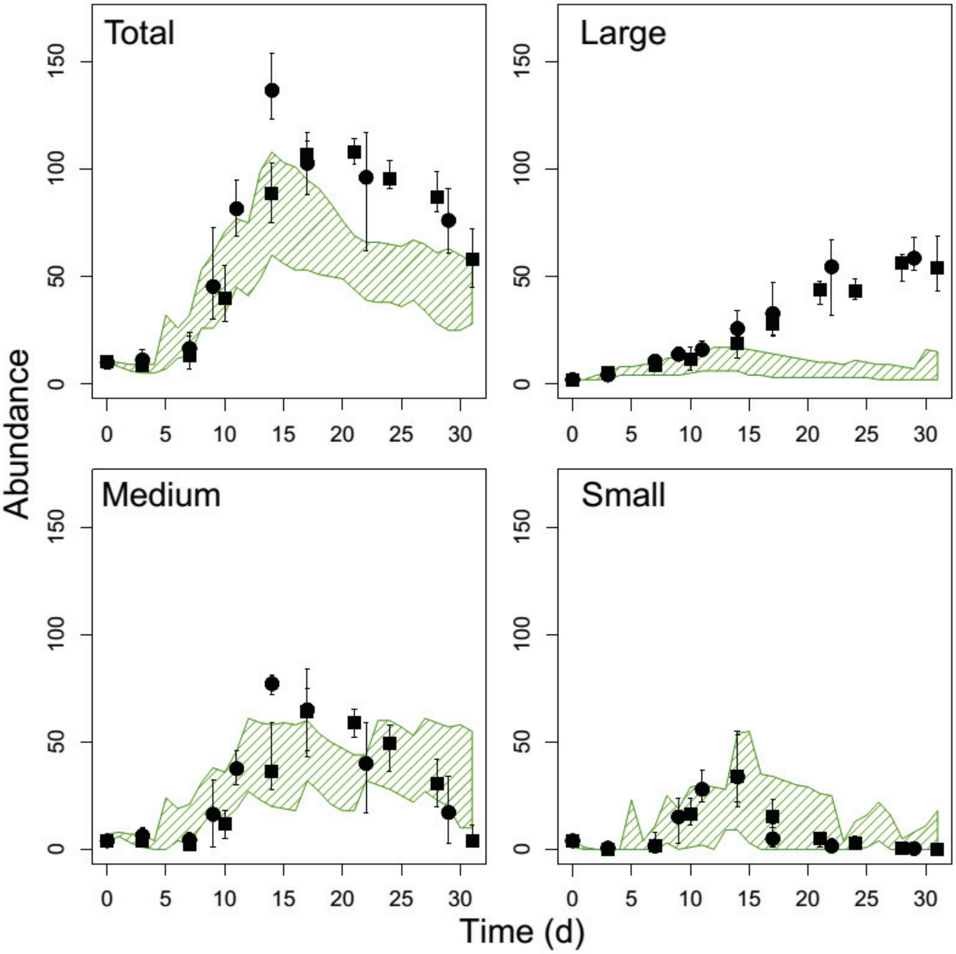


Figure B3: *Total population density (A) and densities of large (B), medium (C) and small (D) individuals for D. magna using a DEBkiss IBM with additional food starvation mortality. Black bullets and squares show the observed dynamics for populations without additional stress in two experiments. Green areas show the best predictions.*

C

SUPPORTIVE INFORMATION CHAPTER 5

C.1. Literature sources for DEBkiss parameters

Asellus: (Maltby 1991, 1995; Arakelova 2001; Galic et al. 2012)

Brachionus: (Halbach 1970a; Dumont et al. 1975; Saunders III and Lewis Jr 1988; Hansen and Bjornsen 1997; Mohr and Adrian 2000; Jensen and Verschoor 2004)

Chaoborus: (Stenson 1978; Van Wijngaarden et al. 2006)

Daphnia: (MacArthur and Baillie 1929; Dumont et al. 1975; Tillmann and Lampert 1984; Ebert 1992; Glazier 1992; Trubetskova and Lampert 1995; Jager and Zimmer 2012; Mulder and Hendriks 2014)

Gammarus: (Nilsson 1977; Welton and Clarke 1980; Gee 1988; Ward 1988; McCahon and Pascoe 1990; Maltby 1995)

C.2. Derivation of multi-species predation equation

Disc equation for multiple prey:

$$C_j = C_{max} W_i \frac{(PD_j/K_j)}{1+\sum_{j=1}^{n}(PD_j/K_j)} \qquad \text{(Rose et al 1999)}$$

Where C_j = the biomass of prey *j* eaten by predator *I* (g); C_{max} = the maximum food uptake by predator *i* (g d^{-1}); W_i = the weight of the predator *i* (g); PD*j* = the density of prey *j* (prey L^{-1}); K_j = the half-saturation coefficient for prey *j* (prey L^{-1}); n = the numer of prey species for predator *i*.

Data available for *Daphnia* and *Brachionus* are based on:

$$PR = \frac{a_j^{'} N_j}{1+\sum_{i=1}^{m} a_j^{'} T_{hj} N_j} \qquad \text{(Krylov 1992)}$$

Where *PR* = predation rate (prey predator^{-1} d^{-1}); a'_j = attack rate on prey *j* (L d^{-1}); *Nj* = density of prey *j* (prey L^{-1}); T_{hj} = handling time of prey *j* (d); n = the number of prey species.

To implement multi-species predation in the IBMs, we need the biomass of prey eaten. However, we only have the parameters for the Krylov 1992 equation. Therefore, we combine both equations using the half-saturation coefficient *K*. The half-saturation coefficient *K* in a type II functional response function corresponds with the food density at which the food uptake is exactly 50% of the maximum food uptake. For the Krylov 1992 equation, the maximum food uptake corresponds to the limit of the function i.e. $\frac{1}{T_h}$. Therefore the Krylov 1992 equivalent of K is equal to $\frac{1}{2T_h}$. The corresponding food density *N* is calculated by:

$$K = \frac{1}{2T_h} = \frac{a^{'}N}{1+a^{'}T_h N} \Rightarrow K = \frac{1}{a_j^{'}T_h}$$

Combined with the Disc equation, the amount of prey biomass C_j eaten can be calculated:

$$C_j = C_{max}W_i f \text{ with } f = \frac{\left(PD_{ij}^{V}/K_{ij}\right)}{1+\sum_{j=1}^{n}\left(PD_{ij}^{V}/K_{ij}\right)} = T_{hj}\frac{N_j a_j^{'}}{1+\sum_{j=1}^{n} N_i a_j^{'} T_{hj}}$$

C.3. Communication protocol

Communication between components in the ChimERA model

Two considerations were taken into account for the design of the communication between the different model programs via the server. Firstly, without a minimum of synchronization imposed upon the system by the server program, it will be very hard to get a robust system. Synchronization implies here that all models/clients should receive a signal to perform their computations and all models/clients inform the server program when they are ready. Only when all model/clients are ready, the system should proceed with the next step. Secondly, the different types of data that need to be exchanged require a two-step process. Some data are state data that can be exchanged as soon as all (new) states are calculated. Other data however might refer to fluxes between models that are calculated only within one model but need to be accounted for in other models before their new state can be calculated. These data can only be send to model 2 when calculations in model 1 have been done. The two-step process thus involves first calculating changes in all models, then exchanging deltas (loss or increase terms) and then calculating the final next state.

Main structure

With the generic architecture presented here, the complete system can be split up in an arbitrary number of modules. Models have an associated client, that handles communication with the server. For the ChimERA model, one client handles the communication with the fate model, one client the communication with the two detritus feeders and one client the communication with the grazers and their predator (Figure C1).

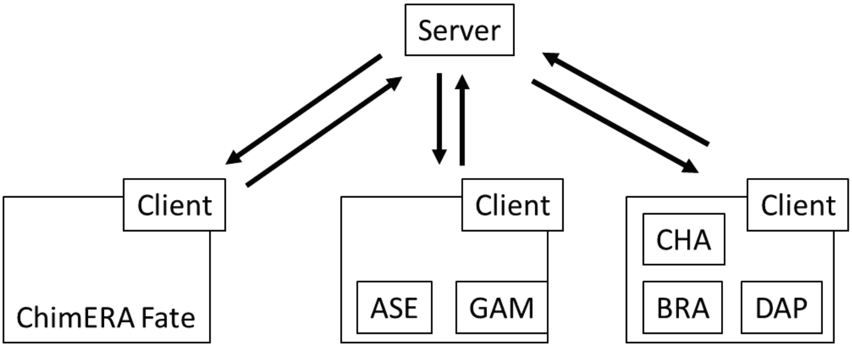


Figure C1: *Setup of the integrated ChimERA model. The server communicates with three clients. One client coordinates the chimERA fate model and the two other clients coordinate the DEBkiss IBMs: one client for the two detritus feeders (ASE and GAM for Asellus and Gammarus, respectively) and one client for the two grazers and the predator (BRA. DAP and CHA for Brachionus, Daphnia and Chaoborus, respectively).*

Table C1: *Data exchanged between the communication server and clients.*

Name	Type	Definition	Unit
PHY	STATE	Phytoplankton concentration	mg ww L^{-1}
CPW	STATE	Concentration chemical in water	mol L^{-1}
PGL	DELTA	Loss (flux) of phytoplankton due to grazing	mg ww L^{-1} h^{-1}
DET	STATE	Detritus (in water) concentration	mg ww L^{-1}
DGL	DELTA	Loss (flux) of detritus due to grazing	mg ww L^{-1} h^{-1}

Communication at connection time

When first connecting with the sever, clients need to specify which data it needs to receive, distinguishing between state variables and fluxes. The two types are indicated by &STATE and &DELTA, and should be followed by the character @ and zero or more (comma-separated) keywords for the data, e.g., '&STATE@PHY,ZOOP1,ZOOP2&DELTA@GRA*'. Note that it should be possible to include models that do not need input from other models, so signalling: '&STATE@&DELTA@*' or just one of the two types: '&STATE@&DELTA@GRA*'

Communication during simulation

The basic loop for each time step is worked out below. The following synchronized steps occur, starting at the moment all models' state variables have just been updated. Exclamation marks are used to indicate the start of a command given by the server, or the following reply coming from the client.

