Frederik DE LAENDER

Predicting effects of chemicals on freshwater ecosystems: model development, validation and application

Thesis submitted in fulfilment of the requirements for the degree of
Doctor (Ph.D.) in Applied Biological Sciences

Proefschrift voorgedragen tot het bekomen van de graad van
Doctor in de Toegepaste Biologische Wetenschappen

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Voorspelling van de effecten van chemicaliën op zoetwater-ecosystemen: modelontwikkeling, validatie en toepassing

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Mountains should be climbed with as little effort as possible and without desire.
The reality of your own nature should determine the speed.
If you become restless, speed up.
If you become winded, slow down.

quote I from '*Zen and the art of motorcycle maintenance*'

by Robert M. Pirsig

In our highly complex organic state we advanced organisms respond to our environment with an invention of many marvellous analogues. We invent earth and heavens, trees, stones and oceans, gods, music, arts, language, philosophy, engineering, civilization and science. We call these analogues reality. And they are reality. We mesmerize our children in the name of truth into knowing that they are reality. We throw anyone who does not accept these analogues into an insane asylum. But that which causes us to invent the analogues is Quality. Quality is the continuing stimulus which our environment puts upon us to create the world in which we live. All of it. Every last bit of it.

quote II from '*Zen and the art of motorcycle maintenance*'

by Robert M. Pirsig

This research was supported by a fellowship of the Flemish Institute for the Promotion of Innovation by Science and Technology (IWT). This research was conducted at the Laboratory of Environmental Toxicology and Aquatic Ecology (Ghent University), Jozef Plateaustraat 22, 9000 Gent, Belgium. T: +32 (0)9 264 7932, F: +32 (0)9 264 3766, e-mail : Frederik.delaender@Ugent.be, URL: http://www.milieutoxicologie.ugent.be/

Voorwoord

Ik denk niet dat er nog wetenschappers rondlopen waarvan men kan zeggen dat ze volledig alleen werken. De periode van afzondering in de kelder en bijhorende stofjas zijn definitief passé. Dat heb ik mogen merken de afgelopen 4 jaar. Tijdens dit doctoraatsonderzoek heb ik de mogelijkheid gekregen om samen te werken met mensen van topniveau.

Colin, ik ben bij jou toegekomen als een pas afgestudeerde, nog groen achter de oren. Ik herinner me (nu met plezier) mijn eerste *fuzzy* schrijfsels die je op je bureau kreeg. Als ik daar deze thesis naast leg, denk ik dat we allebei kunnen zeggen dat een en ander grondig veranderd is. Je inspiratie en veeleisendheid hebben hun effect niet gemist. Bedankt.

Peter, mijn promotor van over de plas! Onze samenwerking startte 5 jaar geleden, tijdens mijn ingenieursthesis. Mede dankzij jouw enthousiasme heb ik toen beslist om mee te dingen naar een IWT-beurs, waar dit boekje de vrucht van is. *Ondanks* de massa water tussen ons zijn we er in geslaagd samen te werken op een efficiënte manier. *Dankzij* de massa water tussen ons heb ik ook Québec eens van dichtbij gezien. Dank voor je doorgedreven verbeterwerk (tot en met de kleinste komma in de best verstopte vergelijking!), ze hebben deze thesis naar een hoger niveau getild.

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Plateau-people, steek de handen in de lucht want dit boekje is ook voor jullie. De keuken, de taarten, de culinaire hoogstandjes in restaurant "*le Brug*" waar we telkens samen van genoten, jullie dwaze grappen, het geduld met mijn dwaze grappen, de discussies op leven en dood: ze hebben allemaal deugd gedaan. Brita, Karel, Marc, Lien, Karen, Peter G (opper macro-invertebraat), Marlies, Marianne (allround problem-solver), Wim, Guido, Luis, Leen, Jan-Bart, Emmy, Marjolein, Rahmat, Ans, Niels, Martine. Nee, "bureau-maten", ik ben jullie niet vergeten. Michiel V (ooit zal ik het Oostends volledig beheersen, heb geduld, voorwaar ik zeg u), Michiel C en Roel, onze collectieve staat van opperste concentratie heeft een relaxerend effect. Ook de vele ex-plateau'ers verdienen een bloemetje: Marjan, Marijana, Nele, Yblin, Andy, Annelies, Eleni, Spiros, Anke, An, Bart, Peggy, Hilde, Barbara, Gijs, Jill, Koen.

Raphael, thanks a lot for being a good friend the last couple of years now. I really enjoyed the many good conversations we had, and I can only hope there are more to come. You're up next, and I know you're ready.

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Almost last but definitely not least: Coupure/Valencia-mensen (Dries, Koen, Kathelijne, Jordi, An, Tineke, David, Bob, plus aanhangsels), Beukelaarstraat'ers (Tobias, Wouter, Nick, Jan, plus appendices), Lede/Erpe-mannen en vrouwen (Annelies, Tine, Thijs, Seb, Saar, Dries), Brussel-club (Anneleen, Bram, Kristof, Lies), collega's op zaterdagochtend (André, Edwin, Peter): merci voor jullie belangstelling.

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List of abbreviations

AF	Application factor
EC_x	Concentration resulting in x% effect
$EC_{50, Cmax}$	Effect concentration leading to a 50 % reduction in consumption rate
$EC_{50, PSmax}$	Effect concentration leading to a 50 % reduction in photosynthesis rate
EU	European union
HC_y	Hazardous concentration for y% of the species
LC_x	Lethal concentration for x% of the tested organisms
LC_{50}	Lethal concentration for 50% of the organisms, as determined in the acute toxicity assay
LCR	Ratio of "lethal effect concentration" to "chronic effect concentration"
LOEC	Lowest observed effect concentration
MEC	Measured environmental concentration
NOEC	No observed effect concentration
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
r_{PZ}	Difference between logarithm of EC_{10} for phytoplankton and logarithm of EC_{10} for zooplankton
RQ	Risk quotient
r_{ZF}	Difference between logarithm of EC_{10} for zooplankton and logarithm of EC_{10} for fish
slope	Slope of the respective concentration-effect function
SSD	Species sensitivity distribution
TGD	Technical guidance document
tox	Toxicant concentration
μ	Mean
σ	Standard deviation

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Introduction

The research presented in this dissertation is situated in the field of ecotoxicology, i.e. the science which aims at understanding the exposure and the effects of stressors on different levels of biological organisation in the environment. Ecotoxicology is an interdisciplinary science that combines biology, ecology, chemistry, toxicology and physiology with quantitative techniques adopted from mathematics and statistics.

The subject of this work is ecological effect assessment. This branch of ecotoxicology is especially concerned with the effects chemicals may have on ecosystems. When combined with results from exposure assessments, it allows to predict the risk of chemicals in the environment. Based on this risk, for example water quality criteria for chemicals can be derived as a concentration of a given chemical which is unlikely to result in adverse effects on ecosystems.

Current approaches for the derivation of ecological risk and water quality criteria mostly rely on results from laboratory single-species ecotoxicity tests. Results from single-species tests reflect the direct effect of a chemical on one isolated species in a laboratory setting. Single-species toxicity test results obtained with a number of species are then extrapolated to assess the effect of the considered chemical on an ecosystem-level. Methods to perform these extrapolations consider species as isolated entities and do not take into account ecological interactions between populations of species. However, populations do interact with each other through processes such as consumption and competition. For example, phytoplankton species may compete for available nutrients, and experience grazing pressure from zooplankton.

It has been shown that effects at the ecosystem-level are determined by (1) ecological interactions and (2) direct effects. Hence, ecological effects predicted using current methods will most likely be inaccurate. It is therefore questionable whether ecological effect assessments relying on these inaccurate predictions can result in an accurate assessment of chemical risk to aquatic ecosystems.

Dynamic ecosystem modelling is a technique aimed at mathematically describing the different processes within an ecosystem. It is based on differential equations quantifying the growth of many different populations while accounting for ecological interactions between these populations. When applied to the field of ecotoxicology, such models are combined with toxic effect sub-models which allow the incorporation of direct chemical effects. As such, direct effects and ecological interactions can be combined into one predictive framework. This enables us to perform virtual experiments to examine the effects of many different chemicals on different ecosystems. Unfortunately, no information is available about which toxic effect sub-model should be used so that the ecosystem model accurately predicts ecological effects.

A prerequisite for the use of any model is that its predictions are validated using results from independent experimental ecosystem studies. In the open literature, articles describing such

validation exercises are scarce. Moreover, these exercises only validate predictions in a qualitative way, or include populations in the model which are not present in the considered experimental ecosystem study. Also, the calibration of ecosystem models on observed population dynamics has received more attention than the accurate prediction of ecological effects.

In this thesis, a new ecosystem modelling approach is developed which is suited for the prediction of ecological effects of chemicals on ecosystems. After having examined the predictive capacity of ecosystem models with different toxic effect sub-models, the approach will be validated using experimentally observed effects described in literature. The validated ecosystem model will be applied in two theoretical studies and in one practical ecosystem study. The aim of these three studies is to elucidate the ecological significance of some assumptions underlying effect extrapolation methods currently used in risk assessment procedures. The last chapter gives an overview of the conclusions drawn in this thesis and proposes a number of suggestions for future research. This work consists of 10 chapters covering the following topics:

- Chapter II: methodologies to examine the relationship between ecosystem effects and single-species toxicity test results are reviewed
- Chapter III: the developed ecosystem model is described
- Chapter IV: the methodology to derive ecological effects from ecosystem model predictions is described
- Chapter V: the predictive capacity of ecosystem models with different toxic effect sub-models is examined
- Chapter VI: ecosystem model predictions are validated using experimentally observed effects described in literature
- Chapter VII: a theoretical model application is performed to test if ecological interactions change sensitivity distributions for chemicals
- Chapter VIII: a second theoretical model application is performed to test if ecosystem structure is equally or more sensitive than ecosystem function
- Chapter IX: a practical model application is performed to derive the ecological significance of different percentiles of an SSD for copper in a planktonic ecosystem
- Chapter X: general conclusions and suggestions for further research.

Chapter I
General introduction
and conceptual framework

I. 1. Pollutants in surface waters

Together with the exponential growth of the world's human population, the use of freshwater has grown over the past 100 years (Gleick, 1998; Jackson *et al*., 2001). Freshwater is an important resource because it provides a wide range of sociological, agricultural, and industrial benefits (Postel and Carpenter, 1997). From the total human appropriation of freshwater (≈ 7000 km^3 yr^{-1}), almost 50% is used for agricultural purposes (Postel and Carpenter, 1997). This is illustrated by the concurrence of the increase in irrigated area and the increase in freshwater use (Fig I.1). Next to agriculture, industry is another important source of freshwater consumption, representing nearly 10% of the global human freshwater use.

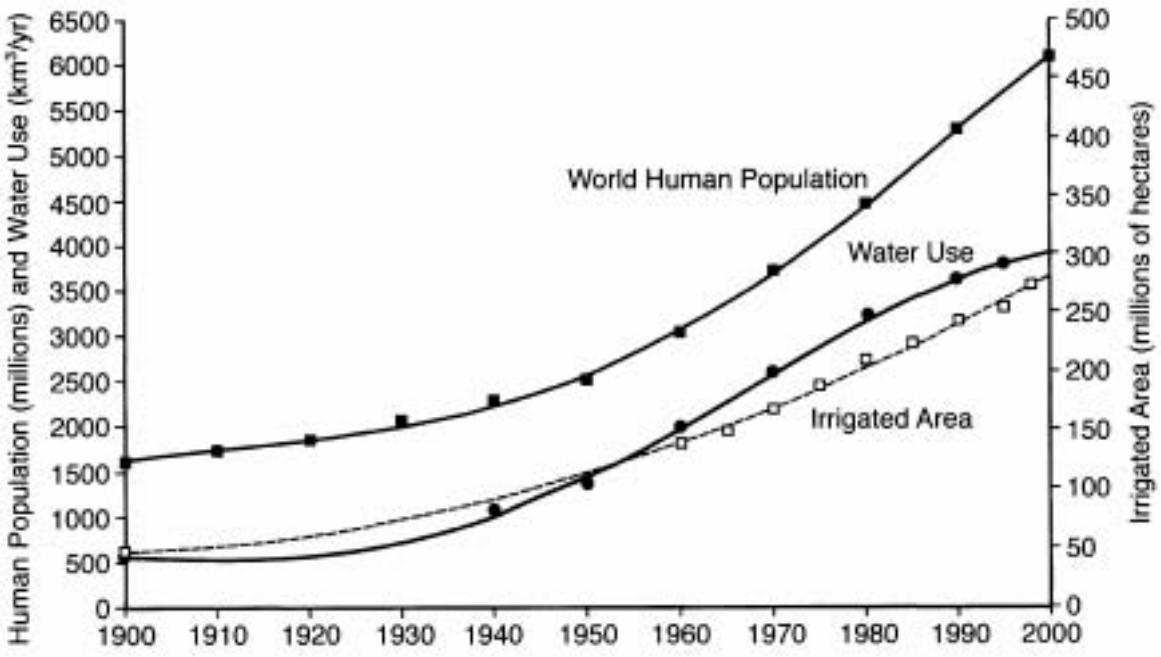

Figure I.1: Global data for human population number, water withdrawal, and irrigated land area. This graph has been reprinted from Jackson *et al.* (2001) and contains updated data from Gleick (1998).

One of the consequences of this increased water use is the presence of chemical pollutants in surface water. The relationship between human activity and polluted surface water is being re-experienced in countries where modern industrial activity has only recently started (Ntengwe, 2006; Zhang and Zhang, 2006).

Within the variety of chemicals which are commonly categorized as pollutants, two classes can be distinguished: macro- and micropollutants. Examples of macropollutants are acids, salts, nutrients, and natural organic matter (Schwarzenbach *et al.*, 2006). Those substances primarily originate

from agricultural, industrial, and municipal sources and occur in the aquatic environment in the µg L^{-1} to mg L^{-1} range. Their behaviour and effects in the aquatic environment has been intensively studied which makes their role in environmental problems relatively well understood (Jackson *et al*., 2001). Biological effects of acidification in surface waters for example, include the increased mortality of sensitive aquatic species, such as fish (Staurnes *et al*., 1996), while elevated levels of nutrients and organic matter can lead to excessive primary production (Schindle, 1971), oxygen depletion, and toxic algal blooms.

Although micropollutants are present in lower concentrations than macropollutants (from ng L^{-1} to µg L^{-1}; Schwarzenbach *et al.*, 2006), the number of micropollutants in the aquatic environment is higher than the number of macropollutants. Therefore, the contribution of micropollutants to surface water pollution may be higher than that of macropollutants. Worldwide fluxes of macropollutants in surface waters are limited to $22 \cdot 10^6$ tons $year^{-1}$, while for micropollutants this is $450 \cdot 10^6$ tons $year^{-1}$ (McGinn, 2002; FAO, 2006).

The chemical structures of these micropollutants exhibit considerable variation. This is reflected by the numerous applications of these chemicals (Table I.1). These pollutants exhibit a wide variety of modes of action in biological systems and some have slow degradation kinetics which prolongs their presence in the environment. The locations where they enter the aquatic environment, as well as the locations where they are transported to via the network of rivers and streams can thus be exposed.

Because micropollutants are highly dispersed in space and time, a large number of organisms may be exposed to these pollutants. Given that the chemical formulations of these pollutants indicate a variety of mode of actions, biological systems may experience adverse effects. Therefore, research aimed at understanding effects of such chemicals on different levels of biological organisation is of primary importance. Any measure taken to prevent the chemical pollution of surface and groundwater resources resulting from such research, will not only improve the health of aquatic life, but will also benefit both the production of clean water and safe food for human consumption (Reddy and Behera, 2006; Schwarzenbach *et al.,* 2006).

Table I.1: Examples of ubiquitous micro pollutants found in surface waters, re-drafted after Schwarzenbach et al (2006).

origin/usage	class	selected examples	related problems	reference
industrial chemicals	solvents	tetrachloromethane	drinking-water contamination	ECETOC, 1999
	intermediates	methyl-t-butylether		
	petrochemicals	BTEX (benzene, toluene, xylene)		
industrial products	additives	phtalates		MacDonald et al., 2000
	lubricants	PCBs	biomagnification, long-range transport	
	flame retardants	polybromilated diphenylesters		Eriksson et al., 2001
consumer products	detergents	nonylphenol ethoxylates	endocrine active transformation product	Ahel et al., 1994
	pharmaceuticals	antibiotics	bacterial resistance, nontarget effects	Kolpin et al., 2002
	hormones	ethinyl estradiol	feminization of fish	Geyer et al., 2000
	personal-care products	uv-filters	multitude of (partially unknown) effects	Daughton and Ternes, 1999
biocides	pesticides	DDT	toxic effects and persistent metabolites	Iwata et al., 1994 and Bignert et al., 1998
		atrazine	effects on primary producers	Solomon et al., 1996
	nonagricultural biocides	tributyltin	endocrine effects	Tanabe, 1999
		triclosan	nontarget effects, persistent degradation product	Lindstrom et al., 2002
geogenic/	metals	lead, cadmium, mercury		Nriagu and Pacyna, 1988
natural chemicals	inorganics	arsenic, selenium, fluoride, uranium	risks for human health	WHO, 2004
	taste and odor	geosmin, methylisoborneol	drinking-water-quality problems	
	cyanotoxines	microcystins		Svrcek and Smith, 2004
	human hormones	estradiol	feminization of fish	Eggen et al., 2003
disinfection/oxidation	disinfection by-products	trihalomethanes, haloacetic acids, bromate	drinking-water-quality, human health problems	Richardson et al., 2002
transformation products	metabolites from all above	metabolites of perfluorinated compounds	bioaccumulation despite low hydrophobicity	Martin et al., 2004
		chloroacetanilide herbicide metabolites	drinking-water-quality problems	Hladik et al., 2005

I. 2. Ecological risk assessment

The interest in the biological consequences of the increasing degree of pollution, although initiated in the 1950s, really took-off in the 1970s (Truhaut, 1977). Scientists with a toxicological background started setting up experiments to reveal the effects of chemicals on measurable traits of animals and plants. This new science, at the crossroads of ecology and toxicology, was termed 'ecotoxicology', and was defined as 'the branch of toxicology concerned with the study of toxic effects, caused by natural and synthetic pollutants, to the constituents of ecosystems - animal (including humans), vegetable, and microbial constituents - in an integrated context' (Truhaut, 1977). More recent definitions tend to exclude humans as the object of study (e.g., Forbes and Forbes, 1994; Walker *et al.*, 2001).

From the early 1980s on, a sub-discipline within ecotoxicology developed with the aspiration of 'estimating the likelihood of a specified adverse effect or ecological event due to a defined exposure to a stressor' (Newman and Unger, 2003). This discipline has been termed 'ecological risk assessment'. Although the health of humans and the state of the environment are in many cases interconnected, human and ecological risk assessment have evolved separately, mainly due to the different study objects treated by both assessments. Human risk assessments study human populations, while ecological risk assessments study ecosystems. Golley (1993) defines 'ecosystem' as an aggregation of interacting populations of three or more species ('community') within an abiotic environment. In many publications, the term 'environmental risk assessment' is used instead of 'ecological risk assessment'. However, the former is more closely associated with the practical application of the knowledge gained by ecological risk assessments, and may involve parts of the human risk assessment procedure.

As previously suggested, 'ecological risk' of a chemical comprises the comparison of its exposure and biological effects (Newman and Unger, 2003). More precisely, the predicted (PEC) or measured (MEC) environmental concentration of a chemical is compared with the concentration which is not expected to result in adverse effects on ecosystems (predicted no effect concentration, PNEC). This comparison can be expressed in a risk quotient:

Risk Quotient (RQ) = (PEC or MEC)/ PNEC

If RQ is > 1, there is an indication that the considered chemical might pose a risk to ecosystems (Fig I.2). Because of the complexity of determining PECs, MECs and PNECs, exposure and effect assessment are classically treated separately.