1. Server sends: !calcDelta* to all (waiting) clients and waits for replies from all. No data accompanies this command. The client allows its model to run up to the moment the next/new state is calculated. It has to stop then, because the calculation of the final new state may require information exchange with other models.
2. All clients send: !delta* to the server, to signal that they are ready. When a client produced information that is needed in other models, the standard data string (possibly composed) with this information should be appended to !delta, e.g. !delta#1&GRA@1,2,3,4,5,6,7,8,9,10*. After sending confirmation/data, clients wait.
3. When all confirmations have arrived at the server, the server starts the exchange of delta data by building for all clients that expressed the need for these data, the pure (and possibly composed) delta data strings, and subsequently sending them. The information from the server has the format !delta<data-string>*, with data-string in the format we decided upon, e.g. !delta#1&PGL@1,2,3,4,5,6,7,8,9,10&DGL@9,8,7,6,5,4,3,2,1,0* or !delta#1&PGL@1,2,3,4,5,6,7,8,9,10*. In the data string, any arbitrary data can be included. These data – not needed by other models – will not be exchanged between component models, but only stored and visualized by the server. E.g., !delta#1&PGL@1,2,3,4,5,6,7,8,9,10&MYDATA@9,8,7,6,5,4,3,2,1,0*.
4. Server waits until all the clients that needed delta data have signalled that these data have been received, e.g. by replying !received*
5. All clients now wait until the server sends them the message !calcState*. Upon receiving this message the clients make their model do the necessary thing to set the new state of the model (e.g. subtracting or adding fluxes calculated in other compartments/models).
6. When new state has been set in the models, all clients have to signal that they are ready, by sending !state* to the server. In case clients provide state data that are needing in other models, the (possibly composed) state data are appended: !state<data-string>* e.g. !state#1&PHY@1,2,3,4,5,6,7,8,9,10*. After sending confirmation, clients wait. Also here, in the data string, any arbitrary data can be included. These data – not needed by other models – will not be exchanged between component models, but only stored and visualized by the server. E.g., !state#1&PHY@1,2,3,4,5,6,7,8,9,10&MYDATA@9,8,7,6,5,4,3,2,1,0*.
7. When all these confirmations have arrived at the server, the server starts the exchange of state data by building for all clients that expressed the need for these data, the pure (and possibly composed) state data strings, and subsequently sending them.

8. Server waits until all the clients that needed state data have signalled that these data have been received, e.g. by replying !received*
9. Etc. continuing with step 1. This is the moment the server might be interrupted, leaving the system in a resumable state.

Some specials steps need to be taken when initializing the whole system. Initialization can be done by a variant of the steps 5 to 8 (related to setting state):

10. After the connections have been made (open socket connection created between server and client) all clients wait until the server sends them the message !initialize*. Upon receiving this message the clients make their model do the necessary initializations to set the state of the model at time 0 (this refers to state that can be initialized without information on the initial state of other models).
11. When initializations are done in the models, all clients have to signal that they are ready, by sending !initialized* to the server. In case clients provide state data that are needed in other models, the (possibly composed) state data are appended, e.g., !initialized#1&PHY@1,2,3,4,5,6,7,8,9,10*. After sending confirmation, clients wait. (we could also just copy/reuse the !state* protocol).
12. When all these confirmations have arrived at the server, the server starts the exchange of state data by building for all clients that expressed the need for these data, the pure (and possibly composed) state data strings, and subsequently sending them.
13. Server waits until all the clients that needed state data have signalled that these data have been received, e.g. by replying !received*. After this, all models have their complete up to date state related to t = 0.

D

SUPPORTIVE INFORMATION CHAPTER 6

Table D1: *Physico-chemical properties of the three selected chemicals. MW = molecular weight; VP = vapour pressure; WS = water solubility;* K_{ow} *= octanol-water partition coefficient;* HL_{water} *= half-life in water.*

Chemical	MW (g mol-1)	VP (Pa)	WS (mg L-1)	Log K_{OW}	HL_{water} (h)
Carbendazim	191.2	6.48 * 10-8	8	1.52	720
Chlorpyrifos	350.6	2.27 *10-3	0.73	4.96	24
Pyrene	202.3	6 * 10-4	0.132	5.18	1700

Table D2: *LC50 values and TKTD parameters for the five species considered in the food web.* LC_{50} *values are reported in µg/L.* k_D *= dominant rate constant (d-1);* k_K *= killing rate constant (L µg-1 d-1); z = internal threshold for effects (µg L-1); ns = Not sensitive at the concentrations tested.*

Parameter	*Asellus*	*Brachionus*	*Chaoborus*	*Daphnia*	*Gammarus*
Carbendazim					
EC_{50}	350	ns	ns	91	55
k_D	$4.6 * 10^{-4}$	$1 * 10^{-6}$	$1 * 10^{-6}$	$2.5 * 10^{-3}$	0.05
k_K	$4.7 * 10^{-3}$	$1 * 10^{-6}$	$1 * 10^{-6}$	0.46	0.021
z	6.7	$1 * 10^{5}$	$1 * 10^{5}$	0.3	7.5
Chlorpyrifos					
EC_{50}	8.58	ns	0.3	0.82	0.23
k_D	$9 * 10^{-4}$	$1 * 10^{-6}$	53	0.3	0.7
k_K	27	$1 * 10^{-6}$	0.23	0.35	2
z	0.003	$1 * 10^{5}$	$4.4 * 10^{-4}$	$6.1 * 10^{-8}$	0.006
Pyrene					
EC_{50}	303.5	ns	ns	68	27.1
k_D	0.021	$1 * 10^{-6}$	$1 * 10^{-6}$	0.969	0.23
k_K	0.022	$1 * 10^{-6}$	$1 * 10^{-6}$	0.008	0.11
z	3.5	$1 * 10^{5}$	$1 * 10^{5}$	0	8.4

REFERENCES

Abbriano RM, Carranza MM, Hogle SL, Levin RA, Netburn AN, Seto KL, et al. Deepwater Horizon Oil Spill: A Review of the Planktonic Response. Oceanography. 2011;24(3):294–301.

Adams J, Gee J, Greenwood P, McKelvey S, Perry R. Factors affecting the microdistribution of Gammarus pulex (Amphipoda): an experimental study. Freshw Biol. 1987;17(2):307–16.

Arakelova KS. The evaluation of individual production and scope for growth in aquatic sow bugs (Asellus aquaticus). Aquat Ecol. 2001;35(1):31–42.

Augusiak J, Van den Brink PJ, Grimm V. Merging validation and evaluation of ecological models to "evaludation": A review of terminology and a practical approach. Ecol Modell. 2014;280:117–28.

Baird DJ, Barber I, Bradley M, Soares AMVM, Calow P. A comparative study of genotype sensitivity to acute toxic stress using clones of Daphnia magna straus. Ecotoxicol Environ Saf. 1991;21(3):257–65.

Baird DJ, Maltby LL, Greig-Smith PW, Douben PET. ECOtoxicology: Ecological dimensions. Chapman & Hall Ecotoxicology Series; 1996.

Beketov M a, Liess M. The influence of predation on the chronic response of Artemia sp. populations to a toxicant. J Appl Ecol. 2006;43(6):1069–74.

Beketov MA, Liess M. Ecotoxicology and macroecology - Time for integration. Environ Pollut. 2012;162:247–54.

Bellas J, Saco-Alvarez L, Nieto O, Beiras R. Ecotoxicological evaluation of polycyclic aromatic hydrocarbons using marine invertebrate embryo-larval bioassays. Mar Pollut Bull. 2008;57(6-12):493–502.

Bontje D, Kooi BW, Liebig M, Kooijman SALM. Modelling long-term ecotoxicological effects on an algal population under dynamic nutrient stress. Water Res. 2009;43(13):3292–300.

Bradbury SP, Feijtel TCJ, Van Leeuwen CJ. Meeting the scientific needs of ecological risk assessment in a regulatory context. Environ Sci Technol. 2004;38(23):463a – 470a.

Bray JR, Curtis JT. An Ordination of the upland forest community of southern Wisconsin.pdf. Ecol Monogr. 1957;27(4):325–49.

Bridges CM. Long-term effects of pesticide exposure at various life stages of the southern leopard frog (Rana sphenocephala). Arch Environ Contam Toxicol. 2000;39(1):91–6.

Brock TCM, Arts GHP, Maltby L, Van den Brink PJ, Maltby ÀL, Brink PJ Van Den. Aquatic risks of pesticides, ecological protection goals, and common aims in european union legislation. Integr Environ Assess Manag. 2006;2(4):e20–46.

Brock TCM, Roessink I, Belgers JDM, Bransen F, Maund SJ. Impact of a Benzoyl Urea Insecticide on Aquatic Macroinvertebrates in Ditch Mesocosms with and without Non-Sprayed Sections. Environ Toxicol Chem. 2009;28(10):2191–205.

Cairns J. Putting the eco in ecotoxicology. Regul Toxicol Pharmacol. 1988;8(2):226–38.

Caquet T, Deydier-Stephan L, Lacroix G, Le Rouzic B, Lescher-Moutoue F. Effects of fomesafen, alone and in combination with an adjuvant, on plankton communities in freshwater outdoor pond mesocosms. Environ Toxicol Chem. 2005;24(5):1116–24.

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, et al. Biodiversity loss and its impact on humanity. Nature. 2012;486(7401):59–67.

Chapman PM, Fairbrother A, Brown D. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. Environ Toxicol Chem. 1998;17(1):99–108.

Chapman PM. Integrating toxicology and ecology: putting the "eco" into ecotoxicology. Mar Pollut Bull. 2002;44(1):7–15.

Chuvieco E, Aguado I, Yebra M, Nieto H, Salas J, Martín MP, et al. Development of a framework for fire risk assessment using remote sensing and geographic information system technologies. Ecol Modell. 2010;221(1):46–58.

Clements WH, Rohr JR. Community responses to contaminants: Using basic ecological principles to predict ecotoxicological effects. Environ Toxicol Chem. 2009;28(9):1789–800.