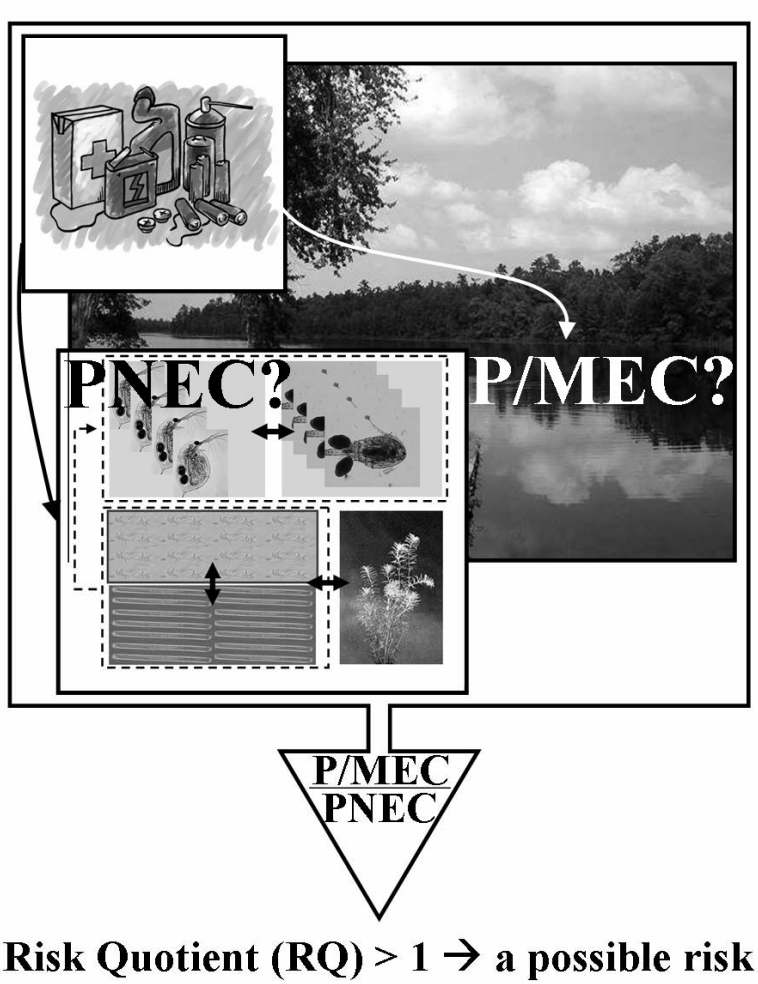

Figure I.2: The risk quotient-approach in ecological risk assessments: A predicted or measured exposure concentration (PEC or MEC) and predicted no effect concentration (PNEC) are calculated and compared. The former represents the predicted (or measured) concentration of the chemical in the environment, the latter represents a concentration which is unlikely to cause adverse effects to ecosystems in the environment.

I. 2. 1. Exposure assessment

In exposure assessments, the behaviour of a substance in the environment is studied, starting with its introduction in the environment (emission), and including various processes of transport and transformation (Newman and Unger, 2003). Where applicable, the partitioning within and between biota (e.g., bioaccumulation, biomagnification,…) is also taken into account. The ultimate goal of an exposure assessment is the derivation of the predicted (or measured) environmental concentrations (PECs or MECs) of the chemical for different environmental compartments, i.e. air, soil, water, and sediment. Again because of the scope of this work, 'PEC' or 'MEC' are consistently used throughout the rest of this dissertation to denote the PEC or MEC for the water compartment.

I. 2. 2. Effect assessment - current practice

Apart from predicting the behaviour of a chemical in the environment, an ecological risk assessment also requires the assessment of ecological effects. The European Chemicals Bureau proposes a concentration-effect approach for ecological effect assessments (TGD, EU 2003). The concentration-effect assessment is concerned with deriving a predicted no effect concentration (PNEC), i.e. a concentration which is expected to cause no adverse ecological effects.

Ecological effects of chemicals are the result of a complex interplay between various factors. The effects of exposing an ecosystem to a given chemical concentration during a given period of time are a function of (1) the inherent sensitivity of the species present; (2) physical and chemical water characteristics; and (3) indirect effects due to ecological interactions between species (Fig I.3).

(1) The inherent sensitivity of a species for a chemical can be examined in a single-species toxicity test. In such an assay, the direct effect of a chemical on a measurement endpoint of a small population of one species is evaluated. With 'measurement endpoint' is meant: the trait of a species which is used to evaluate chemical's effects. In theory, the number of possible measurement endpoints is as large as the number of traits a species has. However, in practice, the most commonly used measurement endpoints include survival, growth, and reproduction (Hoffman *et al*., 1995). In many single-species tests, a concentration-effect approach is followed: the effect of different chemical concentrations on the measurement endpoint is observed in order to establish a concentration - effect relationship. This allows the estimation of the EC_x, the concentration at which x% effect is observed. This EC_x reflects the sensitivity of the tested species for the considered chemical: the lower an EC_x of a species is, the higher its sensitivity for the considered chemical. Additionally, a no observed effect concentration (NOEC) can be derived. This is the highest tested concentration which did not result in significant effects on the examined endpoint. Note that EC_x and NOEC are quantities that can also be used in other experimental settings than single-species toxicity tests. As will be discussed hereunder, this inherent sensitivity is mediated by the physical and chemical characteristics of the surrounding water. In most single-species tests, these water characteristics are standardized to enhance reproducibility of test results.

(2) A plethora of water characteristics have been found to influence the result of single-species tests and thus the sensitivity of a species. Water temperature is an example of a physical quantity which can influence chemical effects on organisms (Heugens *et al*., 2001). Dissolved Organic

Carbon (DOC) and pH are examples of chemical characteristics which can influence the bioavailability of metals to organisms (De Schamphelaere and Janssen, 2002). The observed effect of a chemical on a species thus results from its inherent sensitivity after correction for physical and chemical water characteristics. Note that this observed effect is termed the 'direct effect' of the chemical on the considered species at given water characteristics. If water characteristics are set as desired, this direct effect can also be observed in a single-species toxicity test.

(3) Indirect effects of chemicals originate from consumer-resource interactions (Strauss, 1991; Wootton, 1994) between species. Hence, the study of indirect effects requires the presence of multiple species. As a result, multi-species experiments are needed to address this issue, since single-species test results merely reflect direct effects. Indirect effects are well-studied, and are generally considered in terms of 'top–down' (influence of higher on lower trophic levels) and 'bottom–up' mechanisms (influence of lower on higher trophic levels). The direct effects of a chemical on grazers (e.g. increased mortality) leading to an increased abundance of less sensitive prey species is an example of a top-down indirect effect. A decreased abundance of a less sensitive predator because of direct effects on its more sensitive prey population is an example of a bottom-up indirect effect. Reports of bottom–up indirect effects are less ubiquitous (Posey *et al*., 1999) than reports of top–down indirect effects (e.g., Kneib, 1991; Posey and Ambrose, 1994; Menge, 1995; Brett and Goldman, 1996; Hay, 1997; Havens, 1995). When chemicals directly affect 'keystone species' or 'ecosystem engineers' (Paine 1966; Mills *et al.*, 1993; Jones *et al.*, 1994), trophic cascades may extend far beyond the closely associated species by modifying important ecosystem functions (e.g., decomposition rates, oxygen dynamics and nutrient cycling). A review of indirect effects has been performed by Fleeger *et al*. (2003). This review concludes that indirect effects are a major issue for many types of chemicals in various types of ecosystems.

Given the interplay of different biotic and abiotic factors, the most realistic way to assess effects of chemicals on aquatic ecosystems is to make direct observations in enclosed ecosystems (Cairns, 1983; Clements and Kiffney, 1994). However, because of the inherent stochasticity of biological processes, the reproducibility of such observations is expected to be low (Schindler, 1998). Associated with this limited reproducibility is the low statistical power to detect chemical effects.

As a compromise between the reproducibility of a laboratory-setting and the realism of ecosystem enclosures, artificial ecosystems have been proposed. These are termed microcosms or mesocosms, depending on their dimensions and complexity. Apart from statistical power issues, advantages of

these experimental settings are (1) that they involve multiple species, which allows studying indirect effects of chemicals; and (2) that water characteristics more closely resemble field conditions. Unfortunately, these advantages cannot compensate for the disadvantages of high costs and long study duration. Therefore, micro- and mesocosms are still not used in routine and as a result, data sets from such experiments are scarce. Therefore, risk assessors, whether they are regulators or scientists, often have to rely on single-species toxicity test results to derive a PNEC. Depending on the type and number of single-species toxicity test results available, one of two approaches is applied: application factors (AFs) or species sensitivity distributions (SSDs).

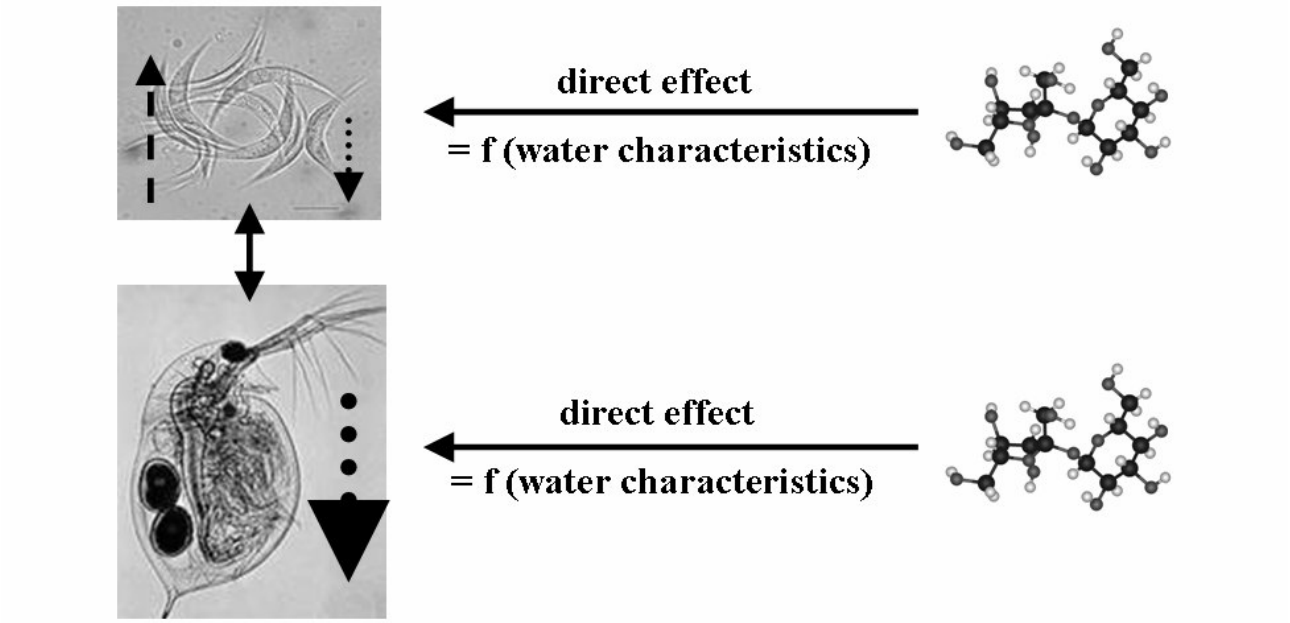

Figure I.3: Direct and indirect chemical effects. The dotted arrows represent a decrease in abundance resulting from direct chemical effects, which are influenced by physical and chemical water characteristics. The size of the arrow represents the magnitude of the effect. This decrease can be observed in a single-species test. In a multi-species experiment, the direct effect of the chemical on the zooplankton species at concentrations lower than those causing effects on the phytoplankton species eventually results in an increase in phytoplankton abundance because of a reduced grazing pressure (dashed upward arrow). The latter is called an 'indirect effect' and cannot be observed in a single-species test.

When only short-term, or 1 to 3 long-term single-species toxicity test results from 3 trophic levels are available, EU guidance documents prescribe to divide the lowest test result by an 'application factor' ('AF', TGD, EU 2003) to derive the PNEC. The use of AFs is inherently associated with the choice of an arbitrary number to represent the uncertainty when extrapolating a single-species effect concentration (EC_x) established in a laboratory, to a PNEC. Because of the lack of scientific arguments to motivate its use, this approach has been criticized (Chapman *et al.*, 1998; Forbes *et al.*, 2001). The use of these AFs has been found to result in a rather conservative approach for TBT and LAS (Selck *et al.*, 2002). Those authors found that the ratio between the lowest NOEC from

enclosure experiments and PNECs calculated using extrapolation of single-species test results, ranged from 1.5 to 6.7 for TBT, and from 10 to ca. 1733 for LAS.

If a larger number of long-term single-species toxicity test results is available, the SSD approach can be followed. Test results are ranked in increasing order and their cumulative probabilities are calculated assuming a statistical distribution type (parametric) or using nonparametric techniques such as bootstrapping. The resulting cumulative distribution is called the 'species sensitivity distribution' (Fig I.4). A chemical concentration corresponding to a lower percentile 'y' of such an SSD is called the 'hazardous concentration for y % of the tested species' (HC_y). The value of 'y' is almost always set at 5. In a regulatory context, the HC_5 may additionally be divided by an additional application factor (1 to 5) (TGD, EU 2003) to derive a PNEC.

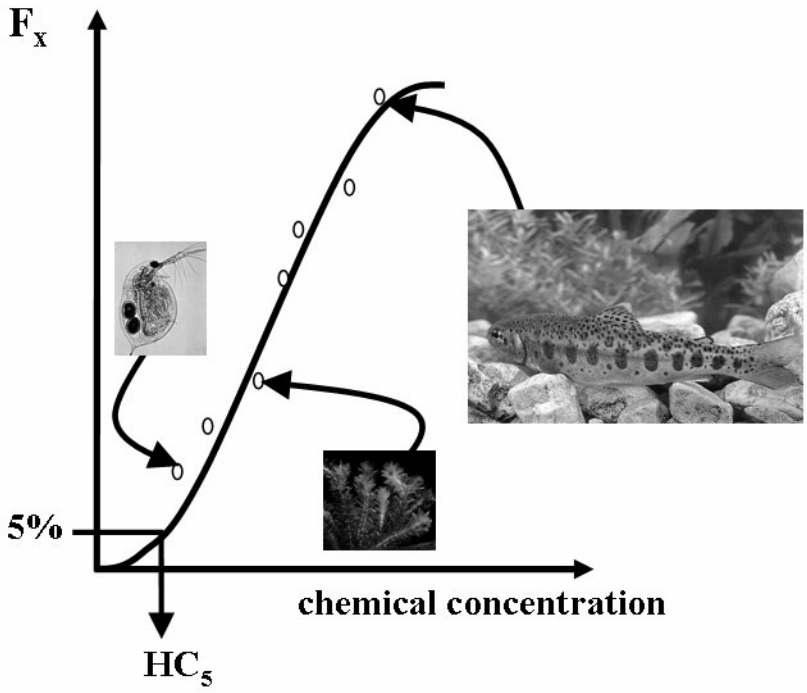

Figure I.4: The species sensitivity distribution (SSD) approach in ecological risk assessment: single-species toxicity test results (e.g., EC_{10}s; the concentration at which a measurement endpoint is affected for 10%) from different species (in this case, a macrophyte, a cladoceran, and a fish) are ranked and a cumulative probability function is fitted to these data. Note that also non-parametric methods can be used instead of an *a priori* defined probability function. A lower percentile from such an SSD is called the hazardous concentration for y% of the species (HC_y) and is used to derive a predicted no effect concentration (PNEC). In this example, as in the vast majority of SSD applications, y is set at 5.

I. 3. Ecological effect assessment - limitations of current practice

Ever since their introduction, both extrapolation techniques (AFs and SSDs) have been criticized for their underlying assumptions and arbitrariness (e.g., Forbes and Forbes, 1993). The latter is typically more closely associated with AFs, and the former more with SSDs. The AF approach assumes that by protecting the most sensitive species, ecosystems are protected against adverse effects. Unfortunately, the lack of an underlying theory for the AF approach impedes estimation of the uncertainty associated with the resulting PNEC, i.e. it is unknown how conservative this PNEC is likely to be. Hence, findings that SSD- and AF-PNECs are conservative (e.g. Selck *et al*., 2002) are interesting from a regulatory point of view, yet they can not replace a sound scientific basis for extrapolation techniques.

As demonstrated by Forbes and Calow (2002), the SSD methodology is based on a set of assumptions, of which 6 are related to the way the methodology is applied in practice, i.e. 'P-assumptions', and 3 to the theoretical background of the SSD concept, i.e. 'T-assumptions' (Table I.2; Forbes and Calow, 2002).

Table I.2: Assumptions associated with the SSD concept and its use (re-drafted from Forbes and Calow, 2002).

Assumptions behind the theory
T1. Interactions between species do not influence the sensitivity distribution
T2. All species are weighted equally
T3. Structure is the target of concern
Assumptions in the application
P1. The sample of species used to construct the sensitivity distribution is an unbiased sample of the target group of species about which conclusions are to be drawn.
P2. The endpoint is ecologically relevant.
P3. The chosen level of protection is appropriate.
P4. Chosen confidence limits around the protection level are appropriate.
P5. The shape of the distribution is appropriate.

I. 3. 1. Assumptions of the underlying theory of the SSD concept (T-assumptions)

Amongst the three assumptions on which the theoretical background of the SSD concept is founded (Table I.2), the key assumption is that ecological interactions do not influence the SSD (T1). The other assumptions (T2 and T3) are somehow related to T1. For example, arguments to criticize the assumption that all species are weighted equally in an SSD (T2) are based on the

distinct ecological roles of those species (e.g. Forbes and Calow, 2002). It is argued that species performing specific ecological functions, e.g., keystone species (Paine 1966), should receive higher weights than functionally more redundant species. As such, this argument also serves to urge for an incorporation of ecological interactions in the SSD concept. The assumption that ecosystem structure is equally or more sensitive than ecosystem function (T3), is equivalent with the statement that species composition is equally or more sensitive than the underlying processes involving fluxes of energy and matter (Cairns and Pratt, 1995). These fluxes of energy and matter represent consumer-resource interactions, i.e. ecological interactions. Until now, only the assumption that ecosystem structure is equally or more sensitive than ecosystem function (T3), has been tested, and found to be valid for TBT and LAS (Selck *et al*., 2002). The validity of the crucial assumption T1, i.e. that ecological interactions do not influence the SSD, has never been examined.

I. 3. 2. Assumptions about the application of the SSD concept (P-assumptions)

Papers discussing P-assumptions show that there are many degrees of freedom when actually applying the SSD concept. Distribution type, number and nature of toxicity test results, taxonomical composition, and chosen percentile HC_y are characteristics of the SSD application which may influence the resulting PNEC. The most commonly used distribution-types are the log-normal (Wagner and Lokke, 1991) and log-logistic distributions (Aldenberg and Slob, 1993). However, numerous distribution types (empirical, log-normal, log-logistic distribution, or others) and methods to derive the HC_5 (bootstrap, Bayesian techniques, or others) exist. The influence of the applied method and distribution type on the resulting HC_5 has been examined (e.g., Wheeler *et al*., 2002; Verdonck *et al*., 2001; Aldenberg and Jaworska, 2000; van der Hoeven, 2004; Newman *et al*., 2000; Jagoe and Newman, 1997). These studies indicate that application of the various methods to the same set of data, can result in HC_5s differing by a factor of three (Grist *et al*., 2002). Also the taxonomic composition of the SSD has been shown to influence the HC_5. Maltby *et al*. (2005) found that SSDs for specific taxonomic groups (vertebrates, arthropods, nonarthropod invertebrates) were different from one another. Duboudin *et al*. (2004a) found that the HC_5 is influenced more by intra-species variability and taxonomical composition of the SSD than by the statistical method used to derive the HC_5.

Finally, the choice for the HC_5 instead of other percentiles has been evaluated by Versteeg *et al*. (1999). Those authors showed that, for 11 different substances, HC_ys higher and lower than the HC_5 may also be protective. The finding that the HC_5 almost never bears its intended significance, i.e. that more (or less) than 95% of the species within an ecosystem will be affected when exposed

to HC_5 (Kefford *et al.,* 2005), can help to understand the results by Versteeg *et al.* (1999). The fact that both higher and lower percentiles than the HC_5 can protect ecosystems, indicates that the ecological significance of the HC_5 is not the 'hazardous concentration for 5% of the species'. Hence, the selection of the HC_5 results from a tradition in statistical testing rather than from experimentally derived ecological thresholds.
Given the multitude of choices for each of the different SSD characteristics, combination calculus learns that the ways to actually apply the SSD are even more abundant. This makes recommendations about how to apply the SSD methodology difficult and case-specific.

In literature, P-assumptions have received more attention (e.g., Kefford *et al*., 2005; Maltby *et al*., 2005; Duboudin *et al*., 2004a; Forbes *et al*., 2001; Hose and Van den Brink, 2004) than T-assumptions (Selck *et al*., 2002; Balczon and Pratt, 1994). The reasons for this may be that (1) there are more P-assumptions than T-assumptions; and (2) the examination of P-assumptions seems to bear more relevance in the light of ecological/environmental effect assessments. However, it should be clear that an ill-suited theoretical foundation can never serve its goal, despite a proper application. Therefore, we feel that discussions on T-assumptions are under-represented in literature.