Coors A, Meester L De. Synergistic, antagonistic and additive effects of multiple stressors: predation threat, parasitism and pesticide exposure in Daphnia magna. J Appl Ecol. 2008;45:1820–8.

Daam MA, Van den Brink PJ. Implications of differences between temperate and tropical freshwater ecosystems for the ecological risk assessment of pesticides. Ecotoxicology. 2010;19(1):24–37.

Day KE, Kaushik NK, Solomon KR. Impact of Fenvalerate on Enclosed Freshwater Planktonic Communities and on in situ Rates of Filtration of Zooplankton. Can J Fish Aquat Sci. 1987;44(10):1714–28.

De Hoop L, De Troch M, Hendriks a J, De Laender F. Modeling toxic stress by atrazine in a marine consumer-resource system. Environ Toxicol Chem. 2013;32(5):1088–95.

De Laender F, De Schamphelaere KAC, Vanrolleghem P a, Janssen CR. Is ecosystem structure the target of concern in ecological effect assessments? Water Res. 2008a;42(10-11):2395–402.

De Laender F, De Schamphelaere KAC, Vanrolleghem P a, Janssen CR. Validation of an ecosystem modelling approach as a tool for ecological effect assessments. Chemosphere. 2008b;71(3):529–45.

De Laender F, De Schamphelaere KAC, Vanrolleghem PA, Janssen CR. Do we have to incorporate ecological interactions in the sensitivity assessment of ecosystems? An examination of a theoretical assumption underlying species sensitivity distribution models. Environ Int. 2008c;34(3):390–6.

De Laender F, Janssen CR. The ecosystem perspective in ecotoxicology as a way forward for the ecological risk assessment of chemicals. Integr Environ Assess Manag. 2013;9(3):e34–8.

De Laender F, Melian CJ, Bindler R, Van den Brink PJ, Daam M, Roussel H, et al. The contribution of intra- and interspecific tolerance variability to biodiversity changes along toxicity gradients. Ecol Lett. 2014b;17(1):72–81.

De Laender F, Morselli M, Baveco H, Van den Brink PJ, Di Guardo A. Theoretically exploring direct and indirect chemical effects across ecological and exposure scenarios using mechanistic fate and effects modelling. Environ Int. 2015;74:181–90.

De Laender F, Taub FB, Janssen CR. Ecosystem functions and densities of contributing functional groups respond in a different way to chemical stress. Environ Toxicol Chem. 2011;30(12).

De Laender F, van den Brink PJ, Janssen CR, Di Guardo A. The ChimERA project: Coupling mechanistic exposure and effect models into an integrated platform for ecological risk assessment. Environ Sci Pollut Res. 2014a;21:6263–7.

De Laender F, Van Sprang P, Janssen CR. A re-evaluation of fifteen years of european risk assessment using effect models. Environ Toxicol Chem. 2013;32(3):594–601.

De Lange HJ, Sala S, Vighi M, Faber JH. Ecological vulnerability in risk assessment - A review and perspectives. Sci Total Environ. 2010;408(18):3871–9.

Del Arco AI, Parra G, Rico A, Van den Brink PJ. Effects of intra- and interspecific competition on the sensitivity of aquatic macroinvertebrates to carbendazim. Ecotoxicol Environ Saf. 2015;120:27–34.

Deneer JW. Toxicity of mixtures of pesticides in aquatic systems. Pest Manag Sci. 2000 Jun;56(6):516–20.

Di Guardo A, Morselli M, Morabito G, Van den Brink PJ, De Laender F. European-wide ecological scenarios for the exposure assessment of chemicals in aquatic systems. Prep.

Di Toro DM, McGrath JA, Hansen DJ. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and Tissue. Environ Toxicol Chem. 2000;19(8):1951–70.

Dohmen GP, Preuss TG, Hamer M, Galic N, Strauss T, Van den Brink PJ, et al. Population-level effects and recovery of aquatic invertebrates after multiple applications of an insecticide. Integr Environ Assess Manag. 2015;9999(9999):1–15.

Dom N, Penninck M, Knapen D, Blust R. Discrepancies in the acute versus chronic toxicity of compounds with a designated narcotic mechanism. Chemosphere. 2012;87(7):742–9.

Dumbauld BR, Brooks KM, Posey MH. Response of an estuarine benthic community to application of the pesticide carbaryl and cultivation of Pacific oysters (Crassostrea gigas) in Willapa Bay, Washington. Mar Pollut Bull. 2001;42(10):826–44.

Dumont HJ, Balvay G. The dry weight estimate of Chaoborus flavicans (Meigen) as a function of length and instars. Hydro. 1979;64(2):139–45.

Dumont HJ, Velde I, Dumont S. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia. 1975;19(1):75–97.

Ebert D. A food-independent maturation treshold and size at maturity in Daphnia magna. Limnol Oceanogr. 1992;37(4):878–81.

EC. Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Consolidated text including amendments. 2004;

EC. Directive 2000/60/EC of the European parliament and of the council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official J Eur Communities. 2000;L327:1–72.

EC. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/4. Official J Eur Communities. 2007;L136(3).

Fleeger JW, Carman KR, Nisbet RM. Indirect effects of contaminants in aquatic ecosystems. Sci Total Environ. 2003;317(1-3):207–33.

FOCUS. FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC; rev. 2. FOCUS Working Group on Surface Water Scenarios (EC Document Reference SANCO/4802/2001). 2001;245.

Foit K, Kaske O, Liess M. Competition increases toxicant sensitivity and delays the recovery of two interacting populations. Aquat Toxicol. 2012;106-107:25–31.

Forbes VE, Calow P, Grimm V, Hayashi T, Jager T, Palmqvist A, et al. Integrating population modeling into ecological risk assessment. Integr Environ Assess Manag. 2009a;6(1):191–3.

Forbes VE, Calow P, Sibly RM. Are current species extrapolation models a good basis for ecological risk assessment? Environ Toxicol Chem. 2001;20(2):442–7.

Forbes VE, Calow P, Sibly RM. The extrapolation problem and how population modeling can help. Environ Toxicol Chem. 2008;27(10):1987–94.

Forbes VE, Calow P. Promises and problems for the new paradigm for risk assessment and an alternative approach involving predictive systems models. Environ Toxicol Chem. 2012;31(12):2663–71.

Forbes VE, Calow P. Species sensitivity distributions revisited: A critical appraisal. Hum Ecol Risk Assess. 2002;8(3):473–92.

Forbes VE, Hommen U, Thorbek P, Heimbach F, Van den Brink PJ, Wogram J, et al. Ecological models in support of regulatory risk assessments of pesticides: developing a strategy for the future. Integr Environ Assess Manag. 2009b;5(1):167–72.

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

Gabsi F, Schäffer A, Preuss TG. Predicting the sensitivity of populations from individual exposure to chemicals: the role of ecological interactions. Environ Toxicol Chem. 2014b;33(7):1449–57.

Galic N, Ashauer R, Baveco H, Nyman AM, Barsi A, Thorbek P, et al. Modelling the contribution of toxicokinetic and toxicodynamic processes to the recovery of Gammarus pulex populations after exposure to pesticides. Environ Toxicol Chem. 2014;33(7):1476–88.

Galic N, Baveco H, Hengeveld GM, Thorbek P, Bruns E, van den Brink PJ. Simulating population recovery of an aquatic isopod: Effects of timing of stress and landscape structure. Environ Pollut. 2012;163:91–9.

Galic N, Hommen U, Baveco JMH, van den Brink PJ. Potential application of population models in the European ecological risk assessment of chemicals. II. Review of models and their potential to address environmental protection aims. Integr Environ Assess Manag. 2010;6(3):338–60.

Gama-Flores JL, Sarma SSS, Nandini S. Effect of cadmium level and exposure time on the competition between zooplankton species Moina macrocopa (Cladocera) and Brachionus calyciflorus (Rotifera). J Environ Sci Health A Tox Hazard Subst Environ Eng. 2006;41(6):1057–70.

Gee JHR. Population dynamics and morphometries of Gammarus pulex L.: evidence of seasonal food limitation in a freshwater detritivore. Freshw Biol. 1988;19:333–43.

Gergs A, Zenker A, Grimm V, Preuss TG. Chemical and natural stressors combined: from cryptic effects to population extinction. Sci Rep. 2013;3(2036).

Gilbert JJ. Competition between Rotifers and Daphnia. Ecology. 1985;66(6):1943–50.

Gilbert JJ. Suppression of rotifer populations by Daphnia: A review of the evidence, the mechanisms, and the effects on zooplankton community structure. Limnol Oceanogr. 1988;33(6):1286–303.

Girling AE, Pascoe D, Janssen CR, Peither A, Wenzel A, Schafer H, et al. Development of methods for evaluating toxicity to freshwater ecosystems. Ecotoxicol Environ Saf. 2000;45(2):148–76.

Glazier DS. Effects of Food, Genotype, and Maternal Size and Age on Offspring Investment in Daphnia Magna. Ecology. 1992;73(3):910–26.

Grimm V, Ashauer R, Forbes V, Hommen U, Preuss TG, Schmidt A, et al. CREAM: a European project on mechanistic effect models for ecological risk assessment of chemicals. Environ Sci Pollut Res Int. 2009;16(6):614–7.

Grimm V, Berger U, Bastiansen F, Eliassen S, Ginot V, Giske J, et al. A standard protocol for describing individual-based and agent-based models. Ecol Modell. 2006;198(1-2):115–26.

Grimm V, Martin BT. Mechanistic effect modeling for ecological risk assessment: Where to go from here? Integr Environ Assess Manag. 2013;9(3):e58–63.

Grimm V, Railsback SF. Individual-based Modeling and Ecology. Princeton University Press; 2013.

Grimm V, Thorbek P. Population models for ecological risk assessment of chemicals: Short introduction and summary of a special issue. Ecol Modell. 2014;280:1–4.

Gunderson AR, Armstrong EJ, Stillman JH. Multiple Stressors in a Changing World: The Need for an Improved Perspective on Physiological Responses to the Dynamic Marine Environment. Ann Rev Mar Sci. 2016;8(1):13–41.

Håkanson L, Peters RH. Predictive Limnology: Methods for Predictive Modelling. Amsterdam: SPB Academic Publishing bv.; 1995.

Halbach U. Die Ursachen der Temporalvariation von Brachionus calyciflorus Pallas (Rotatoria). Oecologia. 1970a;4:262–318.

Halbach U. EinfluB der Temperatur auf die Populationsdynamik des planktischen Rädertieres Brachionus calyciflorus Pallas. Oecologia. 1970b;207:176–207.

Hanazato T. Pesticide effects on freshwater zooplankton : an ecological perspective. Environ Pollut. 2001;112:1–10.

Hansen PJ, Bjornsen PK. Zooplankton grazing and growth: Scaling within the 2-2,000-mu m body size range. Limnol Oceanogr. 1997;42(4):687–704.

Hjorth M, Vester J, Henriksen P, Forbes V, Dahllöf I. Functional and structural responses of marine plankton food web to pyrene contamination. Mar Ecol Prog Ser. 2007;338:21–31.

Hoang TC, Klaine SJ. Influence of organism age on metal toxicity to Daphnia magna. Environ Toxicol Chem. 2007;26(6):1198–204.

Hommen U, Baveco JM, Galic N, van den Brink PJ. Potential Application of Ecological Models in the European Environmental Risk Assessment of Chemicals I: Review of Protection Goals in EU Directives and Regulations. Integr Environ Assess Manag. 2010;6(3):325–37.

Hommen U, Forbes V, Grimm V, Preuss TG, Thorbek P, Ducrot V. How to use mechanistic effect models in environmental risk assessment of pesticides: Case studies and recommendations from the SETAC workshop MODELINK. Integr Environ Assess Manag. 2016;12(1):21–31.

Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, et al. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature. 2012;486:105–8.

Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, et al. Effects of Biodiversity on Ecosystem Functioning: a Consensus of Current Knowledge. Ecol Monogr. 2005;75(1):3–35.

Hose GC, Van den Brink PJ. Confirming the species-sensitivity distribution concept for endosulfan using laboratory, mesocosm, and field data. Arch Environ Contam Toxicol. 2004;47(4):511–20.

Jager T, Albert C, Preuss TG, Ashauer R. General Unified Threshold Model of Survival - a Toxicokinetic-Toxicodynamic Framework for Ecotoxicology. Environ Sci Technol. 2011;45(7):2529–40.

Jager T, Barsi A, Hamda NT, Martin BT, Zimmer EI, Ducrot V. Dynamic energy budgets in population ecotoxicology: Applications and outlook. Ecol Modell. 2014;280:140–7.

Jager T, Martin BT, Zimmer EI. DEBkiss or the quest for the simplest generic model of animal life history. J Theor Biol. 2013;328:9–18.

Jager T, Zimmer EI. Simplified Dynamic Energy Budget model for analysing ecotoxicity data. Ecol Modell. 2012;225:74–81.

Jensen TC, Verschoor AM. Effects of food quality on life history of the rotifer Brachionus calyciflorus Pallas. Freshw Biol. 2004;49(9):1138–51.

Jonker MJ, Svendsen C, Bedaux JJM, Bongers M, Kammenga JE. Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose-response analysis. Environ Toxicol Chem. 2005;24(10):2701–13.

Kattwinkel M, Liess M. Competition matters: Species interactions prolong the long-term effects of pulsed toxicant stress on populations. Environ Toxicol Chem. 2013;33(7):1458–65.

Knillmann S, Stampfli NC, Beketov M a, Liess M. Intraspecific competition increases toxicant effects in outdoor pond microcosms. Ecotoxicology. 2012a;21:1857–66.

Knillmann S, Stampfli NC, Noskov Y a, Beketov M a, Liess M. Interspecific competition delays recovery of Daphnia spp. populations from pesticide stress. Ecotoxicology. 2012b;21:1039–49.

Kooijman S a LM, Metz JAJ. On the dynamics of chemically stressed populations: the deduction of population consequences from effects on individuals. Ecotoxicol Environ Saf. 1984;8:254–74.

Kooijman SALM. Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge University Press; 2010.

Krylov PI. Density-dependent predation of Chaoborus flavicans on Daphnia longispina in a small lake: the effect of prey size. Hydrobiologia. 1992;239(3):131–40.

Liebig M, Schmidt G, Bontje D, Kooi BW, Streck G, Traunspurger W, et al. Direct and indirect effects of pollutants on algae and algivorous ciliates in an aquatic indoor microcosm. Aquat Toxicol. 2008;88(2):102–10.

Liess M, Foit K. Intraspecific competition delays recovery of population structure. Aquat Toxicol. 2010;97(1):15–22.

Liess M. Population response to toxicants is altered by intraspecific interaction. Environ Toxicol Chem. 2002;21(1):138–42.

Liljendahl-Nurminen A, Horppila J, Eloranta P, Malinen T, Uusitalo L. The seasonal dynamics and distribution of Chaoborus flavicans larvae in adjacent lake basins of different morphometry and degree of eutrophication. Freshw Biol. 2002;47(7):1283–95.

Lopes I, Baird DJ, Ribeiro R. Avoidance of copper contamination by field populations of Daphnia longispina. Environ Toxicol Chem. 2004;23(7):1702.

MacArthur JW, Baillie WHT. Metabolic activity and duration of life. J Exp Zool. 1929;53(2):221–42.

MacIsaac HJ, Gilbert JJ. Competition between Rotifers and Cladocerans of Different Body Sizes. Oecologia. 1989;81:295–301.

Mackay D, Shiu WY, Ma KC. Illustrated Handbook of Physical chemical properties and environmental fate for organic chemicals. Chelsea MI, USA: Lewis Publishers; 1992.

Mackay D. Multimedia Environmental models, the fugacity approach. 2nd ed. Chelsea MI, USA: Lewis Publishers; 2001.

Maltby L, Brock TCM, Van Den Brink PJ. Fungicide risk assessment for aquatic ecosystems: Importance of interspecific variation, toxic mode of action, and exposure regime. Environ Sci Technol. 2009;43(19):7556–63.

Maltby L, Hills L. Spray drift of pesticides and stream macroinvertebrates: experimental evidence of impacts and effectiveness of mitigation measures. Environ Pollut. 2008;156(3):1112–20.

Maltby L. Pollution as a Probe of Life-History Adaptation in Asellus aquaticus (Isopoda). Oikos. 1991;61(1):11.

Maltby L. Sensitivity of the Crustaceans gammarus pulex (L.) and Asellus aquaticus (L.) to short-term exposure to hypoxia and unionized ammonia: Observations and possible mechanisms. Water Res. 1995;29(3):781–7.

Martin B, Jager T, Nisbet R. Limitations of extrapolating toxic effects on reproduction to the population level. Ecol Appl. 2014;24(8):1972–83.

Martin BT, Jager T, Nisbet RM, Preuss TG, Grimm V. Predicting population dynamics from the properties of individuals: a cross-level test of dynamic energy budget theory. Am Nat. 2013a;181(4):506–19.

Martin BT, Jager T, Nisbet RM, Preuss TG, Hammers-Wirtz M, Grimm V. Extrapolating ecotoxicological effects from individuals to populations: a generic approach based on Dynamic Energy Budget theory and individual-based modeling. Ecotoxicology. 2013b;22(3):574–83.

Martin BT, Zimmer EI, Grimm V, Jager T. Dynamic Energy Budget theory meets individual-based modelling: a generic and accessible implementation. Methods Ecol Evol. 2012;3(2):445–9.

McCahon CP, Pascoe D. Episodic Pollution - Causes, Toxicological Effects and Ecological Significance. Funct Ecol. 1990;4(3):375–83.

Mohr S, Adrian R. Functional responses of the rotifers Brachionus calyciflorus and Brachionus rubens feeding on armored and unarmored ciliates. Limnol Oceanogr. 2000;45(5):1175–80.

Moore JW. the Role of Algae in the Diet of Asellus Aquaticus L . and Gammarus Pulex L . J Anim Ecol. 1975;44(3):719–30.

Moore M V. Density-dependent predation of early instar Chaoborus feeding on multispecies prey assemblages. Limnol Oceanogr. 1988;33(2):256–68.

Morselli M, Semplice M, Laender F De, Brink PJ Van Den, Di A. Importance of environmental and biomass dynamics in predicting chemical exposure in ecological risk assessment. Sci Total Environ. 2015;526:338–45.

Morselli M, Semplice M, Villa S, Di Guardo A. Evaluating the temporal variability of concentrations of POPs in a glacier-fed stream food chain using a combined modelling approach. Sci Total Environ. 2014;493:571–9.

Mulder C, Hendriks a. J. Half-saturation constants in functional responses. Glob Ecol Conserv. Elsevier B.V.; 2014;2:161–9.

Muyssen BTA, Janssen CR. Age and exposure duration as a factor influencing Cu and Zn toxicity toward Daphnia magna. Ecotoxicol Environ Saf. 2007;68(3):436–42.

Newman MC, Ownby DR, Mezin LCA, Powell DC, Christensen TRL, Lerberg SB, et al. Applying species-sensitivity distributions in ecological risk assessment: Assumptions of distribution type and sufficient numbers of species. Environ Toxicol Chem. 2000;19(2):508–15.

Nikkilä a, Penttinen S, Kukkonen J V. UV-B-Induced acute toxicity of pyrene to the waterflea Daphnia magna in natural freshwaters. Ecotoxicol Environ Saf. 1999;44(3):271–9.

Nilsson LM. Incubation time , growth and mortality of the amphipod conditions Gammarus pulex under laboratory. Oikos. 1977;29:93–8.

Nisbet RM, Muller EB, Lika K, Kooijman SALM. From molecules to ecosystems through dynamic energy budget models. J Anim Ecol. 2000;69(6):913–26.

OECD. Test No. 202: Daphnia sp. Acute Immobilisation Test. OECD Guidel Test Chem Sect 2. OECD Publishing; 2004.

Peretyatko A, Symoens J-J, Triest L. Impact of macrophytes on phytoplankton in eutrophic peri-urban ponds, implications for pond management and restoration. Belgian J Bot. 2007;140(1):83–99.