In summary, it can be concluded that the two discussed extrapolation methods lack a sound scientific basis or rely on unproven assumptions which ignore ecological interactions occurring in ecosystems. Because numerous micro- and mesocosm studies have demonstrated the occurrence of indirect effects, it is suspected that the assumptions underlying both approaches will not always be valid. Hence, predictions of ecological effects by an extrapolation approach will most likely be inaccurate. It is therefore questionable whether ecological effect assessments relying on such inaccurate predictions can result in accurate assessments of chemical risk to aquatic ecosystems. An underestimation of the risk of a chemical will result in adverse effects on aquatic life. An overestimation of this risk can result in extra time needed for refinements of risk assessments and possibly in unnecessary remediation costs.

I. 4. Effect assessments using ecosystem models: a possible solution?

I. 4. 1. Ecosystem models in ecotoxicology

To address the need for alternative methods which can incorporate ecological interactions in ecological effect assessments of chemicals, ecosystem models have been developed. Although the term 'ecosystem model' can bear different meanings (e.g., conceptual models, fuzzy logic-based toolboxes), the type of ecosystem model most often used for ecological effect assessments is a dynamic ecosystem model and consists of three parts, as shown in Fig I.5 (Bartell *et al.*, 1988; DeAngelis *et al.*, 1989; Bartell *et al.*, 1999; Hanratty and Liber, 1996; Traas *et al.*, 1996; Traas *et al.*, 1998; Traas *et al.*, 2004a):

- a bioenergetic food web model
- a model for nutrient and detritus cycling
- toxic effect sub-models

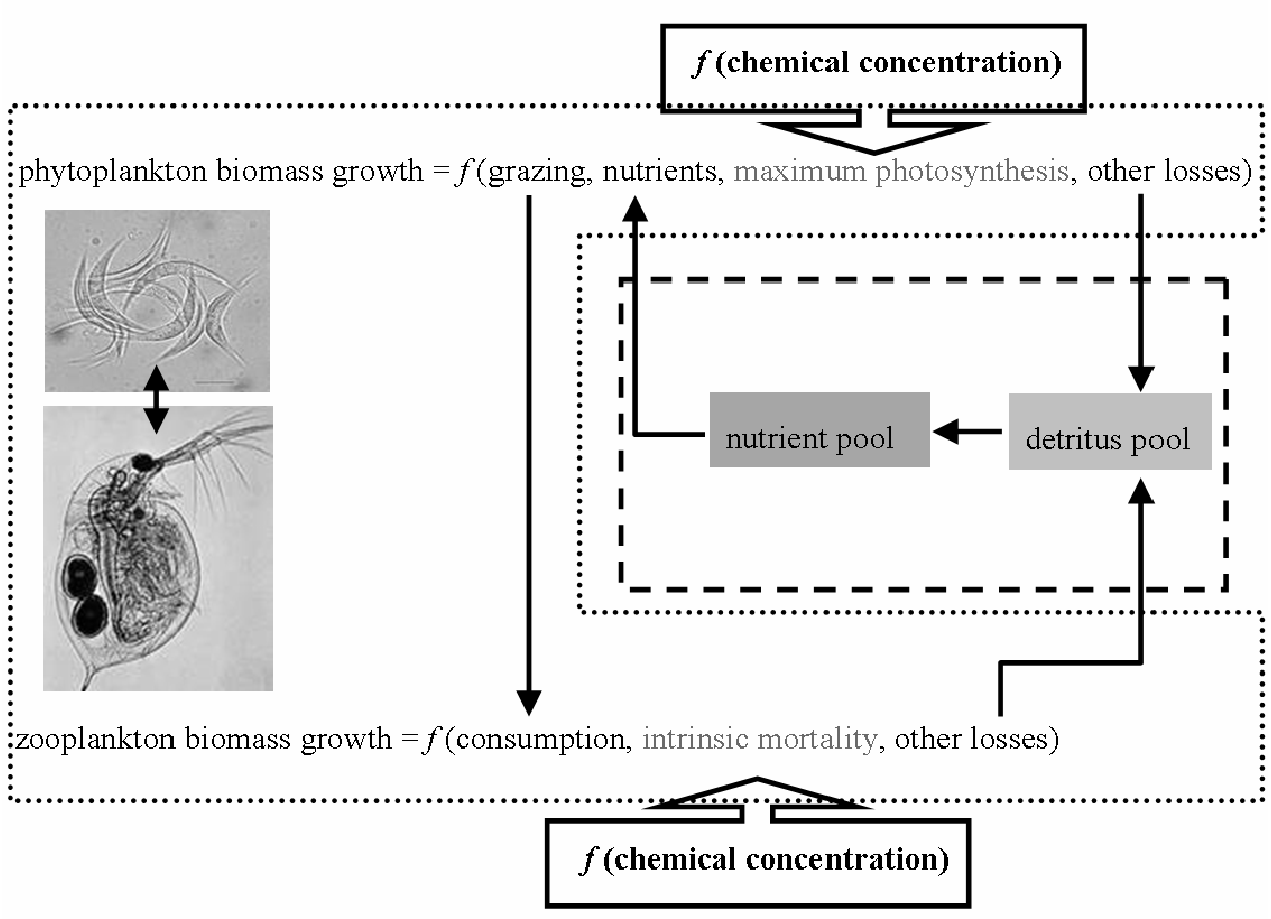

Figure I.5: An example of an ecosystem model consisting of (1) a bioenergetic food web model (within the dotted line); (2) a model for nutrient and detritus cycling (within the dashed line); (3) toxic effect sub-models (within bold line).

Across published examples of such ecosystem models, the food web models used are similar and rely on differential equations describing the growth of populations on a biomass basis. The variables which are governed by differential equations are termed 'state variables'. The different terms in the differential equations are called 'rates'. Differential equations for the state variable 'phytoplankton biomass' may include processes like photosynthesis, excretion, respiration, intrinsic mortality, sinking, and consumption by zooplankton. Differential equations for zooplankton biomass may include consumption of phytoplankton, defecation, respiration, excretion, intrinsic mortality, and predation by fish. Differential equations for fish biomass may include the same processes as those for zooplankton, except that consumption of phytoplankton is mostly replaced by consumption of zooplankton, if appropriate.

Also the model for nutrient and detritus cycling is similar across different publications. Similar to the biomass of populations, nutrients and detritus are also considered as state variables. Detritus mass increases by processes such as excretion by zooplankton and fish, and sinking of phytoplankton. Detritus mass converts into nutrient mass by mineralisation according to stoichiometric laws.

Toxic effect sub-models are used to calculate direct chemical effects on selected parameters of the different populations. These parameters represent the endpoints affected by the chemical. For example, if a chemical directly affects the intrinsic mortality of a population, the parameter 'intrinsic mortality rate' of this population will be a function of the chemical concentration. This function is called a 'toxic effect sub-model' in this dissertation, because it is embedded in the ecosystem model. Indirect effects result from the combination of (1) direct effects; and (2) ecological interactions which are represented within the food web model (see also Fig I.3). Two types of toxic effect sub-models have been used in ecosystem models: highly realistic toxicokinetic models (Bartell *et al.*, 1988; Traas *et al.*, 1996; Traas *et al.*, 2004b), as well as more simple external concentration-effect functions (Traas *et al*., 2004a; Bartell *et al*., 1999). The toxicokinetic sub-models suffer from parameter uncertainty, e.g., uptake and elimination rates, which are difficult to measure (Sijm and van der Linde, 1995). The more straightforward concentration-effect functions have the advantage that they are derived in frequently performed single-species toxicity tests. Consequently, laboratory generated L/EC_{50}s are parameters of such toxic effect sub-models (Bartell *et al*., 1999; Traas *et al*., 2004a). The type of concentration-effect function used varies between ecosystem models. While Bartell *et al*. (1999) use linear concentration-effect functions, Traas *et al*. (2004a) use logistic concentration-effect functions. Which type of toxic effect sub-model is more appropriate to obtain accurate predictions of ecological effects is currently unexplored.

I. 4. 2. The potential of ecosystem models in ecotoxicology

In the context of ecotoxicology, ecosystem models are applied for two major reasons: (1) for predictive ecological effect assessments; and (2) to increase our understanding of how ecological principles co-determine ecological effects caused by chemicals.

Ecosystem models have been used to predict a maximal chemical concentration which does not result in adverse ecological effects (e.g., Hanratty and Liber, 1996; Naito *et al*., 2002; Naito *et al*., 2003). Simulations of the behaviour in time of population's biomass (i.e. population dynamics) at different chemical concentrations are compared with population dynamics in untreated (control) systems. The highest exposure concentration at which the difference between the dynamics in both treatments is less than a given cut-off value is defined as the no observed effect concentrations (NOEC) for that population, i.e. the population-NOEC. This cut-off value is usually set at 20 % (e.g., Naito *et al.*, 2003) because it is the observed natural variability of community characteristics in the field (Suter II, 1993). The lowest population-NOEC in an ecosystem can serve as an ecosystem-NOEC, i.e. a concentration which does not adversely affect the biomass of the populations present. The advantage of such a measure is that it summarizes the information on the sensitivity of an ecosystem for biomass changes of its populations into one number. It can thus be used as a basis for water quality criteria derivation.

Additionally, ecosystem models have been used in parallel with micro- and mesocosm studies to provide additional explanations of the observed phenomena (e.g., Traas *et al*., 2004a; Taub, 1997). For example, the importance of indirect effects can be examined with ecosystem models. Direct effects on populations are given by the toxicity test results, used as input for the toxic effect sub-models. Net effects on populations predicted by an ecosystem model are the combined result of direct and indirect effects. If these predictions are in agreement with the net effects observed in the micro- and mesocosm studies, statements can be made on the importance of indirect chemical effects. This can be done by comparing the net effect on a population with the direct effect on this population (e.g., Naito *et al*., 2003). The use of an ecosystem model allows this comparison to be made at concentrations which are not tested in the micro- or mesocosm study.

I. 4. 3. Possible bottlenecks for the use of ecosystem models in ecotoxicology

Studies which quantitatively compare predicted with observed population-NOECs are almost non-existing. However, such a validation is needed to support the firmness of the results (and decisions based on these results) originating from the use of such models.

Current literature indicates that predictions of ecosystem-NOECs obtained from ecosystem models are rather conservative. The Lake Suwa version of the Comprehensive Aquatic Systems Model (CASM_SUWA) was used to predict ecosystem-NOECs for a variety of chemicals in various ecosystems (Naito *et al*., 2003). Predicted ecosystem-NOECs were compared with ecosystem-NOECs observed in experimental micro- and mesocosm studies. It was found that for most chemicals, predicted ecosystem-NOECs were one to two orders of magnitude lower than observed ecosystem-NOECs. From this it can be concluded that CASM_SUWA overestimates ecological effects.

A first reason for the low accuracy may be the use of one ecosystem model, which specifically represents the Lake Suwa ecosystem, to predict effects in a variety of - sometimes very different - ecosystems. This approach somehow contradicts the rationale for ecosystem modelling, i.e. the need for incorporating relevant ecological interactions in ecological effect assessments. For example, the presence of fish populations in the CASM_SUWA is problematic when using this model to make predictions about chemical effects in ecosystems without fish.

A second reason for the low predictive power may be the use of unsuited toxic effect sub-models. Indeed, which type of toxic effect sub-model should be used in an ecosystem model to obtain accurate predictions of ecological effects is currently unexplored. Because toxic effect sub-models predict the direct chemical effects on populations, they cause indirect effects to appear. Hence, they form the basis of the prediction of the effects on the different populations within an ecosystem.

A third reason why ecosystem models may not predict the expected ecological effects, is the use of non-representative single-species toxicity test results as parameters of toxic effect sub-models. For example, Hanratty and Liber (1996) have predicted diflubenzuron effects in a microcosm which was conducted concurrently. Although the modelled ecosystem was representative for the experimental ecosystem, predictions of effects on population dynamics were poor. These authors attributed the discrepancies between model predictions and observations to the use of non-representative single-species toxicity test results for *Daphnia magna*, an important zooplankton species in the studied ecosystem.

A fourth reason may be that the use of ecosystem models in predictive ecological effect assessments is hindered by the complexity of ecological systems (Egler, 1970). It has been debated that ecosystems may not obey any law, because there are too many variables which may affect ecosystem behaviour (Lawton, 1999). The behaviour of an experimental ecosystem depends to a large extent on the organisms involved and the environmental boundary conditions set in the experiment. For this reason, Van Straalen (2003) argues that the observed behaviour of an ecosystem can not be replicated, let alone predicted. However, despite the difficulty of exactly predicting ecosystem behaviour, patterns in the behaviour of ecosystems can be predicted more easily. Sommer *et al.* (1986) have studied reported seasonal successions of planktonic events in 24 lakes and constructed a linguistic model based on this information. This model describes patterns of population dynamics which are typically observed across many different lake types. This generality makes the linguistic model much simpler than the complex ecosystem behaviours which are classically described in other ecosystem studies. However, this linguistic model will not be able to explain the exact population dynamics in one specific ecosystem. Yet, it can be argued that the exact prediction of population dynamics is not a primary goal of ecological effect assessments. Effect assessors are interested in how common patterns of biomass dynamics, as those given by Sommer *et al.* (1986), change as a result of exposure to a chemical. It may thus be that too much effort is put in calibrating ecosystem models with the aim of exactly reproducing population dynamics of one given ecosystem. Publications aimed at deriving chemical effects on patterns of population dynamics have not been found.

Based on the current knowledge, it is difficult to say whether the reasons summarized in the above lead to differences between predicted and experimentally observed ecosystem-NOECs, or whether the ecosystem modelling approach itself is not suited for ecological effect assessments. This leads to the question: can ecosystem models be used in ecological effect assessments?

I. 5. Problem formulation - Rationale for this thesis

Current approaches used in ecological effect assessments of chemicals are based on the extrapolation of single-species toxicity test results to ecosystem effects. Such extrapolations rely on a set of assumptions and pragmatic 'rules of thumb'. The validity of these is largely unexplored. Hence, the ecological significance of predicted no effect concentrations (PNECs) resulting from these approaches is poorly understood.

The extent to which ecosystem models can be used as an alternative to these extrapolation approaches is unknown. However, validation efforts have been carried out in which predicted and observed NOECs are compared. Yet, those validation studies:

- include populations in the model which are different from those present in the studied ecosystem,
- do not always use representative single-species toxicity test results,
- may not use an appropriate toxic effect sub-model,
- focus on the exact replication of population dynamics instead of on the prediction of ecological effects,
- are aimed at quantitatively validating ecosystem-NOECs, while only qualitatively validating population-NOECs.

I. 6. Goals of this thesis - Outline

Schemes of the outline of this thesis are given in Figs I.6 and I.7.

I. 6. 1. Relationship between single-species toxicity test results and ecosystem effects

Because current ecological effect assessments have to rely on the relationship between single-species toxicity test results and effects on ecosystems, **chapter II** reviews different studies which are designed to examine this relationship. Rather than gathering available information on this relationship, the conceptual background of these studies is critically evaluated. Based on this evaluation, a selection of the reviewed studies is made, and the therein reported relationships between single-species toxicity test results and ecosystem effects are compared.

I. 6. 2. Model development and validation

In **chapter III and IV**, the construction of a new ecosystem model is described. A dynamic ecosystem model is constructed in such a way that it can be customized to represent different lentic (i.e. non-running) aquatic ecosystems. Also toxic effect sub-models can be customized. The ecosystem model aims at accurately predicting ecological effects, rather than pursuing the exact replication of observed population dynamics. Model equations, the methodologies to account for variability in model parameters, and processing of predictions to obtain ecological effects are elaborated. As an example, the application of the ecosystem model is demonstrated in aquatic microcosms exposed to copper.

Chapter V seeks to determine which type of toxic effect sub-model is most appropriate for the ecosystem model presented in the previous chapter. To this end, the ecosystem model described in the previous chapter is configured with different toxic effect sub-models. The capacity of the model to predict experimentally observed population-NOECs and ecosystem-NOECs is tested for the different configurations.

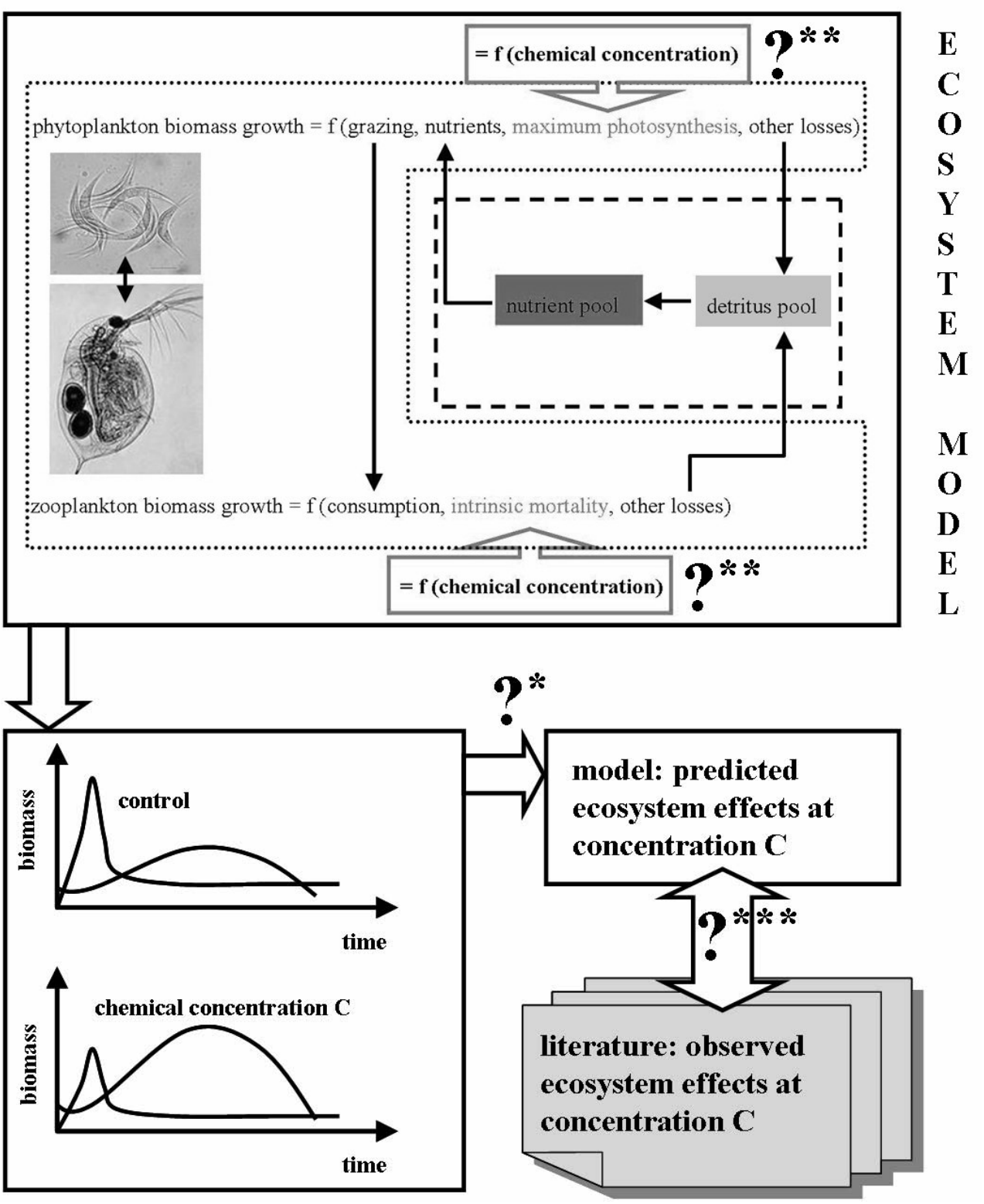

Figure I.6: Overview of the issues addressed in chapters III to VI of this dissertation. Details on the structure of the ecosystem model are given in the caption of Fig I.5.
***Chapter III and IV: How can an ecosystem model predict ecosystem effects without exactly predicting the population dynamics in this system?**
****Chapter V: Which toxic effect sub-model should be incorporated in such an ecosystem model to increase the accuracy of the predictions?**
*****Chapter VI: Can the resulting ecosystem model be used in ecological effect assessments?**

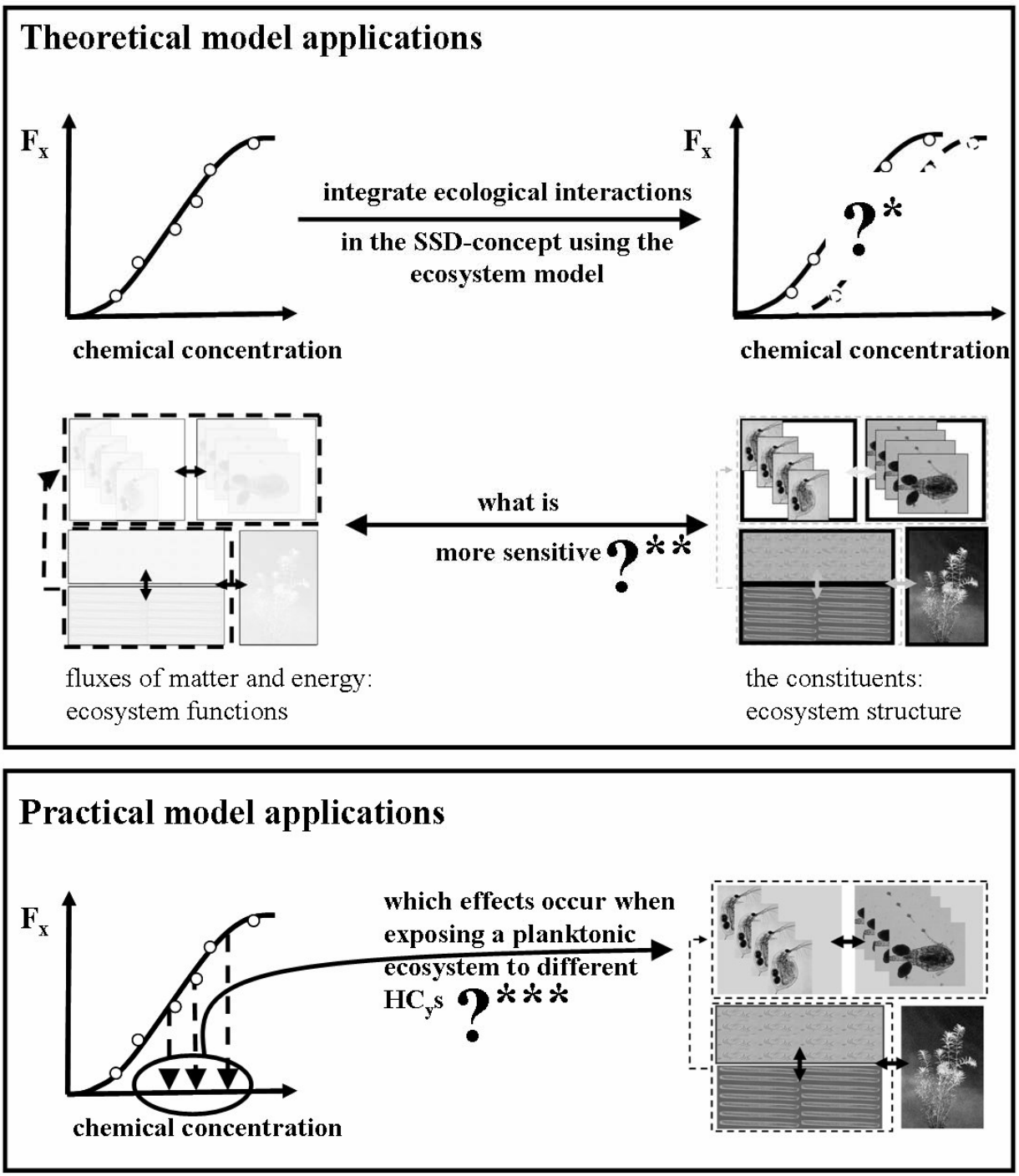

Figure I.7: Overview of the issues addressed in chapters VII to IX of this dissertation.
***Chapter VII: Is it correct to assume that ecological interactions do not affect the species sensitivity distribution?**
****Chapter VIII: What is more sensitive: ecosystem function or structure?**
*****Chapter IX: What is the ecological significance of different HC_y-values?**

In **chapter VI**, the configuration which gave best predictions in **chapter V** is used for validation using 11 other datasets described in literature. The goal of this chapter is to explore the capacity of ecosystem models to aid in ecological effect assessments. To this end, predictions of ecological effects of different chemicals in different ecosystems are compared with observations in micro- and mesocosm studies reported in literature, bearing in mind to:

- include the same populations in the ecosystem model as in the observed ecosystem;
- use the proper toxic effect sub-model with representative single-species toxicity test results;
- focus on the prediction of ecological effects rather than on exact population dynamics;
- also validate predicted population-NOECs in a quantitative way.

In this way, the following question is addressed: can ecosystem models be used in ecological effect assessments?

I. 6. 3. Theoretical model applications

In a second research line, the validated ecosystem model is used to test the assumptions underlying extrapolation approaches currently used in ecological effect assessments. In **chapters VII and VIII**, assumptions T1 and T3 of the SSD concept (Table I.2) are tested. To this end, a hypothesis-testing approach is followed:

Chapter VII: Null hypothesis: T1 is valid, i.e. ecological interactions do not influence the sensitivity distribution.

Chapter VIII: Null hypothesis: T3 is valid, i.e. ecosystem structure is as or more sensitive than ecosystem function.

These hypotheses are tested for a simple freshwater ecosystem individually exposed to 1000 hypothetical toxicants. Amongst these 1000 toxicants, different toxicant types are represented. 'Toxicant type' is defined here on the basis of the trophic levels directly targeted by the toxicant. For example, a toxicant primarily targeting zooplankton (e.g., an insecticide) and a toxicant primarily targeting phytoplankton (e.g., a herbicide) will be considered as different toxicant types.

This approach allows to interpret the validity of the tested assumptions, i.e. to answer the questions: "for which toxicant types are T1 and T3 valid?". Results obtained in these chapters can provide scientific arguments in deciding whether or not to use the SSD approach for an ecological effect assessment of a specific chemical.

I. 6. 4. Practical model applications

In **chapter IX**, the ecosystem model is used to evaluate the ecological significance of the hazardous concentration for y % of the tested species (HC_y) for different values of 'y' in a freshwater planktonic ecosystem exposed to copper. The ecosystem model is used to estimate effects of different HC_ys of copper on ecosystem structure and function.

I. 6. 5. Conclusion

In the **final chapter X**, the information obtained in this dissertation is reviewed and summarized in a set of conclusions. By comparing these conclusions with the problem statements from this introductory chapter, possibilities for further research are suggested.

Questions addressed by this dissertation (see also Figs I.6 and I.7)

→ Chapter II: What is meant with 'ecosystem effects' and how has their relationship with single-species toxicity test results been examined until now?

→ Chapter III and IV: Can an ecosystem model accurately predict ecosystem effects if it is not calibrated on observed population dynamics?

→ Chapter V: Which toxic effect sub-model should be incorporated in such an ecosystem model to increase the accuracy of the predictions?

→ Chapter VI: Can the resulting ecosystem model be used in ecological effect assessments?

→ Chapter VII and VIII: Which assumptions associated with current approaches for ecological effect assessments are valid and for which chemicals?

→ Chapter IX: What is the significance of different y-values HC_y in a planktonic ecosystem?

→ Chapter X: Conclusions and research perspectives

Chapter II

Relating single-species toxicity test results to ecosystem effects: a review of current methodologies

Chapter II

Relating single-species toxicity test results to ecosystem effects: a review of current methodologies

Abstract - Most methodologies used in ecological effect assessments rely on assumptions about the relationship between single-species toxicity test results and ecosystem effects. This relationship is mostly examined by (1) experimental ecosystem studies and (2) ecosystem models. After having characterized the available single-species toxicity test results and studies on ecosystem effects, we review these two methods. Between 1990 and 2006, 75% of the single-species toxicity tests conducted are short-term tests (< 5d) with animals using immobility or mortality as an endpoint. Most frequently studied ecosystem effects are changes in abundance or biomass of populations. Ecosystem studies indicate that EC_xs for a population's biomass or abundance are generally within a factor two of single-species immobility EC_xs. However, this conclusion partly originates from the focus on effects on invertebrates in ecosystem studies with insecticides. Results from the few modelling studies found apparently contradict with the findings from experimental ecosystem studies. The conservatism of the model predictions and the focus on toxicants for which prey are more sensitive than predators in the considered modelling studies can explain the difference between results from experimental ecosystem studies and results from modelling studies.

redrafted from

De Laender F., De Schamphelaere, K.A.C., Vanrolleghem, P.A., Janssen, C.R. Relating single-species toxicity test results to ecosystem effects: a review of current methodologies. Ecotoxicology and Environmental Safety, submitted.

II. 1. Introduction

Water quality criteria for chemicals aim at establishing environmental concentrations which do not adversely affect the structure and functions of ecosystems (EU, 2003). To this end, ecological effect of chemicals have to be assessed. The most realistic way to study ecological effects of a chemical is to expose enclosed ecosystems to different concentrations of the chemical and assess the effects at the community level. To increase reproducibility of such tests, multi-species toxicity tests such as microcosm and mesocosm tests have been conducted. Those tests still enable the evaluation of indirect effects of chemicals resulting from ecological interactions (Fleeger *et al*., 2003) without contaminating real ecosystems. However, the routine use of mesocosm tests to assess the impact of chemicals on ecosystems is not feasible because of the associated costs (Newman and Unger, 2003). Hence, the potential adverse effects of chemicals on ecosystems is generally derived using ecotoxicity tests in which a single species is exposed to a series of increasing chemical concentrations. However, the higher reproducibility of these so called "single-species toxicity tests" is at the expense of a lower degree of realism (Schindler, 1998; Sanderson *et al*., 2004). Results from such single-species toxicity tests do not give accurate information on chemical effects at the ecosystem-level. Methodologies to relate single-species toxicity test results to effects on ecosystems have been developed and include the use of application factors (AFs), species sensitivity distributions (SSDs) and ecosystem models. The assumptions used by the latter approach are based on knowledge about ecological mechanisms and processes obtained through quantitative ecological research. In contrast, the first two methodologies rely on assumptions which are largely pragmatic and not always based on sufficient ecological research.

In this chapter, primary studies published between 1990 and 2006 are reviewed which compare (1) single-species toxicity test results to (2) ecosystem effects in the aquatic environment. The meaning of these two terms is reviewed and databases are examined to map out available data. The factors which may drive the relationship between both types of results are discussed. To focus on the influence of environmental factors on these results, genetic differences between field species and standard test species are not discussed here. The different ways in which this relationship is examined are critically evaluated with special attention to sources of variability which may distort this relationship.

II. 2. Definitions

II. 2. 1. Single-species toxicity tests

In a single-species toxicity test, the direct effect(s) of a (mixture of) chemical(s) on a small population of one isolated species is evaluated. In theory, the number of endpoints on which direct effects of chemicals can be examined is as large as the number of traits a species has. Tests are performed under controlled laboratory conditions: temperature, light regimen, physical and chemical water characteristics, and resources are standardized to increase reproducibility.

To examine which types of single-species toxicity test results have been performed most, a search in the aquatic part of the USEPA database ECOTOX was performed (http://cfpub.epa.gov/ecotox/). This search was constrained by selecting "concentration based endpoints", "lab", "environmental OR not reported" as endpoints, study location, and exposure type, respectively. Only data between 1990 and 2006 were considered. Both animal and plant data were collected. Results from multi-species experiments (e.g. microcosm) were deleted *a posteriori*. The collected single-species toxicity test results primarily reflect the effects of chemicals on animals (4450 records found). Only 228 records for plants were found. Although the number of endpoints which can be examined is theoretically unlimited, the main endpoints studied were mortality or immobility (75%) and photosynthesis (25%) for animals and plants, respectively.

Ninety percent of the single-species tests with animals we found in the USEPA database used an exposure time of 5 days or less (Fig II.1). Similar to the variation in studied endpoints for plants, also exposure time of single-species tests with plants exhibits a slightly higher variability than in tests with animals (Fig II.1): ninety percent of the considered single-species tests with plants had an exposure time of 15 days or less.

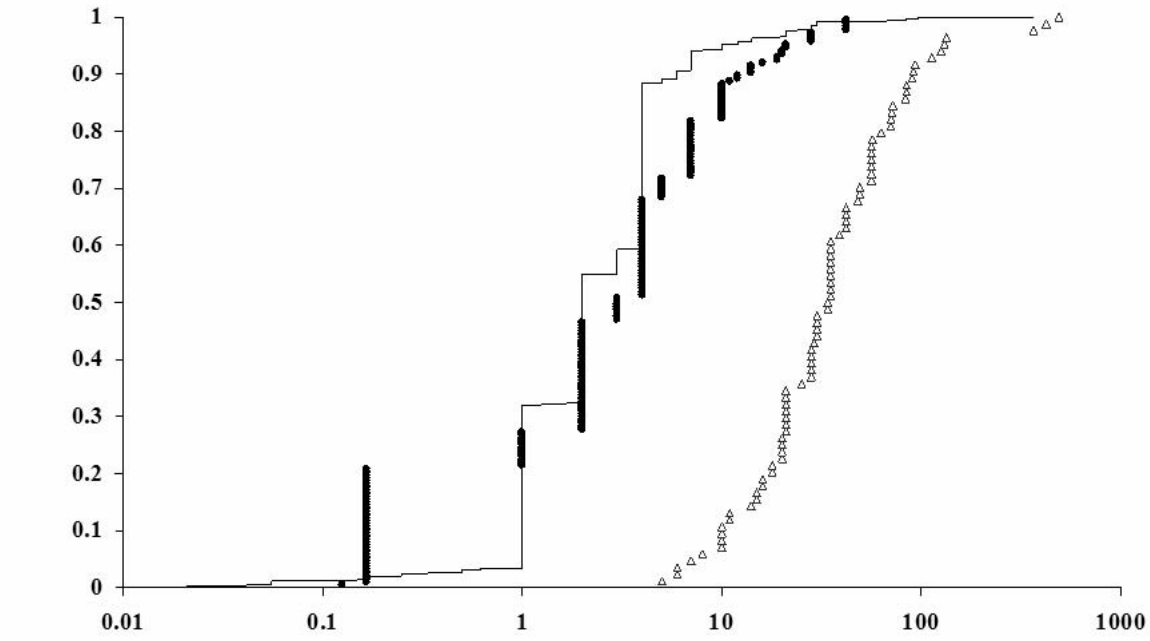

Figure II.1: Probability distributions of the duration of single-species toxicity tests (in days) with plants (black symbols), animals (black line), and ecosystems (open triangular symbols). Data come from the aquatic part of the USEPA database ECOTOX.

II. 2. 2. Ecosystem effects

In the context of ecotoxicology, the term "ecosystem effect" is often used to denote effects on structure and function of an ecosystem. These effects can be studied in artificial ecosystems or in field enclosures. Depending on the study design and its dimensions, environmental conditions are more (microecosystem as in Kersting and van Wijngaerden, 1992) or less controlled (field enclosures as in Brock *et al.*, 2004). A higher reproducibility implies a lower degree of realism (Schindler, 1998). As with single-species tests, a large amount of endpoints can be used to assess chemical effects. Considering their higher spatial and temporal scale, endpoints used in ecosystem studies are expected to be more diverse and more complex than those of single-species. We examined 192 primary studies, published between 1990 and 2006, reporting ecosystem effects of a chemical in aquatic environments [ISI Web Of Science: TS=((mesocosm* OR microcosm* OR enclosure* OR semi-field OR semi field) AND (ecosystem* OR communit*) AND tox* NOT soil AND effect*]. Studies on benthic communities were omitted. References to these studies are given in the appendix (XI.2.1). References to studies explicitly cited in this chapter are integrated in the references at the end of this thesis, as usual. Reported ecosystem endpoints differ in complexity and range from population abundance to case-specific indices such as the quantitative

macro-invertebrate community index (Hickey *et al*., 1999). In nearly half of the collected papers (48%), effects on the abundance or biomass of a species or a trophic level are used as an ecosystem endpoint (Fig II.2). As such, these studies refer to "ecosystem effects" as effects on populations or trophic levels within an ecosystem. This is defensible, since effects on populations within an ecosystem are influenced by the presence or absence of other populations. Different ecosystems can have different populations, making effects on populations ecosystem-specific. Hence, the way populations react to environmental stress is a characteristic of the ecosystem.

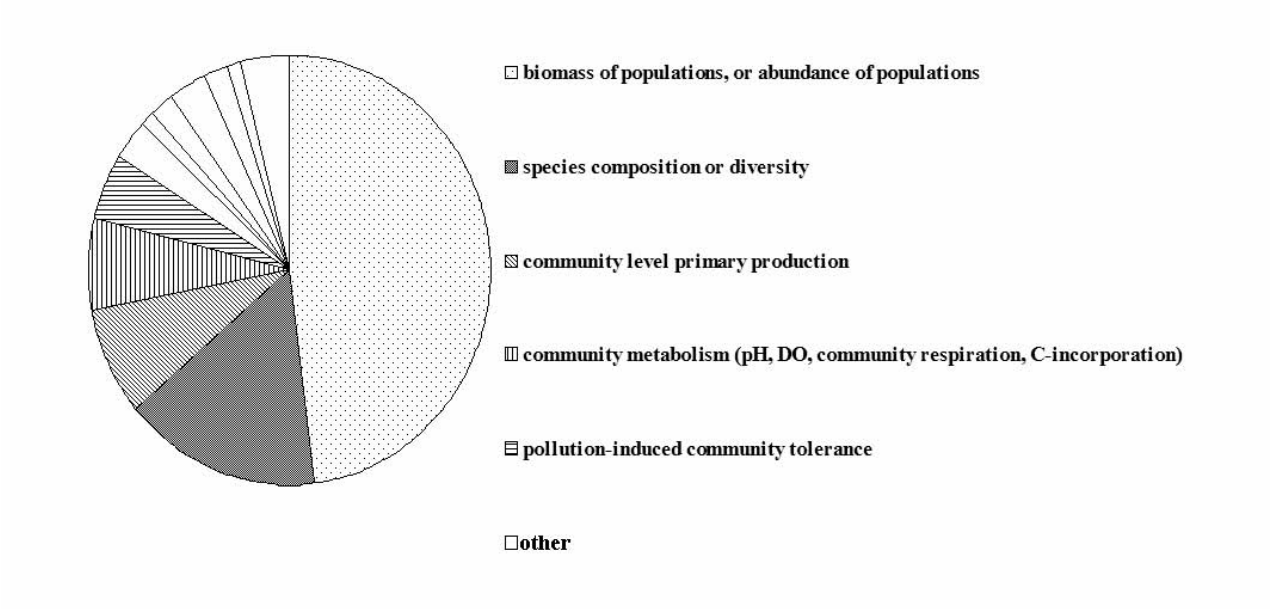

Figure II.2: Ecosystem effects studied in 190 ecosystem studies between 1990 and 2006. Note that "other" consists of several minor classes.

Next to population's biomass and abundance, ecosystem studies also use endpoints describing their organization (e.g. dominance patterns) and functioning (e.g. functional diversity). Examples of such endpoints are diversity indices and rates of primary production. There are fewer ecosystem studies (30%) using such an endpoint than there are ecosystem studies using abundance or biomass as an endpoint.

As expected, the duration of these studies is higher than those used in single-species toxicity tests (Fig II.1). However, from the 192 considered ecosystem studies reviewed here, 50% had a duration of 35 days, or less. Whether this is a sufficiently long period for indirect effects to become apparent, is unclear (Fleeger *et al*., 2003).

From this overview, it can be concluded that ecosystem effects of chemicals are assessed in two ways, depending on the endpoint considered: changes in (1) biomass/abundance of populations and/or trophic levels; and (2) aggregate ecosystem measures. Those two different endpoints will be termed ECO1 and ECO2 endpoints throughout this chapter, respectively.

II. 3. Single-species toxicity test results vs. ecosystem effects: concepts

How a relationship between single-species toxicity test results and ecosystem effects can be interpreted depends on what is meant with the latter (effects on ECO1 or ECO2).

The interpretation of a relationship between single-species toxicity test results and effects on ECO1 endpoints is straightforward. As described earlier, ECO1 endpoints are biomass or abundance-based, and as such are comparable to the majority of single-species toxicity test results. The similarity of both endpoints allows to compare effects quantitatively. For example, an $EC_{50,immobility}$ and an $EC_{50,ECO1}$ both have the same meaning: a 50 % reduction in abundance of a population. The major difference between both results is the experimental design used to derive them.

The factors determining the relationship between effects on ECO1 endpoints and single-species toxicity test results can be divided in biological, physical and chemical, and scale factors. Here, biological factors are defined as the ecological interactions between populations. Biological factors can thus result in indirect effects of chemicals on populations which were intrinsically not directly targeted by the toxicant. Two types of indirect effects have been reported (Fleeger *et al*., 2003): an increased abundance resulting from reduced predation pressure, and an increased or reduced abundance because of altered competition. Physical and chemical factors include temperature and bioavailability-determining water characteristics, both of which may change the sensitivity of species (Heugens *et al*., 2001; De Schamphelaere and Janssen, 2002). Finally, the smaller spatial and temporal scale of a small population of one species in a single-species toxicity test compared to that of an aggregation of species into a food web within an ecosystem, are also expected to influence the relationship between single-species toxicity test results and ECO1 endpoints. The mechanisms of how these factors interact to result in effects on ECO1 endpoints is complex. This is reflected by models which try to account for each of these factors separately. Ecological interactions between populations of species are inherently nonlinear (e.g., Sun *et al*., 1991) since these are density-dependent. Models accounting for some physical and chemical factors (e.g., bioavailability models), use iteration procedures to solve implicit equations (De Schamphelaere and Janssen, 2002).