Pieters BJ, Jager T, Kraak MHS, Admiraal W. Modeling responses of Daphnia magna to pesticide pulse exposure under varying food conditions: intrinsic versus apparent sensitivity. Ecotoxicology. 2006;15:601–8.

Posthuma L, Suter II GW, Traas TP. Species Sensitivity Distributions in Ecotoxicology. Newman MC, editor. Environ. Ecol. Risk Assess. Boca Raton: CRC Press; 2002.

Preston BL. Indirect Effects in Aquatic Ecotoxicology: Implications for Ecological Risk Assessment. Environ Manage. 2002;29(3):311–23.

Preuss TG, Hammers-Wirtz M, Hommen U, Rubach MN, Ratte HT. Development and validation of an individual based Daphnia magna population model: The influence of crowding on population dynamics. Ecol Modell. 2009a;220(3):310–29.

Preuss TG, Hammers-Wirtz M, Ratte HT. The potential of individual based population models to extrapolate effects measured at standardized test conditions to relevant environmental conditions-an example for 3,4-dichloroaniline on Daphnia magna. J Environ Monit. 2010;12(11):2070–9.

Preuss TG, Hommen U, Alix A, Ashauer R, van den Brink P, Chapman P, et al. Mechanistic effect models for ecological risk assessment of chemicals (MEMoRisk)-a new SETAC-Europe Advisory Group. Environ Sci Pollut Res Int. 2009b;16(3):250–2.

R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2012.

Relyea R, Hoverman J. Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecol Lett. 2006;9(10):1157–71.

Rinke K, Vijverberg J. A model approach to evaluate the effect of temperature and food concentration on individual life-history and population dynamics of Daphnia. Ecol Modell. 2005;186(3):326–44.

Rohr JR, Crumrine PW. Effects of an Herbicide and an Insecticide on Pond Community Structure and Processes. Ecol Appl. 2005;15(4):1135–47.

Rohr JR, Kerby JL, Sih A. Community ecology as a framework for predicting contaminant effects. Trends Ecol Evol. 2006;21(11):606–13.

Rooks AC, GAskell P, Maltby LL. Sublethal effects and predator-prey interactions: Implications for ecological risk assessment. Environ Toxicol Chem. 2009;28(11):2449–57.

Rose K a, Rutherford ES, Mcdermot DS, Forney JL, Mills EL, Monographs SE, et al. Individual-Based Model of Yellow Perch and Walleye Populations in Oneida Lake. Ecol Monogr. 1999;69(2):127–54.

Rubach MN, Ashauer R, Buchwalter DB, De Lange HJ, Hamer M, Preuss TG, et al. Framework for traits-based assessment in ecotoxicology. Integr Environ Assess Manag. 2010;7(2):172–86.

SANCO (Santé des Consommateurs). Guidance document on aquatic ecotoxicology in the context of the Directive 91/414/EEC. Brussels (BE); 2002.

Saunders III JF, Lewis Jr WM. Composition and seasonality of the zooplankton community of Lake. J Plankton Res. 1988;10(5):957–85.

SCENIHR, SCHER, SCCS. Preliminary report on Addressing the New Challenges for Risk Assessment. 2012.

SCHER (Scientific Committee on Health and Environmental Risks), SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), SCCS (Scientific Committee on Consumer Safety). Addressing the New Challenges for Risk Assessment. 2013.

Scherer C, Seeland A, Oehlmann J, Müller R. Interactive effects of xenobiotic, abiotic and biotic stressors on Daphnia pulex-Results from a multiple stressor experiment with a fractional multifactorial design. Aquat Toxicol. 2013;138-139:105–15.

Schmitt-Jansen M, Veit U, Dudel G, Altenburger R. An ecological perspective in aquatic ecotoxicology: Approaches and challenges. Basic Appl Ecol. 2008;9(4):337–45.

Schmolke A, Thorbek P, DeAngelis DL, Grimm V. Ecological models supporting environmental decision making: a strategy for the future. Trends Ecol Evol. 2010;25(8):479–86.

Stenson J a E. Differential Predation by Fish on 2 Species of Chaoborus (Diptera, Chaoboridae). Oikos. 1978;31(1):98–101.

Strauss T, Kulkarni D, Preuss TG, Hammers-Wirtz M. The secret lives of cannibals: Modelling density-dependent processes that regulate population dynamics in Chaoborus crystallinus. Ecol Modell. 2016;321:84–97.

Swift MC. Prey capture by the four larval instars of Chaoborus crystallinus. Limnol Oceanogr. 1992;37(1):14–24.

Tillmann U, Lampert W. Competitive Ability of Differently Sized Daphnia Species: An Experimental Test. J Freshw Ecol. 1984;2(4):311–23.

Tollrian R. Neckteeth formation in Daphnia pulex as an example of continuous phenotypic plasticity: morphological effects of Chaoborus kairomone concentration and their quantification. J Plankton Res. 1993;15(11):1309–18.

Trekels H, Van de Meutter F, Stoks R. Habitat isolation shapes the recovery of aquatic insect communities from a pesticide pulse. J Appl Ecol. 2011;48(6):1480–9.

Trubetskova I, Lampert W. Egg size and egg mass of Daphnia magna : response to food availability. Hydrobiologia. 1995;307:139–45.

Turner JT. Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. Aquat Microb Ecol. 2002;27(1):57–102.

UN Human Rigths. Brazilian mine disaster: “This is not the time for defensive posturing” – UN rights experts. Geneva, Switzerland; 2015.

Vala Ragnarsdottir K. Environmental fate and toxicology of organophosphate pesticides. J Geol Soc London. 2000;157(4):859–76.

Van den Brink P, Ter Braak C. Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis. Aquat Ecol. 1998;32(2):163–78.

Van den Brink PJ, Baveco JM, Verboom J, Heimbach F. An individual-based approach to model spatial population dynamics of invertebrates in aquatic ecosystems after pesticide contamination. Environ Toxicol Chem. 2007;26(10):2226–36.

Van den Brink PJ, Blake N, Brock TCM, Maltby L. Predictive value of species sensitivity distributions for effects of herbicides in freshwater ecosystems. Hum Ecol Risk Assess. 2006;12(4):645–74.

Van den Brink PJ, Hattink J, Bransen F, Donk E Van, Brock TCM. Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. Aquat Toxicol. 2000;48(2-3):251–64.

van der Hoeven N, Gerritsen A a. M. Effects of Chlorpyrifos on Individuals and Populations of Daphnia Pulex in the Laboratory and Field. Environ Toxicol Chem. 1997;16(12):2438.

van Leeuwen CJ, Vermeire TG. Risk Assessment of Chemicals: An Introduction. Dordrecht, The Netherlands: Springer Science & Business Media; 2007.

Van Straalen N. Ecotoxicology becomes stress ecology. Environ Sci Technol. 2003;37(17):324a – 330a.

Van Wijngaarden RP a, Brock TCM, Van Den Brink PJ, Gylstra R, Maund SJ. Ecological effects of spring and late summer applications of lambda-cyhalothrin on freshwater microcosms. Arch Environ Contam Toxicol. 2006;50:220–39.

van Wijngaarden RPA, Brock TCM, Douglas MT. Effects of chlorpyrifos in freshwater model ecosystems: the influence of experimental conditions on ecotoxicological thresholds. Pest Manag Sci. 2005;61(10):923–35.

van Wijngaarden RPA, Crum SJH, Decraene K, Hattink J, van Kammen A. Toxicity of Derosal (active ingredient carbendazim) to aquatic invertebrates. Chemosphere. 1998;37(4):673–83.

van Wijngaarden RPA, Maltby L, Brock TCM. Acute tier-1 and tier-2 effect assessment approaches in the EFSA Aquatic Guidance Document: are they sufficiently protective for insecticides? Pest Manag Sci. 2015;71(8):1059–67.

Vanoverbeke J. Modeling individual and population dynamics in a consumer-resource system: Behavior under food limitation and crowding and the effect on population cycling in Daphnia. Ecol Modell. 2008;216(3-4):385–401.

Versteeg DJ, Belanger SE, Carr GJ. Understanding single-species and model ecosystem sensitivity: Data-based comparison. Environ Toxicol Chem. 1999;18(6):1329–46.

Von Ende CN. Phenology of Four Chaoborus Species. Environ Entomol. 1982;11(1):9–16.

Ward PI. Sexual Selection, Natural selecion, and body size in Gammarus pulex (Amphipoda). Am Nat. 1988;131(3):348–59.

Weis JS, Smith G, Zhou T, Santiago-Bass C, Weis P. Effects of Contaminants on Behavior: Biochemical Mechanisms and Ecological Consequences. Bioscience. 2001;51(3):209.

Welton JS, Clarke RT. Laboratory Studies on the Reproduction and Growth of the Amphipod, Gammarus pulex (L.). J Anim Ecol. 1980;49(2):581–92.

Wickwire T, Johnson MS, Hope BK, Greenberg MS. Spatially explicit ecological exposure models: A rationale for and path toward their increased acceptance and use. Integr Environ Assess Manag. 2011;7(2):158–68.

Wilensky U. NetLogo. Evanston, IL: Center for Connected Learning and Computer-Based Modeling; 1999.

Zuur AF, Elena NI, Walker NJ, Saveliev AA, Smith GM. Mixed effects models and extensions in ecology with R. Gail M, Krickeberg K, Samet JM, Tsiatis A, Wong W, editors. Stat. Biol. Heal. New York, USA: Springer Science+Business Media; 2009.

SUMMARY

In Europe, the number of registered chemicals is approximately 100,000 and still increasing. To help make informed decisions about their production, use and disposal, it is important to accurately quantify the risk these chemicals pose to the environment. Chemicals are therefore subjected to an ecological risk assessment. The goal of an ecological risk assessment is to quantify the risk that a given chemical would impair the structure and functioning of natural ecosystems. Traditionally, this is approached by extrapolating the effects measured in a single species toxicity test to ecosystem-level effects. To this end, different extrapolation methods have been developed. For the chemicals that pose the highest risk, model ecosystems are used to assess the ecological risk of the chemical.