The interpretation of a relation between single-species toxicity test results and effects on ECO2 endpoints is problematic. ECO2 endpoints reflect the organization and functioning of populations within an ecosystem, and are therefore inherently incomparable with single-species endpoints. The question how effects on ECO2 endpoints relate to effects on single-species endpoints should therefore be considered as two separate issues (Fig II.3).

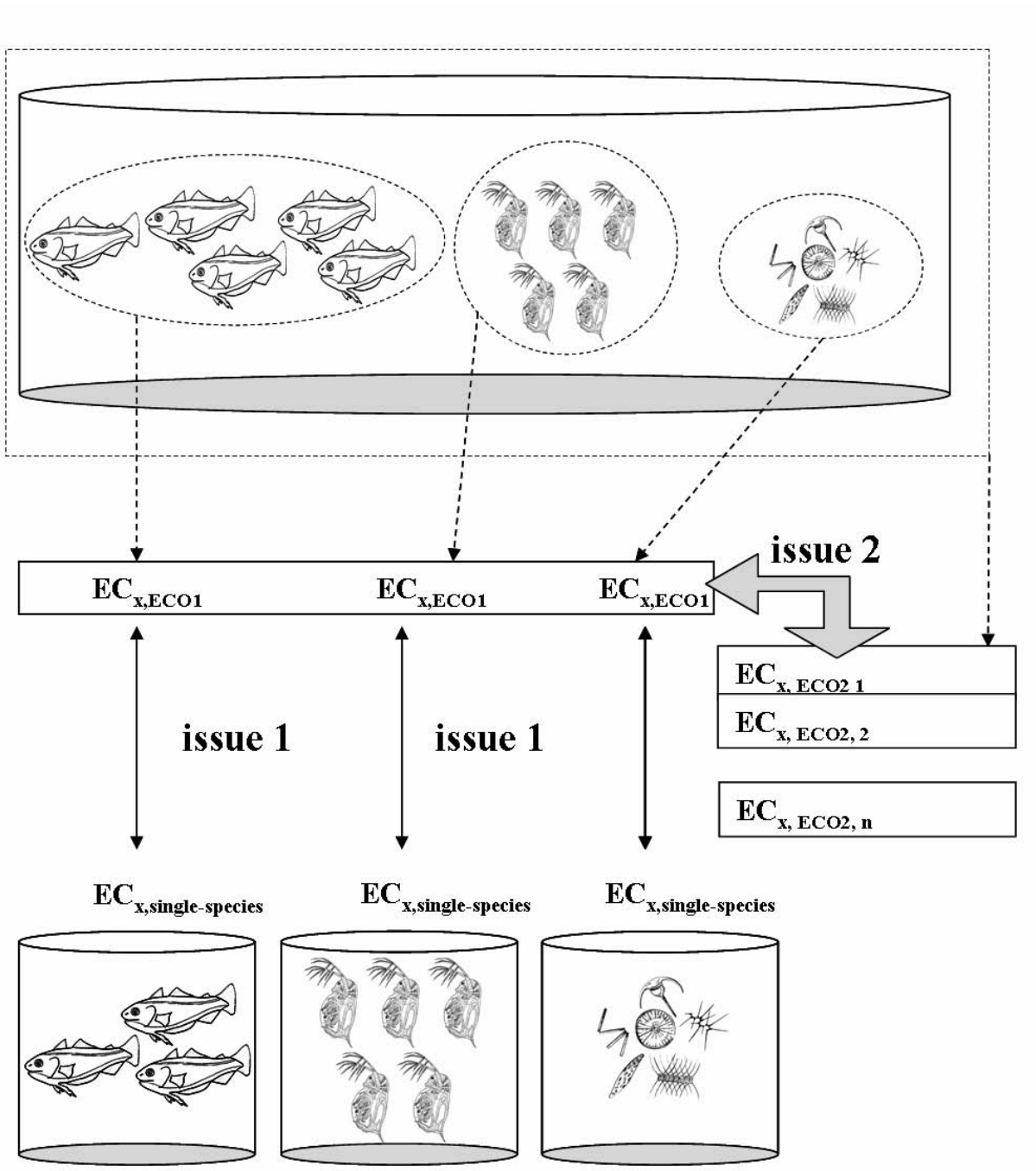

Figure II.3: The two separate issues when studying the relationship between ECO2 endpoints and single-species toxicity test results. Within the dashed square, an ecosystem study is represented. $EC_{x,ECO1}$s are effect concentrations derived with abundance data from this ecosystem study. A first issue is the relationship between those $EC_{x,ECO1}$s and $EC_{x,single\text{-}species}$s, i.e. effect concentrations resulting from single-species tests conducted alongside the ecosystem study. A second issue is the relationship between $EC_{x,ECO1}$s and EC_{xECO2}s, i.e. effect concentrations of n aggregate ecosystem measures, e.g., of ecosystem functions.

The first issue addresses the relationship between single-species toxicity test results and effects on the abundance of populations of the same species within an ecosystem. Note that the latter are termed 'ECO1 endpoints' in this chapter. Considering the complexity of this issue, it should be

treated separately from the second issue. The second issue addresses the relationship between effects on ECO1 endpoints and effects on ECO2 endpoints, i.e. how effects on populations within an ecosystem propagate to effects on overall ecosystem organization and functioning.

II. 4. Single-species toxicity test results vs. ecosystem effects: practical approaches

The relationship between single-species toxicity test results and ecosystem effects can be examined in two ways: by executing experimental ecosystem studies, or by using ecosystem models.

II. 4. 1. Experimental ecosystem studies

When reporting findings from experimental ecosystem studies, it is a common practice to put effect concentrations (ECs) measured for some ecosystem endpoint(s) in perspective by comparing those with (previously published) single-species toxicity test results. In many cases, the motivation for doing so is to examine the relationship between single-species toxicity test results and ecosystem effects. Unfortunately, the outcome of such a comparison is highly case-specific. These comparisons are transparent when considered individually, but their collective significance is unclear. The fact that different ecosystem studies define (and measure) ecosystem effects in a variety of ways and use single-species toxicity test results produced by other authors, impedes any generalization across chemicals. These issues will be elaborated in the following paragraphs.

As previously discussed, different interpretations of the term “ecosystem effects” are given in the literature. Depending on the used interpretation (ECO1 or ECO2), the relation between ecosystem effects and single-species toxicity test results will be different.

Among the 192 articles we considered, only 5 have performed (and reported) single-species toxicity tests concurrent with the experimental ecosystem study. However, inter-laboratory variability originating from differences in sensitivity between clones of the same species and different laboratory practices has been observed (Environment-Canada, 1990). As such, it is unclear to what extent the difference between effects on ECO1 endpoints and effects observed in a single-species toxicity test originates from this inter-laboratory variability or from the factors given in II.3. The extent to which this inter-laboratory variability has influenced the outcome of previous comparisons is unclear.

Another issue that impedes generalizations across studies and chemicals originates from the way in which single-species toxicity test results are compared with ecosystem effects. Some authors compare one single-species test result with effect concentration(s) of one (or more) ecosystem endpoint(s) (e.g., Berard *et al.*, 1999a). In other studies, a given percentile of many single-species toxicity test results is compared with effect concentration(s) of one (or more) ecosystem endpoint(s) (Maltby *et al*., 2005). This percentile is often calculated using the species sensitivity distribution concept (SSD): a statistical distribution is fitted to a set of single-species toxicity test results. Type of distribution and the used toxicity data all may influence the chosen percentile (Duboudin *et al*., 2004a). Verdonck *et al.*, (2001) found that the methodology to derive a percentile from a set of single-species toxicity test results should be chosen based on the number of toxicity test results available. As such, the methodology by which such a percentile is derived will also influence the examined relationship between single-species toxicity test results and effect concentration(s) of one (or more) ecosystem endpoint(s).

In summary, the true mechanisms determining the relationship between single-species toxicity test results and ecosystem effects may thus be distorted by (1) how "ecosystem effects" are defined; (2) inter-laboratory variability of single-species toxicity test results; and (3) how multiple single-species toxicity test results are treated. As a result, the true mechanisms behind the envisaged relationship may get unidentifiable.

In an attempt to minimize possible influences of unwanted sources of variability (1+2+3) on the relationship between single-species toxicity test result and ecosystem effects, we re-analysed the results from the considered ecosystem studies. Only articles which avoided these sources of variability were considered.

II. 4. 1. 1. Experimental ecosystem studies: single-species toxicity test results vs. effects on ECO1

In only 5 of the 192 studies, single-species toxicity tests based on abundance were conducted and reported alongside measurements of effects on ECO1 endpoints, as such eliminating inter-laboratory variability and enhancing comparability between ecosystem effects and single-species toxicity test results. Those studies were: Fairchild *et al*. (1992), van Wijngaarden *et al*. (1996), Schroer *et al*. (2004), van der Hoeven *et al*. (1997), Hose *et al*. (2003). Exposure duration in the ecosystem studies was always longer than in the accompanying single-species tests. Only van der Hoeven *et al.* (1997) and Hose *et al.* (2003) used the same exposure duration in both single-species test and the ecosystem study. Details on the effect concentrations obtained in the 5 studies

are given in the appendix (XI.2.2). In general, exposure durations of the single-species toxicity tests in the USEPA database and exposure duration of those reported in the 5 selected studies showed good agreement.

Results obtained in these studies indicate that for most species, $EC_{x,ECO1}$ is within a factor 2 of $EC_{x,single\text{-}species}$ (Fig II.4). Findings by Roessink *et al.* (2006) indicate that for triphenyltin acetate, invertebrate-SSDs based on $LC_{50,single\text{-}species}$ values have a higher mean than invertebrate-SSDs based on $EC_{x,ECO1}$values measured in microcosms. This contrast with the data presented in Fig II.4 may originate from chronic exposure through the food chain and latency effects of triphenyltin acetate.

Across studies and species, $EC_{50,single\text{-}species}$ values are almost consistently lower than $EC_{50,ECO1}$s. This is in contrast with EC_{10} values, which are comparable for ECO1 endpoints and single-species endpoints. As such, the similarity of $EC_{x,ECO1}$ and $EC_{x,single\text{-}species}$ seems to depend on x. A reason for this might be the higher probability of the occurrence of indirect effects at higher concentrations than at lower concentrations. Since the number of affected single-species endpoints increases with increasing chemical concentration, possible routes for indirect effects also increase. As a result, the probability that $EC_{x,ECO1}$ and $EC_{x,single\text{-}species}$ are different will increase as the exposure concentration increases, i.e. as x increases. All 5 studies which were re-analyzed examined ecosystem effects of insecticides, i.e. substances which primarily target invertebrates. No evidence was found that phytoplankton is directly affected by the studied chemical in 4 of the 5 studies, i.e. by esfenvalerate, chlorpyrifos, or lambda-cyhalothrin. For endosulfan, however, a 96h-EC_{50} of 427.8 µg L^{-1} for photosynthesis-inhibition of the phytoplankton species *Pseudokirchneriella subcapitata* has been reported (Delorenzo *et al*., 2002). This EC_{50} is well above the range of the endosulfan concentrations tested in the ecosystem study conducted by Hose *et al*. (2003) (< 100 µg L^{-1}). Hence, it is unlikely that phytoplankton species would have experienced any direct effect in the 5 considered studies. If $EC_{x,single\text{-}species}$s would have been determined for phytoplankton photosynthesis inhibition in those studies, they would probably have been higher than the exposure concentrations used in the experimental ecosystems. Because indirect effects on phytoplankton abundance are observed by Fairchild *et al*. (1992), an $EC_{x,ECO1}$ for phytoplankton would likely be in the range of the experimental concentrations. Thus EC_{xECO1} would be lower than $EC_{x,single\text{-}species}$ for phytoplankton. If the same holds for the other 4 studies is impossible to say, since phytoplankton abundances are not reported there. It has, however, been reported that phytoplankton blooms are frequently observed in experimental ecosystems exposed to insecticides such as pyrethroids (Hanazato, 2001). From this it can be concluded that, for many insecticides, the $EC_{x,ECO1}$ of phytoplankton is expected to be

lower than the $EC_{x,single\text{-}species}$. The observation made here, i.e. that effects in the field are more severe than effects in the lab, thus partly originates from the focus on effects on invertebrates and vertebrates in ecosystem studies with insecticides.

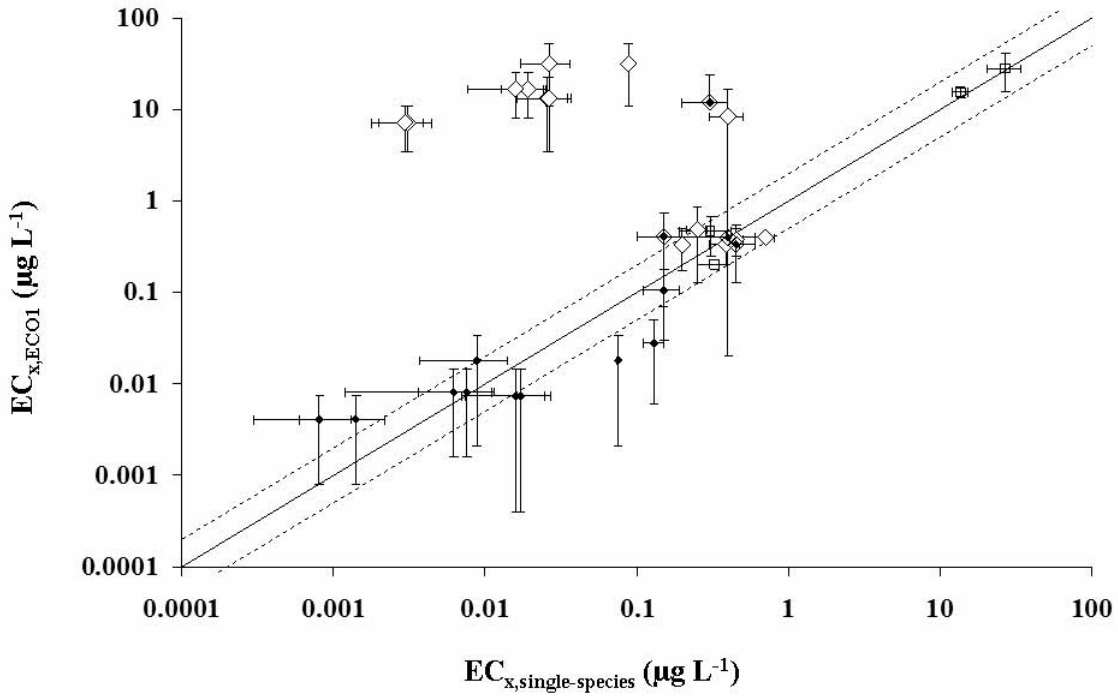

Figure II.4: The relationship between $EC_{x,ECO1}$ and $EC_{x,single\text{-}species}$, i.e. between effect concentrations for the abundance of populations within an ecosystem study and effect concentrations derived in a single-species test, performed concurrently with the ecosystem study. Endpoints of both EC_xs are abundance-based. In most cases, exposure durations differ between both EC_xs. White diamond symbols represent EC_{50}s, black diamond symbols represent EC_{10}s, and square white symbols represent LC_{50}s. Error bars denote upper and lower 95% confidence intervals. The dotted line represents a factor two difference. Data originate from Fairchild *et al.*, 1992; van Wijngaarden *et al.*, 1996; Schroer *et al.*, 2004; van der Hoeven *et al.*, 1997; and Hose *et al.*, 2003. Details on these data are given in the appendix (XI.2.2).

II. 4. 1. 2. Experimental ecosystem studies: effects on ECO1 vs. effects on ECO2

From the 5 studies which were retained after screening, only 3 also examined the relationship between effects on ECO1 and effects on ECO2 endpoints. In these studies this relationship was examined only using relational operators, i.e. ">", "<", or "=". Fairchild *et al.* (1992) concluded that community metabolism was less sensitive for esfenvalerate than population biomass. The NOEC for species composition of a community exposed to the insecticide λ-cyhalothrin was always higher than the established population-EC_xs (Schroer *et al.*, 2004). Conversely, Hose *et al.* (2003) found that the species composition was altered at concentrations which did not affect populations of the same species. A mechanistic approach to relate effects on ECO1 endpoints to effects on ECO2 endpoints was not found. The difficulty in doing so is the inherent difference

between ECO1 endpoints (abundance-based) and ECO2 endpoints (related to ecosystem organization and functioning). One possibility to overcome this difficulty is to compare effects on ECO1 endpoints with effects on associated ECO2 endpoints. For example, in chapter IX, the probability of copper effects on zooplankton and phytoplankton biomass (i.e. both ECO1 endpoints) was lower than and equal to the probability of effects on total ingestion by zooplankton and total photosynthesis by phytoplankton (i.e. both ECO2 endpoints), respectively. The ubiquitous finding that populations within an ecosystem are equally or more sensitive than the ecosystem functions they perform (e.g., Selck *et al.*, 2002; Balczon and Pratt, 1994) also indicates that ECO1 endpoints are more sensitive than ECO2 endpoints.

II. 4. 2. Ecosystem models

Because of the high cost of large-scale studies, mathematical models have been proposed as an alternative to examine ecosystem effects of chemicals. These models mostly consist of a food web in which toxic effect sub-models are incorporated. These toxic effect sub-models vary in complexity, going from highly realistic toxicokinetic models to simple concentration-effect functions, which use single-species test results as parameters. Here, we only focus on ecosystem models with the latter type of toxic effect sub-models, since those are specifically designed to relate single-species test results into ecological effects, i.e. their goal is equivalent with the goal of the 5 ecosystem studies discussed in the previous section. In the literature covering the period between 1990 and 2006, three models were found (Hanratty and Liber, 1996; Bartell *et al.*, 1999; and Traas *et al.*, 2004a) which use single-species L/EC_{50}s to predict ecosystem effects. Not all of those models incorporate all parameters of a single-species toxicity test. Exposure duration of the single-species toxicity test is taken into account in two models (Bartell *et al.*, 1999; and Traas *et al.*, 2004a), while only one model also takes slope into account (Traas *et al.*, 2004a). The advantage of these models is that they explicitly use single-species toxicity test results to predict ecosystem effects. As such, the $EC_{x,single\text{-}species}$ values of the considered populations are unambiguously defined.

The comparison of predicted effects on ECO1 endpoints with single-species toxicity test results in a quantitative way is rare. Naito *et al.* (2003) found that for the concentration at which a 50% fish biomass reduction is predicted, is 1 to 5 orders of magnitude lower than the single-species LC_{50} for this fish species. The sensitivity of zooplankton and/or benthic invertebrates - i.e. food sources for omnivorous fish - for the considered chemicals is proposed as an explanation for this observation (Naito *et al.*, 2003). A second reason may be the conservative effect estimates

produced by the comprehensive aquatic systems model (CASM). Indeed, in the same study, predicted ecosystem-no observed effect concentrations for a range of chemicals were one to two orders of magnitude lower than observed ones. The 5 studies reviewed in this chapter (Fig II.4) only include one study where fish were present. Fairchild *et al.* (1992) report that bluegill population biomass was affected at esfenvalerate concentrations similar to their $LC_{50,single\text{-}species}$. The slightly lower $LC_{50,single\text{-}species}$ obtained for bluegill than for cladocerans (Fairchild *et al.*, 1992) indicates that the bluegill populations were affected at lower concentrations than their food source. As such, indirect effects due to a reduction of food availability, as found for the chemicals examined by Naito *et al.* (2003), is less likely in the case of esfenvalerate. This may explain why Naito *et al.* (2003) found $EC_{x,ECO1}$ to be lower than $EC_{x,single\text{-}species}$ for fish populations while Fairchild *et al.* (1992) found that $EC_{x,single\text{-}species} \approx EC_{x,ECO1}$.

A reason for the scarcity of modelling studies focusing on the relationship between single-species toxicity test results and ecosystem effects might be that ecosystem models are mostly used to derive chemical concentrations which do not result in adverse ecological effects (Hanratty and Liber, 1996; Bartell *et al.*, 1999). Alternatively, ecosystem models have also been used alongside microcosm studies to provide additional explanations for observed phenomena and to evaluate ecosystem effects at concentrations which were not tested in the microcosm (e.g., Traas *et al.*, 2004a).

Because they are primarily used for deriving environmentally 'safe' concentrations, predictions of effects on ECO1 endpoints are rarely quantitatively validated using more than one dataset. Qualitative tests have been conducted (Naito *et al.,* 2003). Yet, more than large-scale experiments, ecosystem models are based on a set of ecological and mathematical assumptions. Therefore, validation of predicted population-level effects is a prerequisite for the use of ecosystem models to address the issues discussed here.

Although these models are not often used in the specific context of the relation between single-species toxicity test results and ecosystem effects, they do provide insight in the mechanisms determining this relation. In applications of all of the three models (Hanratty and Liber, 1996; Bartell *et al.*, 1999), the emphasis is put on revealing patterns of indirect effects which result in effects on populations which were unpredictable from single-species toxicity test results alone.

II. 5. Conclusions

The majority of single-species toxicity test results given in the aquatic part of the USEPA database report acute effects of chemicals on the mobility or survival of animals. Effects of chemicals on these endpoints are mostly examined in tests with a duration of 5 days or less. Single-species toxicity test results with plants are underrepresented in this database.

Across experimental ecosystem study reports, the studied ecosystem effects differ. Depending on the endpoint considered we can distinguish: effects on ECO1 endpoints, i.e. on the abundance or biomass of populations and/or trophic level; or effects on ECO2 endpoints, i.e. on aggregate ecosystem measures. Roughly half of the reviewed studies focus on effects on ECO1 endpoints and have a duration of 35 days, or less. Because they are both based on abundance or biomass, single-species toxicity test results and effects on ECO1 endpoints have the same meaning. However, reported comparisons between single-species toxicity test results and ecosystem effects in ecosystem studies are highly case-specific and their collective meaning is unclear. The reason for this is the presence of unwanted sources of variability in the majority of the articles reviewed here: (1) the fact that effects on ECO2 endpoints are related with inherently incomparable single-species toxicity test results; (2) inter-laboratory variability; and (3) the fact that multiple single-species toxicity test results are treated in a variety of ways following the species sensitivity distribution concept.

Only 5 studies did perform single-species toxicity tests together with ecosystem studies in which effects on ECO1 endpoints were measured. As such, these studies eliminate inter-laboratory variability and enhance the comparability between single-species test results and effects on ecosystem endpoints. Results from these 5 studies indicate that for most species, $EC_{x,ECO1}$ is within a factor 2 of $EC_{x,single\text{-}species}$. However, this conclusion partly originates from the focus on effects on invertebrates in ecosystem studies with insecticides. If more combined $EC_{x,single\text{-}species}$ and $EC_{x,ECO1}$ for phytoplankton species would be available, this conclusion could change, depending on the relative sensitivities of the interacting populations.

Although they are perfectly suited to pursue the relationship between single-species toxicity test results and effects on ECO1 endpoints, ecosystem models are rarely used for this purpose. Clearly, more modelling studies are needed to take advantage of this potential. The few modelling studies we found in literature stress the importance of ecological interactions to understand ecosystem effects, especially for populations which are not directly targeted by the toxicant. Although the ecosystem modelling approach requires additional validation, it can aid in

understanding some of the true mechanisms which determine the relationship between single-species toxicity test results and ecosystem effects.

Chapter III
Equations and assumptions of the constructed ecosystem models

III. 1. Introduction

The findings presented in this thesis result from predictions made with different ecosystem models. Although these models differ in that they represent different ecosystems, their scientific basis is identical. The dynamic ecosystem models developed in this dissertation all rely on the same object oriented basis. This object oriented basis consists of a set of objects, where each object describes the growth of a population (e.g., phytoplankton, zooplankton). Growth is described in terms of biomass concentration using differential equations. In these differential equations, terms proceeded by a minus sign represent biomass losses (e.g. respiration) while positive terms represent biomass gains (e.g. food consumption). The number of populations that can be modelled is unlimited and available objects are phytoplankton, macrophytes, zooplankton, planktivorous fish, and piscivorous fish. To construct a model for a given ecosystem, objects corresponding to the populations present in this system are selected from the object oriented basis. By defining feeding interactions between these objects (i.e. ecological interactions), a customized food web is designed (Fig III.1). Additionally, the growth kinetics of these objects can be adjusted through parameter tuning (e.g., slow growing populations vs. fast growing populations). Also an object for the dynamic behaviour of detritus and nutrients is available. Implementation of the object oriented framework was done in the software package WEST (®, MOSTforWATER NV, Kortrijk, Belgium).

In what follows, the equations underlying these objects are listed and discussed. Equations are based on the AQUATOX model (USEPA, 2002). The USEPA-equations, in turn, were taken from other ecosystem models described in earlier publications by a variety of authors. It is not the intention to refer to all these original articles and books. Only when deemed necessary in terms of reproducibility of the results obtained in this thesis, further references are given.

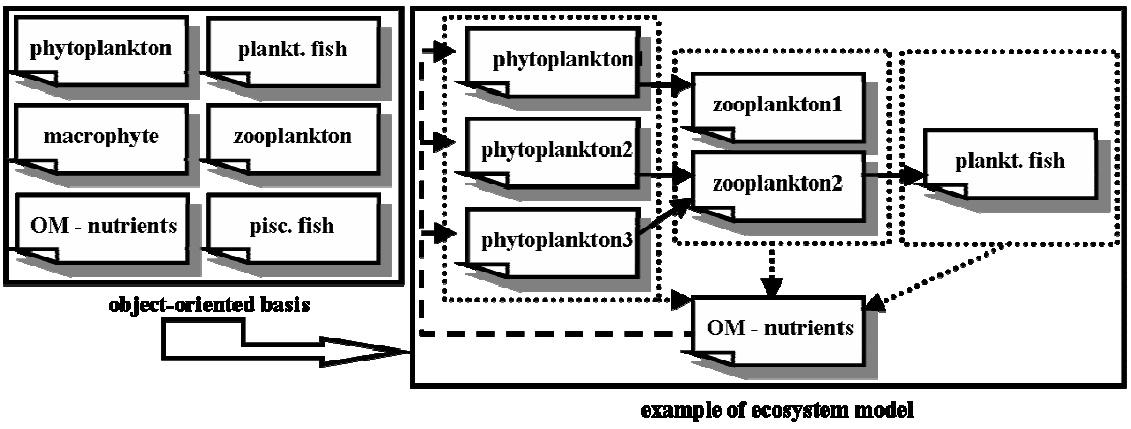

Figure III.1: Illustration of object-oriented based ecosystem modelling. Objects represent populations or nutrient and detritus pools. One object can be used several times and differentiation between two identical objects can be done by parameter tuning (e.g. zooplankton1 and 2). An ecosystem model can be developed by selecting appropriate objects and defining relationships between them (arrows). Bold arrows represent feeding relationships, dashed arrows represent nutrient flows, dotted arrows are detritus flows.

III. 2. Equations

III. 2. 1. Phytoplankton

The main differential equation describing the growth of phytoplankton biomass ($Bio_{phytoplankton}$) is:

$$\frac{dBio_{phytoplankton}}{dt} = photosynthesis - respiration - excretion - mortality - sinking - consumption_{zooplankton}$$

with:

photosynthesis = photosynthesis (mg L^{-1} d^{-1})

respiration = respiratory losses (mg L^{-1} d^{-1})

excretion = excretion (mg L^{-1} d^{-1})

mortality = intrinsic mortality, i.e. not resulting from consumption by zooplankton (mg L^{-1} d^{-1})

sinking = sinking of phytoplankton to the bottom (mg L^{-1} d^{-1})

$consumption_{zooplankton}$ = consumption of phytoplankton by zooplankton (mg L^{-1} d^{-1})

III. 2. 1. 1. Photosynthesis

Photosynthesis is modelled as a maximum rate which is reduced by a limitation factor:

$$photosynthesis = PS_{max} \cdot PS_{limit} \cdot Bio_{phytoplankton}$$

with:

PS_{max} = maximum photosynthesis (d^{-1})

PS_{limit} = limitation factor (-)

$Bio_{phytoplankton}$ = phytoplankton biomass concentration (mg L^{-1})

A value of 1 for PS_{limit} means no limitation, a value of zero means total limitation. When ecosystem models are applied in the context of ecotoxicology, i.e. to study effects of toxicants on ecosystems, the value of PS_{max} depends on a given maximum in control conditions ($PS_{max,0}$) and the toxicant concentration in the ecosystem. Which functions *f* are to be used in such applications, will be discussed in chapter V:

$$PS_{max} = f\left(PS_{max,0},\ toxicant\ concentration\right)$$

Apart from toxicants, also suboptimal light, temperature, and nutrients can limit photosynthesis. These three factors are grouped in an overall limitation factor (PS_{limit}):

$$PS_{limit} = Nutr_{limit} \cdot Temp_{limit} \cdot Light_{limit}$$

$$Light_{limit} = 2.718 \cdot \frac{Photoperiod}{Extinction \cdot Depth} \cdot \left(\exp\left(-\frac{Light}{L_m} \cdot \exp\left(-Extinction \cdot Depth\right) \right) - \exp\left(-\frac{Light}{L_m} \right) \right)$$

$$Light = \frac{Solar}{2}$$

with:

$Nutr_{limit}$ = limitation of photosynthesis due to insufficient nutrient concentrations (-) see III. 2.5.5.4.

$Temp_{limit}$ = limitation due to suboptimal water temperature (-) see III. 2.5.5.1.

$Light_{limit}$ = limitation of photosynthesis due to suboptimal light conditions (-)

Photoperiod = the fraction of day with sunlight (-)

Extinction = extinction of sunlight by organic matter and phytoplankton (m^{-1})

Depth = depth of the reservoir (m)

Light = photosynthetically active fraction of light intensity (cal m^{-2} day^{-1})

L_m = optimal light intensity for phytoplankton photosynthesis (cal m^{-2} day^{-1})

Solar = average daily incident solar radiation (cal m^{-2} day^{-1})

It is assumed that half of the solar radiation is photosynthetically active (Edmondson, 1956). Like in many other ecosystem models (Park, 1974; O'Connor *et al*., 1981; Osidele and Beck, 2004; and others) light limitation is calculated using the Steele equation (Steele, 1966). The light intensity is a driver variable, i.e. it is known a-priori. For the variable 'Extinction', the formula of Chapra (1997) was used. The term for light extinction by phytoplankton and organic matter is based on the product of the concentrations of these variables with their extinction coefficient:

$$Extinction = Extinction_{water} + Extinction_{phytoplankton} + Extinction_{macrophytes} + Extinction_{DOM} + Extinction_{POM}$$

$$Extinction_{phytoplankton} = Ecoeff_{phytoplankton} \cdot Bio_{all\ phytoplankton}$$

$$Extinction_{macrophytes} = Ecoeff_{macrophytes} \cdot Bio_{all\ macrophytes}$$

$$Extinction_{DOM} = Ecoeff_{DOM} \cdot DOM$$

$$Extinction_{POM} = Ecoeff_{POM} \cdot POM$$

$$Bio_{all\ phytoplankton} = \sum_{i=1}^{p} Bio_{phytoplankton_i}$$

$$Bio_{all\ macrophytes} = \sum_{i=1}^{m} Bio_{macrophyte_i}$$

with:

$Extinction_{water}$ = light extinction by water (m^{-1})

$Extinction_{phytoplankton}$ = light extinction by phytoplankton (m^{-1})

$Extinction_{macrophytes}$ = light extinction by macrophytes (m^{-1})

$Extinction_{DOM}$ = light extinction by DOM (m^{-1})

$Extinction_{POM}$ = light extinction by POM (m^{-1})

$Ecoeff_{phytoplankton}$ = extinction coefficient of phytoplankton (m^{-1} mg^{-1} L)

$Ecoeff_{macrophytes}$ = extinction coefficient of macrophytes (m^{-1} mg^{-1} L)

$Ecoeff_{DOM}$ = extinction coefficient of DOM (m^{-1} mg^{-1} L)

$Ecoeff_{POM}$ = extinction coefficient of POM (m^{-1} mg^{-1} L)

DOM = dissolved organic matter (mg L^{-1})

POM = particulate organic matter (mg L^{-1})

$Bio_{all\ phytoplankton}$ = total biomass of all p phytoplankton populations (mg L^{-1})

$Bio_{all\ macrophytes}$ = total biomass of all m macrophyte populations (mg L^{-1})

The calculation of DOM and POM is discussed in the section on nutrient and detritus cycling (III.2.5).

III. 2. 1. 2. Respiration

Respiratory losses consist of an intrinsic respiration rate representing maintenance costs multiplied by an exponential factor for increased respiration because of increased water temperature (Riley, 1963):

$$respiration = Resp0 \cdot \exp(TempResp \cdot Temperature) \cdot Bio_{phytoplankton}$$

with:

Resp0 = intrinsic respiration (d^{-1})

TempResp = exponential coefficient for increased respiration because of increased water temperature ($°C^{-1}$)

Temperature = water temperature (°C)

$Bio_{phytoplankton}$ = phytoplankton biomass concentration (mg L^{-1})

III. 2. 1. 3. Excretion

Excretion, i.e. the release of photosynthate, is totally dependent on photosynthesis and light. If the amount of available light is high ($Light_{limit}$ approaches 1), excretion decreases (e.g., Collins, 1980):

$$excretion = Exc \cdot photosynthesis \cdot \left(1 - Light_{limit}\right)$$

with:

Exc = excretion / photosynthesis ratio (-)

photosynthesis = photosynthesis (mg L^{-1} d^{-1})

$Light_{limit}$ = limitation of photosynthesis due to suboptimal light conditions (-)

III. 2. 1. 4. Mortality

The mortality term does not include consumption by zooplankton. The latter is included in another term which will be discussed further on. It is assumed that non-predatory mortality consists of three parts: (1) intrinsic mortality; (2) increased mortality because of elevated temperature; and (3) increased mortality by suboptimal nutrient and light levels.

$$mortality = (Mort + ExcessT + Stress) \cdot Bio_{phytoplankton}$$

$$ExcessT = \frac{\exp\left(Temperature - T_{max}\right)}{2}$$

$$Stress = 1 - \exp\left(-Emort \cdot \left(1 - Nutr_{limit} \cdot Light_{limit}\right)\right)$$

with:

Mort = intrinsic mortality (d^{-1})

ExcessT = increased mortality because of too high water temperature (d^{-1})

Stress = increased mortality resulting from stress associated with suboptimal nutrients and light levels (d^{-1})

$Bio_{phytoplankton}$ = biomass concentration of considered phytoplankton population (mg L^{-1})

Temperature = water temperature (°C)

T_{max} = maximum water temperature above which photosynthesis is impeded (°C)

Emort = exponential coefficient for stress-related increased mortality (-)

$Nutr_{limit}$ = limitation of photosynthesis due to insufficient nutrient concentrations (-) see III. 2.5.5.4.

$Temp_{limit}$ = limitation due to suboptimal water temperature (-) see III. 2.5.5.1.

III. 2. 1. 5. Sinking

An intrinsic sinking rate is multiplied by a factor reflecting accelerated sinking because of nutrient and light limitation, as observed by Smayda (1974):

$$\text{sinking} = \frac{\text{Sed}}{\text{Depth}} \cdot \text{SedAccel} \cdot \text{Bio}_{\text{phytoplankton}}$$

$$\text{SedAccel} = \exp\left(\text{ESed} \cdot \left(1 - \text{Light}_{\text{limit}} \cdot \text{Nutr}_{\text{limit}} \cdot \text{Temp}_{\text{limit}}\right)\right)$$

with:

Sed = intrinsic sinking velocity (m d^{-1})

Depth = depth of the reservoir (m)

SedAccel = accelerated sedimentation (-)

$Bio_{phytoplankton}$ = phytoplankton biomass concentration (mg L^{-1})

ESed = exponential factor for accelerated sinking (-)

$Light_{limit}$ = limitation of photosynthesis due to suboptimal light conditions (-)

$Nutr_{limit}$ = limitation of photosynthesis due to insufficient nutrient concentrations (-) see III. 2.5.5.4.

$Temp_{limit}$ = limitation due to suboptimal water temperature (-) see III. 2.5.5.1.

III. 2. 1. 6. Consumption

Losses because of zooplankton grazing on phytoplankton (consumption) are discussed in the section on zooplankton (III.2.3).

III. 2. 2. Macrophytes

Growth of macrophyte populations is described using the same equation as for phytoplankton. However, two processes are not incorporated for macrophytes: sinking and consumption by zooplankton. Also, parameter values are different from those of phytoplankton to represent the different growth kinetics of macrophytes. Note that in the AQUATOX model, macrophytes are assumed not to be limited by aqueous nutrient concentrations, as the sediment is thought of as containing nutrients in excess. As much as this may hold in large lakes, experimental ecosystems are often closed and always smaller than real ecosystems. Competition for nutrients between phytoplankton and macrophytes have been reported in such circumstances (e.g. Ozimek *et al.*,

1993). Therefore, photosynthesis of macrophytes also includes the variable 'PS_{limit}' in the presented model.

III. 2. 3. Zooplankton

The main differential equation describing the growth of zooplankton biomass ($Bio_{zooplankton}$) is:

$$\frac{dBio_{zooplankton}}{dt} = consumption - defecation - respiration - excretion - mortality - predation$$

with:

consumption = consumption of resources (mg L^{-1} d^{-1})

defecation = defecation of ingested resources (mg L^{-1} d^{-1})

respiration = respiratory losses (mg L^{-1} d^{-1})

excretion = excretion of dissolved organic matter (mg L^{-1} d^{-1})

mortality = intrinsic mortality (mg L^{-1} d^{-1})

predation = consumption of zooplankton by planktivorous fish (mg L^{-1} d^{-1})

III. 2. 3. 1. Consumption

Zooplankton biomass increases by consumption of two types of food: phytoplankton and particulate organic matter (POM):

$$consumption = consumption_{phytoplankton} + consumption_{POM}$$

$$consumption_{phytoplankton} = \sum_{i=1}^{p} consumption_{phytoplankton_i}$$

with:

$consumption_{phytoplankton}$ = consumption of all p phytoplankton populations (mg L^{-1} d^{-1})

$consumption_{POM}$ = consumption of POM (mg L^{-1} d^{-1})

Similar to the equation for photosynthesis of phytoplankton, consumption by zooplankton is modelled as the product of a maximum consumption rate with limiting factors:

$$consumption_{phytoplankton_i} = C_{max} \cdot SatFeeding_{phytoplankton_i} \cdot Temp_{limit} \cdot Bio_{zooplankton}$$

with:

C_{max} = maximum ingestion rate (d^{-1})

$SatFeeding_{phytoplankton\ i}$ = kinetic factor to express feeding saturation (-)

$Temp_{limit}$ = limitation due to suboptimal water temperature (-) see III. 2.5.5.1.

$Bio_{zooplankton}$ = zooplankton biomass concentration (mg L^{-1})

In the context of ecotoxicology, C_{max} may be a function of a given maximum in control conditions ($C_{max,0}$) and toxicant concentration:

$$C_{max} = f\left(C_{max,0},\ \text{toxicant concentration}\right)$$

The kinetic factor to express feeding saturation is based on the fact that many animals adjust their feeding habits according to the food availability (Park, 1974; Park *et al*., 1980):

$$SatFeeding_{phytoplankton_i} = Pref_{phytoplankton_i} \cdot \frac{Food_{phytoplankton_i}}{\left(\text{Helping variable} + FHalfSat_{phytoplankton_i}\right)}$$

$$Food_{phytoplankton_i} = Bio_{phytoplankton_i} - MinBio_{phytoplankton_i}$$

$$\text{Helping variable} = \sum_{i=1}^{p} Food_{phytoplankton_i} \cdot Pref_{phytoplankton_i} + Pref_{POM} \cdot Food_{POM}$$

$$Food_{POM} = POM - MinPOM$$

with:

$Pref_{phytoplankton\ i}$ = preference of given zooplankton for phytoplankton population i (-)

$Food_{phytoplankton\ i}$ = concentration of phytoplankton population i which is available for consumption by zooplankton (mg L^{-1})

Helping variable = helping variable (mg L^{-1})

$FHalfSat_{phytoplankton\ i}$ = half saturation constant for consumption of phytoplankton population i (mg L^{-1})

$Bio_{phytoplankton\ i}$ = biomass concentration of phytoplankton population i (mg L^{-1})

$MinBio_{phytoplankton\ i}$ = minimum biomass concentration of phytoplankton i to begin feeding (mg L^{-1})

$Pref_{POM}$ = feeding preference of zooplankton for POM (-)

$Food_{POM}$ = fraction of POM which is available for consumption by zooplankton (mg L^{-1})

POM = particulate organic matter concentration (mg L^{-1})

MinPOM = minimum POM concentration to begin feeding (mg L^{-1})

Note that consumption is hampered below a minimum resource concentration, $MinBio_{phytoplankton\ i}$, and / or MinPOM (e.g., Park, 1974). The presence of feeding preference factors in the model allows to choose which resources are preferred by the given zooplankton population. A preference

factor equal to zero for a resource means that the resource is not used by the given zooplankton population.