All current ERA methods however fail to provide an accurate answer to the central question in ecotoxicology: what are the effects of chemical exposure in real-world systems? The major problem when approaching this question is to extrapolate from single species in a controlled environment to the protection goals set by the authorities, which involve population size and ecosystem structure and function. Ecological risk cannot be adequately assessed using procedures that disregard most of the inherent environmental and ecological complexity. In order to more accurately predict the effects of chemicals on communities and ecosystems, more ecology needs to be integrated. One of the most prominent problems with traditional ERA approaches is that they regard individuals as discrete units instead of interacting entities. In reality, however, individuals are not isolated but interact with individuals of the same and/or of another species. These species interactions can alter the direct effects of chemicals but also lead to indirect effects i.e. effects on tolerant species through interactions with sensitive species. Competition and predation are considered the most important interactions to account for. Accurately assessing how species interactions can alter the response to chemical exposure is therefore essential. The **first objective** of this work was therefore to understand how competition and predation interfere with chemical exposure.

This PhD thesis starts with experimentally exploring how intraspecific competition, interspecific competition and predation alter the population dynamics of *Daphnia magna* exposed to pyrene (**Chapter 2**). Predation pressure by *Chaoborus* sp. larvae and intraspecific competition limited the *D. magna* population densities when pyrene exposure was absent. However, predation and intraspecific competition altered the size structure of the *D. magna* population, reducing the amount of small individuals that are most sensitive to pyrene. As a result, pyrene effects were smaller in these populations. Because the competitive advantage of *D. magna* over *B. calyciflorus* was so large, *B. calyciflorus* disappeared quickly from the system and no interactions of interspecific competition with pyrene exposure were observed.

Ecological modelling has been proposed as one of the best options to improve effect assessment, specifically to account for ecological interactions. Individual based models (IBMs) seem particularly suited for use in ERA. Since most ecotoxicological tests focus on the individual level, IBMs are ideal

tools to translate these test results to the population and community level. However, current IBM applications have neglected possible interactions with other species, despite this being one of the main criticisms on current ERA methods. A **second objective** of this thesis was to develop an IBM framework capable of predicting chemical effects on interacting species.

In **Chapter 3**, an individual-based modelling (IBMs) implementation was developed that accounted for interactions between species. IBMs are ideal tools to simulate effects of chemicals on populations but need to be based on a sound theoretical basis. Dynamic energy budget theory based on the keep it simple, stupid principle (DEBkiss) offers a good compromise between complexity and the amount of data required to parameterize the model. IBMs based on the DEBkiss theory were developed and chemical effects on survival were implemented. Two possible methods to calculate the effects of chemicals were included: concentration-response curves (CR) and toxicokinetic toxicodynamic models (TKTD). In order for models to be trustworthy, they need to be validated i.e. their predictions need to be compared with observations. Therefore, in **Chapter 4**, DEBkiss IBMs were parameterized for *D. magna* and *B. calyciflorus* and compared to the outcome of the experiments of Chapter 2. The population dynamics of isolated *B. calyciflorus* were accurately predicted using the DEBkiss IBM. Population dynamics of isolated *D. magna* populations were reasonably predicted but not the size structure of the population. This was attributed to the absence of a reserve compartment in the models, leading to faster starvation when food is limited. Both toxicity models predicted pyrene effects that occurred sooner than observed but the TKTD model approached the observed effects best. The outcome of competition was accurately predicted when both DEBkiss IBMs were coupled to a shared food source: *D. magna* quickly outcompeted *B. calyciflorus*. Using the models to simulate competition when exposed to pyrene resulted in an increase of *B. calyciflorus* when *D. magna* densities decreased. This was not observed in the experiments but was attributed to the predicted effects of pyrene occurring earlier in the simulations than in the experiments.

Ecological risk assessment does not aim to protect one or two species in the laboratory but to protect realistic communities in the field. A **third objective** of this work was to develop an IBM-based modelling approach to account for chemical effects on higher ecological levels in realistic conditions. $\text{ChimERA}_{\text{foodweb}}$ was developed as a novel approach (**Chapter 5**): a food web model based on DEBkiss IBMs. To achieve this, a newly implemented predation interaction was added to the earlier implementation of competition between DEBkiss IBMs. The food web included two grazers (*Brachionus* and *Daphnia*) and their predator (*Chaoborus*) and two detritus feeders (*Asellus* and *Gammarus*). In order to perform simulations for realistic conditions, $\text{ChimERA}_{\text{foodweb}}$ was coupled with $\text{ChimERA}_{\text{fate}}$ to form the integrated ChimERA model. $\text{ChimERA}_{\text{fate}}$ is a dynamic and spatially explicit fate model that predicts environmental concentrations based on environmental variables (hydrodynamics, temperature and trophic state).

The integrated ChimERA model was developed as a tool to perform more realistic ecological risk assessments. To test its application, the ChimERA model was applied to a two-pond system in 15 hypothetical scenarios (**Chapter 6**), differing in water residence time, temperature, trophic state and the applied chemical. These differences in environmental conditions greatly determined the effects of chemical exposure and large differences were predicted between the different scenarios. The physico-chemical properties of the chemical determined the spatiotemporal pattern of the exposure i.e. where and when the concentrations were highest and how fast the chemical disappeared. Concentrations of applied chemicals were highest in scenarios with a high water residence time. Direct effects on *Chaoborus, Daphnia* and *Gammarus* were predicted, as expected from the sensitivities of these species. These effects however differed greatly between environmental scenarios, with more pronounced effects in scenarios with a high trophic state and temperature. Within one scenario, the effects of the chemicals on food web dynamics were heterogeneously distributed in space and time, following the differences in exposure. The most notable indirect effects were a shift in dominance from *Daphnia* and *Gammarus* to *Brachionus* and *Asellus*, respectively. Also, the predator *Chaoborus* was affected indirectly through effects on its prey species *Daphnia*. The effects of the mixture of three chemicals differed between species, where the effects of pyrene dominated for *Daphnia* and the effects of carbendazim and chlorpyrifos for *Gammarus*. These simulations demonstrate how much the outcome of chemical exposure is determined by environmental conditions and how important it is to account for these environmental conditions in ERA through the use of models such as the ChimERA model.

In **Chapter 7**, the main conclusions and proposals for future research directions are given. This work has shown how essential it is to approach the risk assessment of chemicals from an ecological perspective. Species are not isolated entities but interact and influence the outcome of chemical exposure. The ChimERA model developed and applied here is an important proof of principle on how these interactions can be accounted for. More than this, the model also accounted for spatiotemporal differences in exposure and effects of chemicals. The ChimERA model, when further tested and if necessary extended, can serve as a blueprint for future efforts to model how chemicals affect realistic systems.

SAMENVATTING

Meer dan 100,000 verschillende chemicaliën zijn reeds geregistreerd in Europa. Om geïnformeerde keuzes te maken omtrent de productie, het gebruik en het verwerken van deze chemicaliën is het belangrijk om hun risico voor het milieu accuraat in te schatten. Dit wordt gedaan aan de hand van ecologische risico-evaluaties. Ecologische risico-evaluaties hebben als doel het risico van chemicaliën voor de structuur en het functioneren van natuurlijke ecosystemen te kwantificeren. Dit wordt traditioneel benaderd door de effecten gemeten met een toxische test op één soort te extrapoleren naar het ecosysteem-niveau. Hiervoor werden verschillende extrapolatietechnieken ontwikkeld en voor de chemicaliën die het grootste risico vormen worden testen met modelecosystemen uitgevoerd.

Alle huidige ecologische risicoschatting technieken slagen er echter niet in een accuraat antwoord te geven op de centrale vraag in ecotoxicologie: wat zijn de effecten van chemicaliën in realistische ecosystemen? Het grootste probleem is hoe de effecten die gemeten zijn voor, in het beste geval, modelgemeenschappen in gecontroleerde omstandigheden te extrapoleren naar de beschermingsdoelstellingen vastgelegd door overheidsinstanties. Het ecologische risico kan niet accuraat berekend worden met technieken die voorbij gaan aan de inherente complexiteit van ecologie. Om het risico van chemicaliën voor gemeenschappen en het milieu beter in te schatten moet meer rekening gehouden worden met de ecologische realiteit. Eén van de grootste problemen met de huidige technieken voor ecologische risicoschatting is dat organismen als discrete eenheden worden beschouwd. In werkelijkheid interageren organismen met andere organismen van dezelfde en/of een andere soort. Deze soorteninteracties kunnen zowel de directe effecten van chemicaliën op gevoelige soorten beïnvloeden als de indirecte effecten, d.w.z. de effecten die ontstaan door interacties met tolerante soorten. Competitie en predatie worden algemeen beschouwd als de meest belangrijke types van soorteninteracties. Nauwkeurig inschatten hoe deze soorteninteracties de effecten van een chemische stof beïnvloeden is daarom essentieel.

De eerste doelstelling van dit doctoraatswerk was dan ook om te begrijpen hoe competitie en predatie de effecten van chemicaliën kunnen beïnvloeden.

Dit doctoraatsonderzoek begint met experimenteel na te gaan hoe intraspecifieke competitie, interspecifieke competitie en predatie een populatie van *Daphnia magna* beïnvloeden wanneer die blootgesteld wordt aan pyreen (**Hoofdstuk 2**). Lagere densiteiten van *D. magna* werden vastgesteld wanneer de populatie blootgesteld werd aan predatie door *Chaoborus* sp. larven of intraspecifieke competitie. Deze twee soorteninteracties leidden er ook toe dat grote individuen talrijker waren. Grote individuen zijn resistenter tegen pyreen effecten en de effecten van pyreen op de D. magna densiteit waren dan ook kleiner wanneer de populatie ook blootgesteld was aan predatie of intraspecifieke competitie. Omdat *D. magna* een veel sterkere competitor was dan *B. calyciflorus* had interspecifieke competitie, onafhankelijk van de blootstelling aan pyreen, weinig invloed op de *D. magna* densiteiten.

Ecologische modellen worden gesuggereerd als één van de beste opties om het inschatten van de ecologische effecten van chemicaliën te verbeteren. Individu-gebaseerde modellen (IBMs) in het bijzonder zijn uitermate geschikt om de gemeten effecten in standaard ecotoxicologische testen te extrapoleren naar populatie- en gemeenschapsniveau. De huidige toepassingen van IBMs houden echter geen rekening met hoe soorteninteracties de effecten van chemicaliën kunnen beïnvloeden. De tweede doelstelling van dit doctoraat was dan ook om een IBM te ontwikkelen waarmee de interacties tussen soorten kunnen in rekening gebracht worden.

In **hoofdstuk 3** werd een IBM model ontwikkeld om twee soorten in competitie met elkaar te modelleren. IBMs moeten een goede theoretische basis hebben om algemeen toepasbaar te zijn. De “Dynamic energy budget” theorie gebaseerd op het “keep it simple, stupid” principe (DEBkiss) werd gekozen omwille van de goede balans tussen complexiteit en benodigde data om het model te parameteriseren. Als nieuwe aanpak om chemische effecten te voorspellen werden deze DEBkiss IBMs gekoppeld met twee verschillende toxiciteitsmodellen :concentratie-effect curves en toxicokinetische-toxicodynamische (TKTD) modellen. Als modelvalidatie werden de DEBkiss IBMs simulaties vergeleken in **hoofdstuk 4** met de resultaten van de experimenten uit hoofdstuk 2. De populatiedensiteiten van *B. calyciflorus* werden accuraat voorspeld aan de hand van een DEBkiss IBM. De populatiedensiteit van D. magna werd redelijk accuraat voorspeld maar de voorspelde populatiestructuur week af van de geobserveerde. De afwezigheid van een reserve compartiment in de DEBkiss theorie was waarschijnlijk de oorzaak, waardoor de individuen sneller verhongerden wanneer voedsellimitatie optrad in de simulaties in vergelijking met de experimenten. De effecten van pyreen werden door beide toxiciteitsmodellen te snel voorspeld, maar deze afwijking was kleiner voor het TKTD model. Net als in de experimenten nam de *B. calyciflorus* populatie snel af door competitie met *D. magna*. Wanneer de twee soorten in competitie blootgesteld werden aan pyreen werd een toename van *B. calyciflorus* voorspeld. Dit werd niet waargenomen in de experimenten en was het gevolg van de te vroeg voorspelde effecten van pyreen op *D. magna*.

Het doel van ecologische risicoschattingen is niet het beschermen van enkele soorten in het labo maar het beschermen van gemeenschappen in het veld. Een laatste doelstelling van dit doctoraat was dan ook om een model op basis van IBMs te ontwikkelen waarmee de effecten van chemicaliën op gemeenschapsniveau kunnen ingeschat worden in realistische omstandigheden. Als een nieuwe aanpak werd in **hoofdstuk 5** het ChimERA$_{\text{foodweb}}$ model ontwikkeld op basis van de hier ontwikkelde DEBkiss IBMs. Predatie werd geïmplementeerd voor DEBkiss IBMs en gekoppeld aan de eerder ontwikkelde implementatie voor competitie. Het gemodelleerde voedselweb bestond uit twee grazers (*Brachionus* en *Daphnia*), hun predator (*Chaoborus*) en twee detritivoren (*Asellus* en *Gammarus*). Dit voedselwebmodel werd gekoppeld aan ChimERA$_{\text{fate}}$, een omgevingsmodel om de concentratie aan chemicaliën te voorspellen. Het geïntegreerde ChimERA model kan voor een opgegeven landschapsstructuur de effecten van een chemische stof op het voedselweb voorspellen doorheen de tijd op basis van opgegeven omgevingsvariabelen (hydrodynamica, temperatuur en nutriëntenstatus).

Het geïntegreerde ChimERA model werd ontwikkeld om meer realistische ecologische risicoschattingen uit te voeren. Om dit te testen werd het toegepast voor 15 hypothetische scenario's op een landschap van twee geconnecteerde vijvers (**Hoofdstuk 6**). De retentietijd van water, temperatuur, nutriëntenstatus en toegevoegde chemische stof (carbendazim, chlorpyrifos en pyreen) werden aangepast tussen deze 15 scenario's. De verschillen in omgevingsvariabelen bepaalden in grote mate de voorspelde effecten van blootstelling aan chemicaliën en grote verschillen werden dan ook vastgesteld tussen de verschillende scenario's. Chemische concentraties waren het hoogst in scenario's met een hoge retentietijd. De fysicochemische eigenschappen van een chemische stof bepaalden waar en wanneer de concentraties het hoogst waren en hoe snel de stof terug verdween uit de omgeving. Directe effecten van de blootstelling aan chemicaliën werden voorspeld voor de meest gevoelige soorten (*Chaoborus*, *Daphnia* en *Gammarus*). Deze voorspelde effecten vertoonden grote verschillen tussen de gekozen scenario's en waren in het algemeen groter wanneer de nutriëntenstatus en de temperatuur hoog waren. In een scenario waren de voorspelde effecten van de chemicaliën heel heterogeen verdeeld in tijd en ruimte als gevolg van de grote spatiotemporele verschillen in blootstelling,. De meest voorkomende indirecte effect was het veranderen in dominantie van *Daphnia* en *Gammarus* naar respectievelijk *Brachionus* en *Asellus*. Indirecte effecten van blootstelling werden ook voorspeld op de predator *Chaoborus* via effecten op de gevoelige prooisoort *Daphnia*. Bij blootstelling aan het mengsel van alle drie de chemicaliën domineerden de effecten van pyreen voor *Daphnia* en de effecten van carbendazim en chlorpyrifos voor *Gammarus*. Deze scenariosimulaties tonen aan hoe groot de invloed van omgevingsvariabelen is op de effecten van blootstelling aan chemicaliën en hoe belangrijk het dus is om technieken zoals het ChimERA model te ontwikkelen..

In **hoofdstuk 7** wordt een overzicht van de belangrijkste conclusies per hoofdstuk gegeven en worden mogelijke pistes voor verder onderzoek aangereikte. Dit doctoraatsonderzoek heeft aangetoond hoe essentieel het is om ecologische risicoschattingen te benaderen vanuit een ecologische invalshoek. Soorten zijn geen geïsoleerde eenheden maar interageren en bepalen wat de effecten zijn van blootstelling aan een chemische stof. Het hier ontwikkelde ChimERA model is een belangrijk "proof of principle" waarmee werd aangetoond hoe soorteninteracties kunnen in rekening gebracht worden. Meer dan dat, dit model was in staat om spatiotemporele verschillen in blootstelling en effecten van chemicaliën te voorspellen op gemeenschapsniveau. Het ChimERA model, wanneer het nog verder getest en uitgebreid wordt, kan als blauwdruk dienen voor toekomstige modellen en leiden tot meer realistische ecologische risicoschattingen.

DANKWOORD

Mijn doctoraat is het eindresultaat van een bijna vijfjarige, intense reis. Gedurende die reis heb ik lief en leed gedeeld met vele mede-reizigers die ik hier wil bedanken voor de vele mooie ervaringen.

Laat mij eerst beginnen bij de “reisleiders”, Colin en Frederik, want zonder hen was deze reis er niet geweest. Colin, bedankt om mij als verloren gelopen bioloog de kans te geven om te beginnen aan het labo. Ondanks het niet halen van mijn IWT-beurs heb je me toch de kans gegeven om te blijven werken op interessante projecten. Je gezonde scepticisme voor het modelleren zorgde ook altijd voor de nodige reality check. En nog eens extra bedankt om er op het einde nog een serieuze duw aan te geven bij het schrijven, mijn doctoraat is er zeker nog een stuk beter door geworden. Frederik, ik weet niet goed waar te beginnen. Dankzij jou ben ik in dit doctoraat gerold en ik heb niet meer omgekeken. Al van in het begin gaf je me zo veel vertrouwen en vrijheid. We hebben samen veel rondgereisd (Noorwegen, Como, Berlijn) en ik kan je niet genoeg bedanken voor alle kansen die je me gegeven hebt. Je stond, en staat, altijd klaar met goede raad en oplossingen wanneer het wat minder ging. Je had er altijd het volste vertrouwen in wanneer ik aan iets nieuws begon en ik wist dat ik op je kon terugvallen als ik vast zat. En je hield ook altijd de menselijke kant in de gaten. Bedankt om zo geduldig te zijn, zo begrijpzaam, mij zo veel bij te leren en mij te steunen door dik en dun.

De vaste reisgenoten waren natuurlijk de collega’s van de Plateau. En de reis zou niet half zo leuk geweest zijn zonder jullie! De gezellige koffiepauzes (die verassend vaak uitliepen), een “Brugske” doen (of waren het burgers?) over de middag, het pintje na de uren dat we ons af en toe toch eens beklaagd hebben “the day after”, de legendarische SETAC-congressen, het zijn allemaal reiservaringen die ik niet snel ga vergeten. Een aantal personen wil ik hierbij nog eens extra bedanken. Als eerste, mijn roomie Tina. De toffe babbels, de lachmomenten, de gezamenlijk powerpointsessies, ja, ik zal onze “oase van rust“ echt missen. David, Lisbeth en Charlotte, we leggen al dezelfde reisweg af sinds we samen biologie zijn begonnen en het zou absoluut niet hetzelfde geweest zijn zonder jullie. David, mijn vaste bunk buddy, merci om zo vaak mijn klankbord te zijn en altijd klaar te staan, ik weet gewoon dat wij elkaar zullen blijven horen. Maarten, ik heb het gevoel dat ik je eigenlijk al gans mijn leven ken. Ik zal je ongezouten uitspraken zeker missen! En laat me de andere Stickers niet vergeten: Jenny, de gin-tonics met jou erbij waren altijd de beste. Jan, vogels zullen nooit meer dezelfde zijn na jou. Emmanuel, ondertussen ben je toch ook officieel een Sticker. Bedankt voor de vele onvergetelijke uren op het labo en erbuiten! Cecilia, you are my favourite Portuguese person! Thanks for all the great talks and laughs. Tara, altijd lief en zorgzaam. Wanneer is de volgende spelletjesavond met pannenkoeken (ja, ik ben mezelf weer aan het uitnodigen)? Sacha, je dagelijkse bezoekje was een welgekomen verstrooiing tijdens het schrijven. Marianne, Sigrid en Veerle: bedankt voor alle steun, zowel administratief als in de vorm van een snoep en een babbel. Nancy, ik heb niet veel in het labo gestaan in Gent, maar ik kon toch altijd terecht bij je voor praktische vragen. En ik vergeet ongetwijfeld nog veel andere mensen, sorry daarvoor! Ik weet niet waar de reis nu naartoe gaat, maar als mijn toekomstige reisgenoten half zo leuk zijn als jullie, zal ik mezelf heel gelukkig prijzen.