III. 2. 3. 2. Defecation

The fraction of the consumed resource lost as faeces or discarded during consumption is termed 'defecation'. Because in the current modelling framework zooplankton populations have two possible food resources (phytoplankton and POM), defecation consists of two terms:

$$defecation = defecation_{phytoplankton} + defecation_{POM}$$

$$defecation_{phytoplankton} = \sum_{i=1}^{p} defecation_{phytoplankton_i}$$

$$defecation_{phytoplankton_i} = EgestionCoeff_{phytoplankton_i} \cdot consumption_{phytoplankton_i}$$

$$defecation_{POM} = EgestCoeff_{POM} \cdot consumption_{POM}$$

with:

$defecation_{phytoplankton}$ = defecation of all p phytoplankton populations consumed by the given zooplankton population (mg L^{-1} d^{-1})

$defecation_{POM}$ = defecation of POM by the considered zooplankton population (mg L^{-1} d^{-1})

$EgestionCoeff_{phytoplankton\ i}$ = fraction of consumed phytoplankton population i lost through egestion (-)

$consumption_{phytoplankton\ i}$ = consumption of phytoplankton population i (mg L^{-1} d^{-1})

$EgestCoeff_{POM}$ = fraction of consumed POM lost through egestion (-)

$consumption_{POM}$ = consumption of POM (mg L^{-1} d^{-1})

III. 2. 3. 3. Respiration

Respiration is modelled as consisting of two components:

$$respiration = StandardRespiration + DynamicAction$$

$$StandardRespiration = Resp0 \cdot Temp_{limit} \cdot Bio_{zooplankton}$$

$$DynamicAction = Resp \cdot (consumption - defecation)$$

with:

StandardRespiration = standard respiration, i.e. when the organisms in a population are in a resting state (mg L^{-1} d^{-1})

DynamicAction = the additional cost for the processing of consumed resources (mg L^{-1} d^{-1})

Resp0 = intrinsic respiration (d^{-1})

$Temp_{limit}$ = limitation due to suboptimal water temperature (-) see III. 2.5.5.1.

$Bio_{zooplankton}$ = zooplankton biomass concentration (mg L^{-1})

Resp = fraction of energy lost to dynamic action (-)

consumption = consumption by the considered zooplankton population (mg L^{-1} d^{-1})

defecation = defecation by the considered zooplankton population (mg L^{-1} d^{-1})

III. 2. 3. 4. Excretion

Because excretion of dissolved organic matter occurs concurrently with respiration, a proportional relationship between both processes is used by the model (Scavia and Park, 1976):

$$\text{excretion} = \text{Excr} \cdot \text{respiration}$$

with:

Excr = constant relationship between excretion and respiration (-)

respiration = respiration (mg L^{-1} d^{-1})

III. 2. 3. 5. Mortality

If the water temperature is below a given maximum temperature for the considered zooplankton population, non-predatory mortality is only dependent on an intrinsic mortality rate:

$$\text{mortality} = \text{Mort} \cdot \text{Bio}_{\text{zooplankton}}$$

with:

Mort = intrinsic mortality rate (d^{-1})

$Bio_{zooplankton}$ = zooplankton biomass concentration (mg L^{-1})

If water temperature exceeds this maximum temperature, mortality is reflected by:

$$\text{mortality} = \text{Mort} \cdot \text{Bio}_{\text{zooplankton}} + \frac{\exp(\text{Temperature} - \text{T}_{\text{max}})}{2} \cdot \text{Bio}_{\text{zooplankton}}$$

with:

Mort = intrinsic mortality (d^{-1})

$Bio_{zooplankton}$ = zooplankton biomass concentration (mg L^{-1})

Temperature = water temperature (°C)

T_{max} = maximum temperature which is tolerated by the considered zooplankton population (°C)

When ecosystem models are applied in the context of ecotoxicology, i.e. to study effects of toxicants on ecosystems, the value of Mort depends on a given value at control conditions (i.e. without toxicant added, $Mort_0$) and the toxicant concentration in the ecosystem. Which functions are to be used in such applications, will be discussed in chapter V:

$$Mort = f\ (Mort_0,\ toxicant\ concentration)$$

III. 2. 4. Planktivorous and piscivorous fish

The object for planktivorous fish growth relies on equations which have the same structure as those for zooplankton. The food source for planktivorous fish is zooplankton. The 'consumption' term for planktivorous fish equals the 'predation' term in the equation for zooplankton. The equations for planktivorous fish are thus changed to model this different resource. Also, most parameter values of the planktivorous fish and zooplankton are different and reflect different growth kinetics of both groups. An extensive list of these equations is provided at the end of this chapter. The parameters and variables in the equations are identical to those for zooplankton.

The object for piscivorous fish growth also relies on equations which have the same structure as those for zooplankton. The food source for piscivorous fish is planktivorous fish. Most parameter values are different between piscivorous fish and zooplankton, to model the different growth kinetics of both types of populations. An extensive list of these equations is provided at the end of this chapter. The parameters and variables in the equations should be understood in an analogous way as those for zooplankton and planktivorous fish.

III. 2. 5. Organic matter and nutrient cycling

All the different losses described in the previous equations end up in an organic matter (OM) pool. Three types of organic matter are defined: dissolved, particulate, and settled organic matter: DOM, POM, and SOM. This is a great simplification compared with the remineralisation compartment in

USEPA's AQUATOX model, and hence a great reduction in the number of variables and parameters.

III. 2. 5. 1. Dissolved organic matter (DOM)

Because the excretions of all populations consist of dissolved material, they form the basis of the dissolved organic matter pool:

$$\frac{dDOM}{dt} = excretion_{phytoplankton\ and\ macrophyte} + excretion_{zooplankton\ and\ fish} - decomposition_{DOM}$$

$$excretion_{phytoplankton\ and\ macrophyte} = \sum_{i=1}^{p} excretion_{phytoplankton_i} + \sum_{i=1}^{m} excretion_{macrophytes_i}$$

$$excretion_{zooplankton\ and\ fish} = \sum_{i=1}^{z} excretion_{zooplankton_i} + \sum_{i=1}^{f} excretion_{plank.fish_i} + \sum_{i=1}^{c} excretion_{pisc.fish_i}$$

with:

$excretion_{phytoplankton\ and\ macrophyte}$ = excretion of all p phytoplankton and m macrophyte populations (mg L^{-1} d^{-1})

$excretion_{zooplankton\ and\ fish}$ = excretion of all z zooplankton populations, all f planktivorous fish populations, and all c piscivorous fish populations (mg L^{-1} d^{-1})

$decomposition_{DOM}$ = conversion of DOM to nutrients by micro-organisms (mg L^{-1} d^{-1})

The conversion of DOM to nutrients is calculated without explicitly modelling the associated micro-organisms. Rather, a maximum decay rate is defined, which is corrected for suboptimal temperature, dissolved oxygen, and pH:

$$decomposition_{DOM} = DecayMax_{DOM} \cdot DO_{limit} \cdot Temp_{corr} \cdot pH_{limit} \cdot DOM$$

with:

$DecayMax_{DOM}$ = maximum rate of DOM conversion to nutrients (d^{-1})

DO_{limit} = limitation of DOM conversion to nutrients because of too low dissolved oxygen levels (-) see III. 2.5.5.3.

$Temp_{corr}$ = temperature correction of DOM conversion to nutrients (-) see III. 2.5.5.1.

pH_{limit} = limitation of DOM conversion to nutrients because of suboptimal pH (-) see III. 2.5.5.2.

III. 2. 5. 2. Particulate organic matter (POM)

When an organism dies, its organic matter is not instantly dissolved in the water column. Rather, it stays in suspension, where it is converted to nutrients, sinks to the sediment, or is ingested by zooplankton:

$$\frac{dPOM}{dt} = mortality_{phytoplankton\ and\ macrophyte} + mortality_{zooplankton\ and\ fish} - decomposition_{POM} - sedimentation_{POM} - consumption_{POM}$$

$$consumption_{POM} = \sum_{i=1}^{z} consumption_i$$

$$mortality_{phytoplankton\ and\ macrophyte} = \sum_{i=1}^{p} mortality_{phytoplankton_i} + \sum_{i=1}^{m} mortality_{macrophytes_i}$$

$$mortality_{zooplankton\ and\ fish} = \sum_{i=1}^{z} mortality_{zoplankton_i} + \sum_{i=1}^{f} mortality_{plank.fish_i} + \sum_{i=1}^{c} mortality_{pisc.fish_i}$$

with:

$mortality_{phytoplankton\ and\ macrophyte}$ = mortality of all p phytoplankton and m macrophyte populations (mg L^{-1} d^{-1})

$mortality_{zooplankton\ and\ fish}$ = mortality of all z zooplankton populations, all f planktivorous fish populations, and all p piscivorous fish populations (mg L^{-1} d^{-1})

$decomposition_{POM}$ = decomposition of POM (mg L^{-1} d^{-1})

$sedimentation_{POM}$ = sedimentation of POM (mg L^{-1} d^{-1})

$consumption_{POM}$ = consumption of POM by all z zooplankton populations (mg L^{-1} d^{-1})

The equation for POM-decomposition is completely analogous to that of DOM-decomposition:

$$decomposition_{POM} = DecayMax_{POM} \cdot DO_{limit} \cdot Temp_{corr} \cdot pH_{limit} \cdot POM$$

with:

$DecayMax_{POM}$ = maximum rate of POM conversion to nutrients (d^{-1})

DO_{limit} = limitation of POM conversion to nutrients because of too low dissolved oxygen levels (-) see III. 2.5.5.3.

$Temp_{corr}$ = temperature correction of POM conversion to nutrients (-) see III. 2.5.5.1.

pH_{limit} = limitation of POM conversion to nutrients because of suboptimal pH (-) see III. 2.5.5.2.

The sedimentation rate of POM is calculated as follows:

$$\text{sedimentation}_{POM} = \frac{Sed}{Depth} \cdot POM$$

with:

Sed = sedimentation velocity rate (m d^{-1})

Depth = depth of the reservoir (m)

The fraction of particulate organic matter that settles goes to a pool of settled organic matter (SOM).

III. 2. 5. 3. Settled organic matter (SOM)

Apart form settled particulate organic matter, SOM consists of fecal pellets originating from zooplankton and fish, and settled phytoplankton cells. Because fecal pellets sink rapidly (Smayda, 1971), defecation is directly categorized as SOM, instead of POM:

$$\frac{dSOM}{dt} = \text{defecation} + \text{sedimentation}_{POM} - \text{decomposition}_{SOM} + \text{sinking}_{phytoplankton}$$

with:

defecation = defecation of all zooplankton and fish (mg L^{-1} d^{-1})

$\text{sedimentation}_{POM}$ = sedimentation of particulate organic matter (mg L^{-1} d^{-1})

$\text{decomposition}_{SOM}$ = decomposition of SOM (mg L^{-1} d^{-1})

$\text{sinking}_{phytoplankton}$ = sinking of phytoplankton (mg L^{-1} d^{-1})

Again, the equation for SOM-decomposition is completely analogous to that of DOM and POM-decomposition:

$$\text{decomposition}_{SOM} = \text{DecayMax}_{SOM} \cdot DO_{limit} \cdot \text{Temp}_{corr} \cdot pH_{limit} \cdot SOM$$

with:

DecayMax_{SOM} = maximum rate of SOM conversion to nutrients (d^{-1})

DO_{limit} = limitation of SOM conversion to nutrients because of too low dissolved oxygen levels (-) see III. 2.5.5.3.

Temp_{corr} = temperature correction of SOM conversion to nutrients (-) see III. 2.5.5.1.

pH_{limit} = limitation of SOM conversion to nutrients because of suboptimal pH (-) see III. 2.5.5.2.

III. 2. 5. 4. Nutrients

III. 2. 5. 4. 1. Nitrogen

Nitrogen is modelled as inorganic NH_3-N, or NO_3-N. Nitrogen originating from converted organic matter is in the form of NH_3-N. As respiration occurs, biomass is lost and NH_3-N is excreted directly to the water (Horne and Goldman, 1994). In the model by USEPA (2002), respiration is not included in any detritus pool, thus allowing organic matter to be lost from the system. Since the model in this thesis will be used to make predictions in closed experimental systems, we included the respiration-term in the detritus pool. This also allows to follow a mass balance approach for verification of implementation of the model's equations, as discussed further on. To produce new biomass, phytoplankton and macrophyte populations take up nitrogen:

$$\frac{dNH_3\text{-}N}{dt} = (\text{decomposition}_{DOM} + \text{decomposition}_{POM} + \text{decomposition}_{SOM} + \text{respiration}_{\text{phytoplankton and macrophyte}} + \text{respiration}_{\text{zooplankton and fish}}) \cdot \text{Org2Ammonia} - \text{nitrification} - NH_3\text{-N assimilation}_{\text{phytoplankton and macrophyte}}$$

$$\text{respiration}_{\text{zooplankton and fish}} = \sum_{i=1}^{z} \text{respiration}_{\text{zooplankton}_i} + \sum_{i=1}^{f} \text{respiration}_{\text{plank.fish}_i} + \sum_{i=1}^{c} \text{respiration}_{\text{pisc.fish}_i}$$

$$\text{respiration}_{\text{phytoplankton and macrophyte}} = \sum_{i=1}^{p} \text{respiration}_{\text{phytoplankton}_i} + \sum_{i=1}^{m} \text{respiration}_{\text{macrophytes}_i}$$

with:

$\text{decomposition}_{DOM}$ = decomposition of dissolved organic matter (mg L^{-1} d^{-1})

$\text{decomposition}_{POM}$ = decomposition of particulate organic matter (mg L^{-1} d^{-1})

$\text{decomposition}_{SOM}$ = decomposition of settled organic matter (mg L^{-1} d^{-1})

$\text{respiration}_{\text{phytoplankton and macrophyte}}$ = respiration of the p phytoplankton, and m macrophyte populations (mg L^{-1} d^{-1})

$\text{respiration}_{\text{zooplankton and fish}}$ = respiration of the z zooplankton populations, the f planktivorous fish populations, and c piscivorous fish populations (mg L^{-1} d^{-1})

Org2Ammonia = a default conversion factor between organic matter and NH_3-N (-)

nitrification = conversion of NH_3-N to NO_3-N (mg L^{-1} d^{-1})

NH_3-N $\text{assimilation}_{\text{phytoplankton and macrophyte}}$ = assimilation of NH_3-N by phytoplankton and macrophytes during photosynthesis (mg L^{-1} d^{-1})

Nitrification is calculated by multiplication of a maximum rate with limiting factors. Because nitrification primarily occurs at the sediment-water interface (Effler, 1996), it is also corrected for the area to volume ratio of the reservoir:

$$\text{nitrification} = K_{nitri} \cdot \frac{\text{Area}}{\text{Volume}} \cdot DO_{limit} \cdot Temp_{corr} \cdot pH_{limit} \cdot NH_3\text{-N}$$

K_{nitri} = maximum rate of nitrification (m d^{-1})

Area = surface area of reservoir (m^2)

Volume = volume of water in reservoir (m^3)

DO_{limit} = limitation of nitrification because of too low dissolved oxygen levels (-) see III. 2.5.5.3.

$Temp_{corr}$ = temperature correction of nitrification (-) see III. 2.5.5.1.

pH_{limit} = limitation due to suboptimal pH (-) see III. 2.5.5.2.

NH_3-N = ammonia-nitrogen (mg L^{-1})

Denitrification, i.e. the conversion of nitrate into nitrogen gas (N_2), is modelled in a similar way. Because denitrification occurs at low dissolved oxygen levels, the complement of DO_{limit} is used:

$$\text{denitrification} = K_{denitri} \cdot \frac{\text{Area}}{\text{Volume}} \cdot (1 - DO_{limit}) \cdot Temp_{limit} \cdot pH_{limit} \cdot NO_3\text{-N}$$

with:

$K_{denitri}$ = maximum rate of nitrification (m d^{-1})

Area = surface area of reservoir (m^2)

Volume = volume of water in reservoir (m^3)

DO_{limit} = limitation of denitrification because of too low dissolved oxygen levels (-) see III. 2.5.5.3.

$Temp_{corr}$ = temperature correction of denitrification (-) see III. 2.5.5.1.

pH_{limit} = limitation due to suboptimal pH (-) see III. 2.5.5.2.

NO_3-N = nitrate-nitrogen (mg L^{-1})

As such, the nitrate concentration is dependent on nitrification and denitrification:

$$\frac{dNO_3\text{-N}}{dt} = \text{nitrification} - \text{denitrification}$$

III. 2. 5. 4. 2. Phosphorus

Inorganic phosphorus originates from decomposition of organic matter and from respiration-associated excretion (Horne and Goldman 1994). To produce new biomass, phytoplankton and macrophyte populations take up phosphorus:

$$\frac{dPO_4\text{-P}}{dt} = (\text{decomposition}_{DOM} + \text{decomposition}_{POM} + \text{decomposition}_{SOM} + \text{respiration}_{\text{phytoplankton and macrophyte}} + \text{respiration}_{\text{zooplankton and fish}}) \cdot \text{Org2Phos} - PO_4\text{-P assimilation}_{\text{phytoplankton and macrophyte}}$$

$$PO_4\text{-P assimilation}_{\text{phytoplankton and macrophyte}} = \sum_{i=1}^{p} PO_4\text{-P assimilation}_{\text{phytoplankton}_i} + \sum_{i=1}^{m} PO_4\text{-P assimilation}_{\text{macrophytes}_i}$$

with:

$\text{decomposition}_{DOM}$ = decomposition of dissolved organic matter (mg L^{-1} d^{-1})

$\text{decomposition}_{POM}$ = decomposition of particulate organic matter (mg L^{-1} d^{-1})

$\text{decomposition}_{SOM}$ = decomposition of settled organic matter (mg L^{-1} d^{-1})

$\text{respiration}_{\text{phytoplankton and macrophyte}}$ = respiration of the p phytoplankton, and m macrophytes populations (mg L^{-1} d^{-1})

$\text{respiration}_{\text{zooplankton and fish}}$ = respiration of the z zooplankton populations, the f planktivorous fish populations, and c piscivorous fish populations (mg L^{-1} d^{-1})

Org2Phos = a default conversion factor between organic matter and PO_4-P (-)

PO_4-P $\text{assimilation}_{\text{phytoplankton and macrophyte}}$ = assimilation of PO_4-P by phytoplankton and macrophytes during photosynthesis (mg L^{-1} d^{-1})

III. 2. 5. 4. 3. Mass balance check

In the AQUATOX model, some fluxes of organic matter are lost. An example of this is the loss of respiration terms, as described earlier in this chapter. This has been solved in the presented equations. Because of the closed nature of the modelling framework in this thesis, no nutrients are lost when denitrification is zero. This occurs when there is no dissolved oxygen limitation, i.e. in well-aerated systems where DO_{limit} approaches 1. The ecosystem models developed in this dissertation serve to predict ecological effects of chemicals in such well-aerated experimental

systems. Hence, the conservation of nutrients within the system can be assumed and the law of nutrient-mass conservation can be applied. This allows to construct two testing variables, N_{test} and P_{test}, representing the total concentration of nitrogen and phosphorus in the system, respectively. If the model equations are implemented and solved correctly, these variables should have a constant value throughout the complete simulation period:

$$N_{test} = \left(Bio_{phytoplankton\ and\ macrophyte} + Bio_{zooplankton\ and\ fish} + DOM + POM + SOM\right) \cdot Org2Ammonia + NH_3\text{-}N + NO_3\text{-}N$$

$$P_{test} = \left(Bio_{phytoplankton\ and\ macrophyte} + Bio_{zooplankton\ and\ fish} + DOM + POM + SOM\right) \cdot Org2Phos + PO_4\text{-}P$$

with:

$Bio_{phytoplankton\ and\ macrophyte}$ = biomass concentration of the p phytoplankton populations, and of the m macrophyte populations (mg L^{-1})

$Bio_{zooplankton\ and\ fish}$ = biomass concentration of the z zooplankton populations, the f planktivorous fish populations, and c piscivorous fish populations (mg L^{-1})

DOM = dissolved organic matter (mg L^{-1})

POM = particulate organic matter (mg L^{-1})

SOM = settled organic matter (mg L^{-1})

Org2Ammonia = a default conversion factor between organic matter and NH_3-N (-)

NH_3-N = ammonia nitrogen (mg L^{-1})

NO_3-N = nitrate nitrogen (mg L^{-1})

Org2Phos = a default conversion factor between organic matter and PO_4-P (-)

PO_4-P = phosphate phosphorus (mg L^{-1})

III. 2. 5. 5. Limitation and correction terms

The rates of many different processes are limited by a number of water characteristics, such as water temperature, pH, and dissolved oxygen. Therefore, limitation terms are incorporated. A value of 1 for such a limitation term means that there is no limitation. A value of zero means total limitation. One exception is the temperature correction term used for microbial processes, which can also be >1 to reflect increasing activity.