Tijdens mijn doctoraatsreis werd er ook veel samengewerkt met buitenlandse collega's. Aan de mensen van Wageningen, bedankt voor alles, de tijd in Nederland is echt voorbij gevlogen. Andreas en Hans, dit doctoraat zou er niet geweest zijn zonder jullie hulp. De vele mails, skype sessies en blitzbezoeken aan Wageningen, jullie stonden altijd klaar voor me. Ik ga onze gezamenlijke modelleersessies missen. Zoals afgesproken gaan we zeker nog eens een pintje drinken. Paul, bedankt voor de kans om bij jullie op het labo te werken, ik voelde me er echt thuis. De mensen van het labo in Wageningen wil ik zeker ook bedanken: Ana and Andreu, thanks for making the long days in the lab so enjoyable. Dimitri, ik wil je hier nog eens extra bedanken om me op weg te zetten in Wageningen en voor de babbels met onze hoofden boven de microcosmossen. John, Frits, Wendy, bedankt voor alle technische steun.

Ook bedankt aan de mensen van Nijmegen voor de vlotte samenwerking. En Lisette, onze rondreis in de Lofoten zal me zeker altijd bijblijven. Veel succes met je verdediging! To the colleagues from Como: thanks for the great co-operation. Thanks to you, fate models seem less scary now! Melissa, thanks for being my co-PhD student in the ChimERA project, it was great to have somebody to share laughs with.

Tijdens mijn doctoraatsreis kreeg ik ook vaak het gezelschap van enkele oude bekenden. Karen, er is veel gebeurd tussen ons, maar je hebt me zeker door een groot deel van dit doctoraat geholpen. Matthias en Sarah, dankzij jullie was Brugge één van de terugkerende reisbestemmingen. Zoals je weet, Matthias, is het leven van een doctoraatstudent toch hard he! Bedankt voor mij te pushen wanneer ik weer eens te veel slabakte en om gewoon een goeie maat te zijn. Wanneer brouwen we nog eens? Nuyttens en Tjorre, beste vrienden, we kennen elkaar al zo lang en ik vind het echt de max dat we blijven contact houden. Dit doctoraat is er ook gekomen dankzij jullie steun tijdens brunches of spelletjesavonden. Samuel, roomie, merci om zo relaxt te zijn. De vele boardgames, hots-kes en frisbee sessies hebben zeker geholpen om de stress te beperken. Thokie, ik ben echt blij dat we zo goed blijven overeen komen. Wanneer is de volgende roadtrip? Robin en Kevin, merci om er altijd te zijn, al zo lang.

Ten slotte, er is niets zo leuk als te weten dat je na een lange reis altijd welkom bent bij het thuisfront. Mama en papa, merci om me altijd mijn ding te laten doen maar er toch te staan wanneer het nodig is. Ik zou nooit zo ver geraakt zijn zonder jullie, mijn veilige thuishaven. Thomas, Charlotte en Jozefien bedankt om mij te steunen door dik en dun, het heeft echt deugd gedaan om te weten dat jullie er waren.

De reis gaat nu verder, ik weet niet waarheen. Maar ik kijk alvast met een brede glimlach terug op de reeds afgelegde weg.

CURRICULUM VITAE

PERSONALIA

Surname: Viaene
First name: Karel
Address: Koningin Astridlaan 145
B-9000 Gent
E-mail: viaenekarel@hotmail.com
Mobile: 0496/49 70 24
Date of birth: 6 juli 1987
Place of birth: Izegem
Nationality: Belgium

PROFESSIONAL EMPLOYMENT

2011-Present PhD Student at the Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium

EDUCATION

2010 – 2011 Master in de Milieusanering en het Milieubeheer Ghent University

Master thesis: *Long-term changes in the Barents Sea: Modelling the effects of fisheries, organic pollutants, climate and ecological interactions on Gadus morhua.*
Promotor: Prof. Dr. Colin Janssen
Co-promotor: Dr. Ir. Frederik De Laender

With distinction

2008 – 2010 Master in Biology Ghent University
Majors: Ecology – Evolution

Master Thesis: Phylogeny and ecophysiology of *Cylindrotheca closterium* and the consequences for ecosystem functioning.
Promotor: Prof. Dr. Wim Vyverman
Co-promotor: Prof. Dr. Koen Sabbe

With great distinction

2005 –2008 Bachelor in Biology Ghent University

Bachelor thesis: *Speelt de verwantschap tussen soorten een rol in de relatie tussen diversiteit en productiviteit?*
Promotor: Prof. Dr. Koen Sabbe
Co-promotor: Prof. Dr. Wim Vyverman

GRANTS

FWO Travelling Grant for a Long Stay Abroad at Aquatic Ecology and Water Quality Management Group, Wageningen University and Research Centre, Wageningen, The Netherlands. 1 August 2013 - 30 September 2013

FWO Travelling Grant for a Long Stay Abroad at Aquatic Ecology and Water Quality Management Group, Wageningen University and Research Centre, Wageningen, The Netherlands. 18 May 2014 – 18 July 2014

PUBLICATIONS

Viaene KPJ, De Laender F, Van den Brink PJ, Janssen CR. Using additive modelling to quantify the effect of chemicals on phytoplankton diversity and biomass. Science of the Total Environment. 2013; 449:71–80.

Viaene KPJ, Janssen CR, de Hoop L, Hendriks AJ, De Laender F. Evaluating the contribution of ingested oil droplets to the bioaccumulation of oil components--a modeling approach. Science of the Total Environment. 2014; 499:99–106.

De Hoop L, Huijbregts M a J, Schipper AM, Veltman K, De Laender F, Viaene KPJ, et al. Modelling bioaccumulation of oil constituents in aquatic species. Mar Pollut Bull. 2013;76(1-2):178–86.

Viaene KPJ, De Laender F, Rico A, Van den Brink PJ, Di Guardo A, Morselli M, et al. Species interactions and chemical stress: Combined effects of intraspecific and interspecific interactions and pyrene on Daphnia magna population dynamics. Environtal Toxicoly and Chemistry. 2015; 34(8):1751–9.

PLATFORM PRESENTATIONS

Viaene K.P.J., Janssen C.R., De Hoop L., Hendriks A.J. and De Laender F. Oil spills: never mind the droplets. Oral pitching presentation at the 13th VLIZ Young Marine Scientists' Day, Bruges, 15 February 2013

Viaene K.P.J., Janssen C.R., De Hoop L., Hendriks A.J. and De Laender F. Models that ignore oil droplet uptake are sufficiently accurate to predict the bioaccumulation of oil-associated PAHs. Oral presentation at the 18th National Symposium on Applied Biological Sciences, Ghent, 8 February 2013

POSTER PRESENTATIONS

Viaene K.P.J., De Laender F., Van den Brink P.J., Janssen C.R. Inferring the effects of chemicals on biodiversity: a case study with the herbicide linuron. Poster presentation at the 6th SETAC World Congress, Berlin, 21-24 May 2012.

Viaene K.P.J., Janssen C.R., De Hoop L., Hendriks A.J., De Laender F. Models that ignore oil droplet uptake are sufficiently accurate to predict the bioaccumulation of oil-associated PAHs. Poster presentation at the 23th Annual Meeting SETAC Europe, Glasgow, 13-16 May 2013.

De Laender F., Viaene K.P.J., Di Guardo A., Morselli M., Baveco H., Van den Brink P. ChimERA: Coupling exposure and effects into a predictive integrated framework for risk assessment. Poster presentation at the 23th Annual Meeting SETAC Europe, Glasgow, 13-16 May 2013.

Viaene K.P.J., Rico A., Janssen C.R., van den Brink P.J., De Laender F. Ecological interactions alter the response of *Daphnia magna* populations to chemical stress. Poster presentation at the 24th Annual Meeting SETAC Europe, Basel, 11-15 May 2014.

Viaene K.P.J., Focks A., Baveco H., van den Brink P.J., De Laender F., Janssen C.R. Coupled individual based models as a new tool to predict ecological effects in multi-species systems. Poster presentation at the 24th Annual Meeting SETAC Europe, Basel, 11-15 May 2014.

Viaene K.P.J., De Laender F., Di Guardo A., Morselli M., Janssen C.R. The ecological consequences of various chemical emission patterns in food webs. Poster presentation at the 24th Annual Meeting SETAC Europe, Basel, 11-15 May 2014.

Viaene K.P.J., Focks A., Baveco H., van den Brink P.J., Janssen C.R., De Laender F. Using the DEBkiss IBM framework to predict chemical effects on two interacting zooplankton species. Poster presentation at the 25th Annual Meeting SETAC Europe, Barcelona, 3-7 May 2015.

Viaene K.P.J., De Laender F., Janssen C.R. Food web models as a tool for ecological scenario analysis. Poster presentation at the 25th Annual Meeting SETAC Europe, Barcelona, 3-7 May 2015.

ATTENDED CONFERENCES AND WORKSHOPS

Society of Environmental Toxicology and Chemistry (SETAC), 6th World Congress, 20-24 May, Berlin, Germany

SETAC Europe, 23th Annual Meeting, Glasgow, UK.

SETAC Europe, 24th Annual Meeting, Basel, Switzerland.

SETAC Europe, 25th Annual Meeting, Barcelona, Spain.

National Symposium on Applied Biological Sciences, 18th Edition, 8 February 2013, Ghent, Belgium

VLIZ Young Marine Scientists' Day, 13th Edition, 15 February 2013, Bruges, Belgium

MEMBERSHIP OF PROFESSIONAL ORGANIZATIONS

Member of the Society of Environmental Toxicology and Chemistry (SETAC)