III. 2. 5. 5. 1. Temperature limitation and correction

Temperature limitation is modelled using a nonlinear adaptive response to temperature changes (Stroganov function, Park, 1974):

$$Temp_{limit} = VT^{XT} \cdot \exp(XT \cdot (1\text{-}VT))$$

with:

VT = the ratio of (1) the difference between the maximum temperature at which a process will occur and the ambient temperature; and (2) the difference between the maximum temperature at which a process will occur and the optimal temperature (-)

XT = an intermediate variable (-)

If the quantity 'VT' < 0, $Temp_{limit}$ is set to zero. If the water temperature is larger than a predefined reference temperature, acclimation is calculated as follows:

$$Acclimation = XM \cdot (1 - \exp(\text{-}KT \cdot |Temperature - T_{ref}|))$$

with

XM = the maximum acclimation (°C)

KT = a coefficient for decreasing acclimation as water temperature approaches T_{ref} (-)

Temperature = water temperature (°C)

T_{ref} = reference temperature, below which there is no acclimation (°C)

If the water temperature is lower than the reference temperature, acclimation is multiplied with (-1). The quantities VT and XT are calculated as follows:

$$VT = \frac{((T_{max} + Acclimation) - Temperature)}{((T_{max} + Acclimation) - (T_{opt} + Acclimation))}$$

$$XT = WT^2 \cdot \frac{\left(1 + \sqrt{1 + 40 / YT}\right)^2}{400}$$

$$WT = \ln(Q10) \cdot (T_{max} - T_{opt})$$

$$YT = \ln(Q10) \cdot (T_{max} - T_{opt} + 2)$$

with:

T_{max} = maximum temperature for given population (°C)

Acclimation = temperature acclimation (°C)

Temperature = water temperature (°C)

T_{opt} = optimum temperature for given population (°C)

Q10 = rate of change per 10°C temperature change (-)

In Fig III.2, $Temp_{limit}$ is calculated with XM = 10, KT = 5, T_{ref} = 8, T_{max} = 35, T_{opt} = 22, and Q10 = 2, and plotted as a function of temperature.

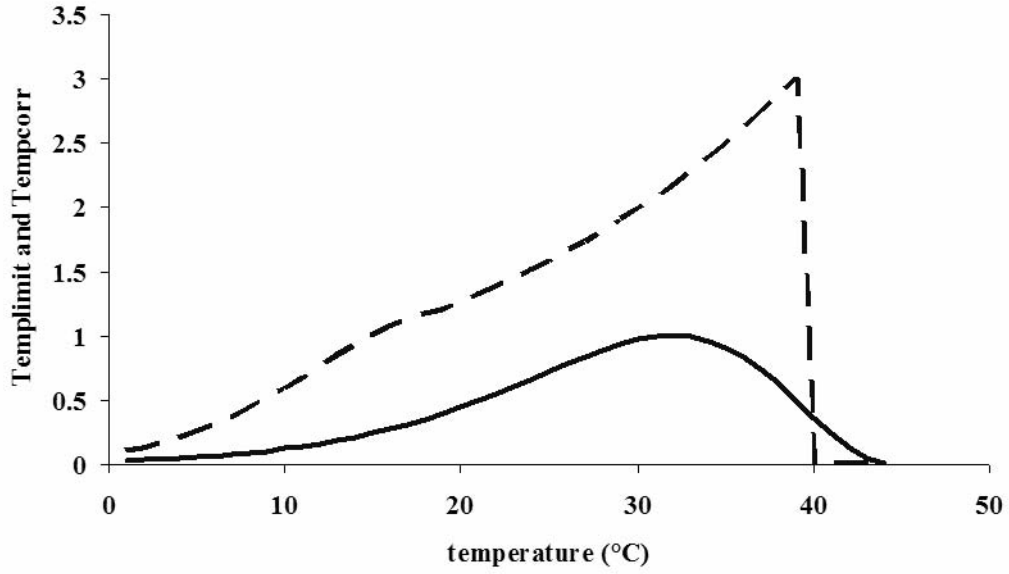

Figure III.2: An example of $Temp_{limit}$ (bold) and $Temp_{corr}$ (dashed) as a function of temperature.

A more straightforward approach is used to calculate the temperature correction for microbial processes (Thomann and Mueller, 1987). If water temperature exceeds a user-defined maximum, temperature limitation is total (i.e. equal to zero). Otherwise, it is calculated as:

$$Temp_{corr} = \theta^{Temperature - T_{obs}}$$

with:

θ = an intermediate variable (-)

Temperature = water temperature (°C)

T_{obs} = the temperature at which the considered process rate was determined (°C)

If the temperature > 19°C, θ is set to 1.047. Otherwise, the following formula is used:

$$\theta = 1.185 - 0.00729 \cdot Temperature$$

An example of $Temp_{corr}$ for microbial processes (with T_{obs} = 14°C) is given in Fig III.2.

III. 2. 5. 5. 2. pH limitation

A pH-correction is applied to a process if pH exceeds a predefined pH-range (Fig III.3). If pH is larger than the upper limit of this range (pH_{max}), pH_{limit} is calculated as follows:

$$pH_{limit} = \exp\left(pH_{max} - pH\right)$$

If pH is lower than the lower limit of the pH-range for the considered process (pH_{min}), the following equation is used:

$$pH_{limit} = \exp\left(pH - pH_{min}\right)$$

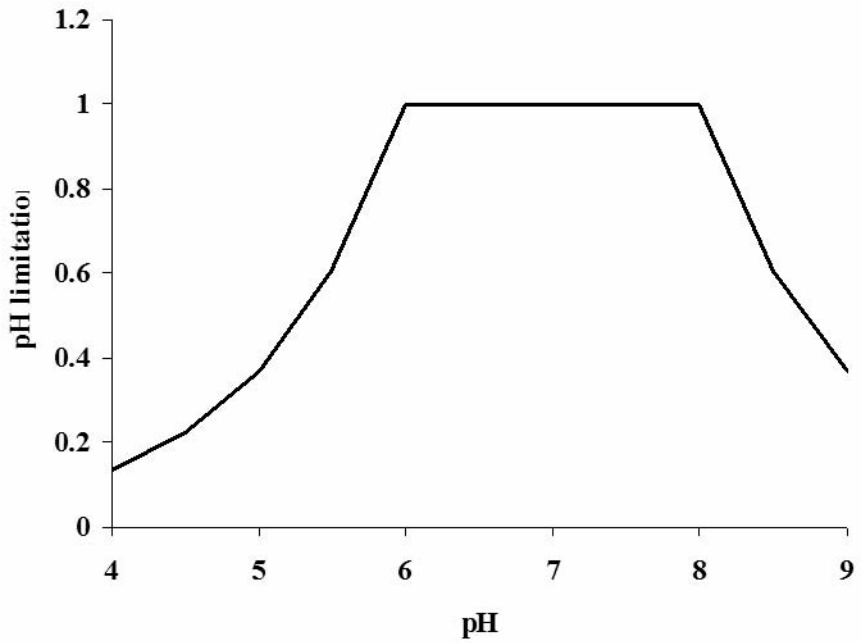

Figure III.3: pH correction as a function of pH, with pH_{min} and pH_{max} equal to 6 and 8, respectively.

III. 2. 5. 5. 3. Dissolved oxygen limitation

Although oxygen dynamics are not implemented in the current modelling framework, oxygen can be treated as a driver variable. This allows to calculate the limitation of certain processes due to too low oxygen levels:

$$DO_{limit} = \frac{Oxygen}{(K_O + Oxygen)}$$

with:

Oxygen = oxygen concentration in the water columns (mg L^{-1})

K_O = Michaelis-Menten constant for oxygen limitation (mg L^{-1})

III. 2. 5. 5. 4. Nutrient limitation

Nutrient limitation ($Nutr_{limit}$) is calculated as the minimum of the limitation factors for nitrogen and phosphorus:

$$Nutr_{limit} = \min (N_{limit}, P_{limit})$$

$$N_{limit} = \frac{N}{(N + K_N)}$$

$$P_{limit} = \frac{P}{(P + K_P)}$$

with:

N_{limit} = limitation of photosynthesis due to insufficient nitrogen concentrations (-)

P_{limit} = limitation of photosynthesis due to insufficient phosphorus concentrations (-)

N = nitrogen concentration (mg N L^{-1})

P = phosphorus concentration (mg P L^{-1})

K_N = Michaelis-Menten constant for nitrogen limitation (mg L^{-1})

K_P = Michaelis-Menten constant for phosphorus limitation (mg L^{-1})

III. 3. Model parameterization

The values assigned to the parameters of the demonstrated ecosystem model differ depending on its application and are listed in the appendix (XI.5 and XI.6).

III. 4. Driver variables

To simulate seasonal fluctuations of light and temperature, photoperiod and water temperature were calculated as a function of Julian date, using the equations proposed by USEPA (2002). The

resulting pattern of those two quantities is given in Fig III.4. Solar irradiance was set at a mean daily value of 200 cal m^{-2} d^{-1}, a typical value for temperate regions (USEPA, 2002).

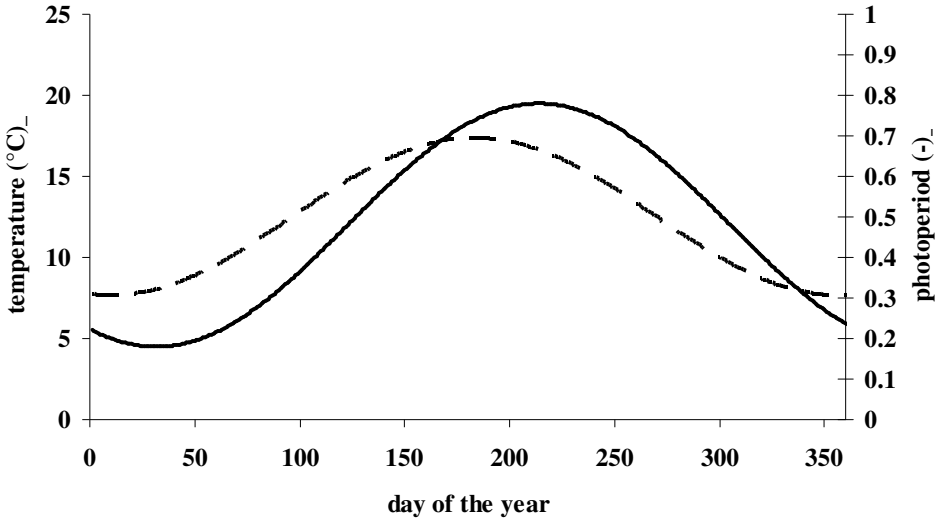

Figure III. 4: Water temperature (bold) and photoperiod (dashed) as a function of day of the year.

III. 5. Sensitivity of predictions for model parameters

To give some insight into the influence that parameters may have on ecosystem model predictions, a sensitivity analysis was performed in WEST (®, MOSTforWATER NV, Kortrijk, Belgium; perturbation factor = 1E-6). The routine followed by WEST is as follows. Biomass dynamics are simulated with a given set of parameters. Next, the value of one parameter is changed and dynamics are simulated again. By comparing the latter dynamics with the former dynamics, the influence of the considered parameter on the biomass of the populations present can be quantified. The extent to which a parameter is changed in the second run is called the perturbation factor. The outcome of such an analysis can be used to check if model equations truly reproduce the mechanisms they represent.

In the ecosystem described in chapter IX, phytoplankton biomass was stimulated most by increasing the parameters PS_{max} and T_{max}, while increasing T_{opt}, Q10, sedimentation parameters (Sed and ESed), and L_m resulted in the most pronounced decrease of phytoplankton biomass. The importance of temperature and light-related parameters reassures that driver variables are indeed causing seasonal changes. Among parameters from other objects, the increase of maximal ingestion rates of zooplankton (C_{max}) proved to cause the highest decrease of phytoplankton. This confirms that zooplankton grazing is an important loss term for phytoplankton.

Zooplankton parameters of which an increase leaded to the highest decrease in zooplankton biomass are $FHalfSat_{phytoplankton\ i}$, $EgestionCoeff_{phytoplankton\ i}$ and Resp. This makes sense, since the two first parameters are directly related with the consumption process, the only process in which biomass can be gained by zooplankton. Indeed, increasing C_{max} also gives rise to an increase in zooplankton biomass. The importance of ecological interactions in the model structure is also reflected by the decrease of zooplankton biomass when sedimentation parameters of phytoplankton are increased. It is sensible that increased sinking of phytoplankton decreases the amount of available food for zooplankton populations which graze on phytoplankton.
In conclusion, the performed sensitivity analysis shows that the main mechanisms seem well-represented by the ecosystem model's equations. However, one should take into account that the performed sensitivity analysis only has a local nature. This means that these results are only valid for the planktonic system from chapter IX and the parameter values used. If the model would be applied in other circumstances, a new sensitivity analysis should be performed.

III. 6. Implementation and use of an ecosystem model

Implementation of the described equations was done in WEST (®, MOSTforWATER NV, Kortrijk). Equations are written in MSL, the programming language of WEST, and grouped per object. Every object represents a population, or the nutrient and detritus pool. Note that the object for the nutrient and detritus pool also calculates the limitation and correction terms. To model an ecosystem, objects are linked. In Fig III.5, the configuration of an ecosystem model consisting of one phytoplankton population, one zooplankton population, and the nutrient and detritus pool is illustrated. The links between these objects are represented by arrows. Note that these links are not biomass transfers. Rather, they pass on information about the values of certain variables from one object to the other. Next to the arrows, the variables whose values are transferred from one object to the other are given. For example, the value of $Bio_{phytoplankton}$ is necessary to calculate the consumption term in the zooplankton object. Therefore, the value of $Bio_{phytoplankton}$ is passed on to the zooplankton object. Once this consumption term is calculated, it is passed on to the phytoplankton object, where it is used as a loss term in the general differential equation of phytoplankton. The values of all other loss terms (of zooplankton and phytoplankton) are passed on to the nutrient and detritus pool. The transfer of the values of $Bio_{zooplankton}$ and $Bio_{phytoplankton}$ to the nutrient and detritus pool is necessary to calculate the extinction of light in the water column, which is used for the derivation of $Light_{limit}$.

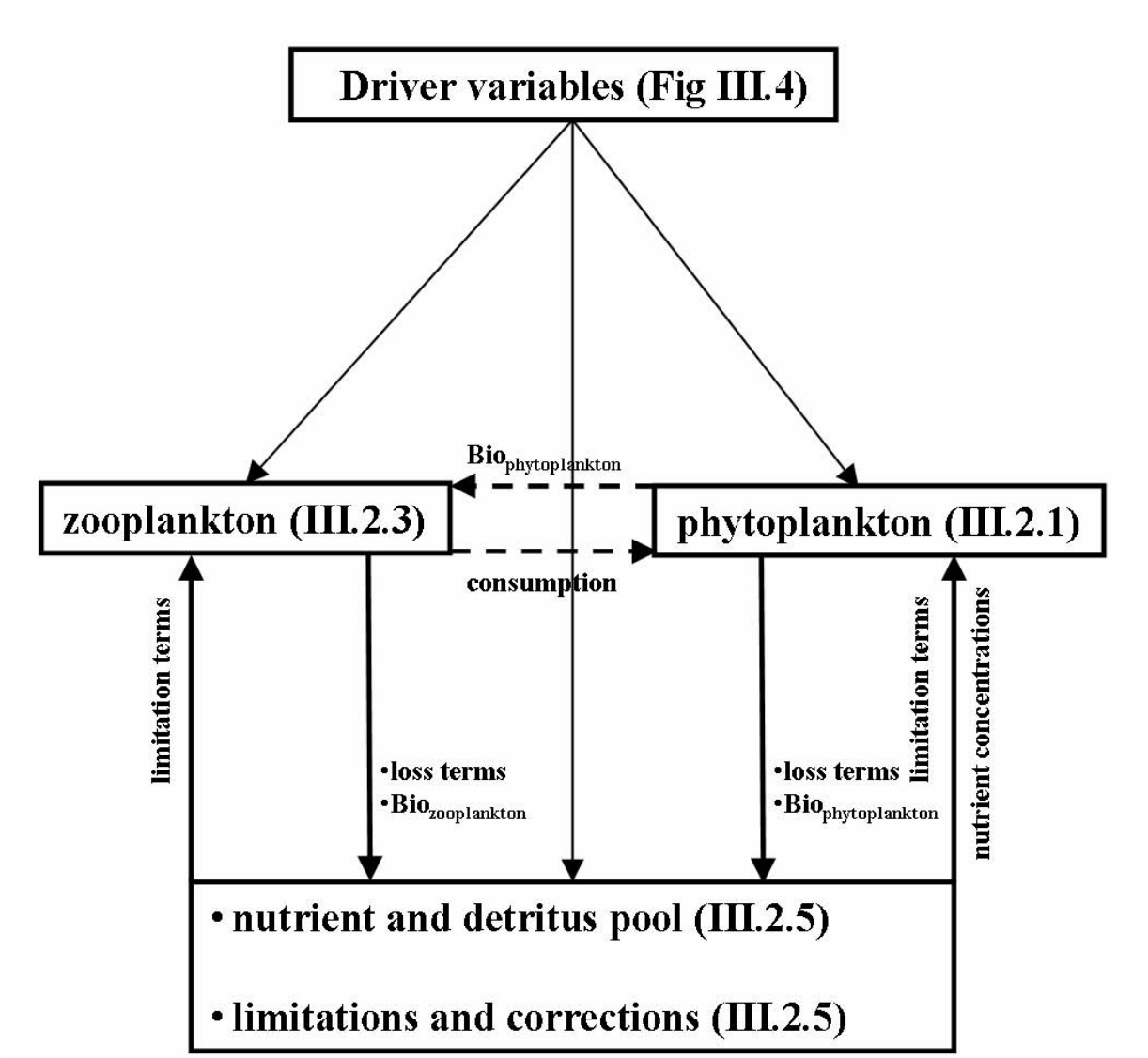

Figure III.5: Configuration of an ecosystem model consisting of one phytoplankton population, one zooplankton population, and the nutrient and detritus pool in WEST(®, MOSTforWATER NV, Kortrijk, Belgium). Driver variables are light , photoperiod and temperature.

In the following chapters, ecosystem models are constructed using the object oriented basis described here. Used parameter values can be found in the appendix of this thesis (XI). In chapters IV to VIII, ecological interactions between populations are set to default interactions (Sommer *et al.*, 1986). Chapter IX involves a practical ecosystem study and ecological interactions are therefore adapted to reflect one particular ecosystem using calibration. In chapter IV, model parameterization and the use of preference factors is illustrated for the case of a simple planktonic ecosystem, consisting of two phytoplankton populations, three zooplankton populations, and one macrophyte.

III. 7. Equations for planktivorous fish

$$\frac{dBio_{plank\,fish}}{dt} = consumption - defecation - respiration - excretion - mortality - predation$$

$$consumption = \sum_{i=1}^{z} consumption_{zooplankton_i}$$

$$consumption_{zooplankton_i} = C_{max} \cdot SatFeeding_{zooplankton_i} \cdot Temp_{limit} \cdot Bio_{plank.fish}$$

$$SatFeeding_{zooplankton_i} = Pref_{zooplankton_i} \cdot \frac{Food_{zooplankton_i}}{(Helping\ variable + FHalfSat_{zooplankton_i})}$$

$$Helping\ variable = \sum_{i=1}^{z} Food_{zooplankton_i} \cdot Pref_{zooplankton_i}$$

$$Food_{zooplankton_i} = Bio_{zooplankton_i} - MinBio_{zooplankton_i}$$

$$defecation = \sum_{i=1}^{z} defecation_{zooplankton_i}$$

$$defecation_{zooplankton_i} = EgestionCoeff_{zooplankton_i} \cdot consumption_{zooplankton_i}$$

$$excretion = Excr \cdot respiration$$

$$respiration = StandardRespiration + DynamicAction$$

$$StandardRespiration = Resp0 \cdot Temp_{limit} \cdot Bio_{plank\ fish}$$

$$DynamicAction = Resp \cdot (consumption - defecation)$$

$$mortality = Mort \cdot Bio_{plank.fish}$$

$$mortality = Mort \cdot Bio_{plank.fish} + \exp\frac{(Temperature - T_{max})}{2} \cdot Bio_{plank.fish}$$

$$Mort = f\ (Mort_0,\ toxicant\ concentration)$$

III. 8. Equations for piscivorous fish

$$\frac{dBio_{pisc.\ fish}}{dt} = consumption - defecation - respiration - excretion - mortality$$

$$consumption = \sum_{i=1}^{f} consumption_{plank.fish_i}$$

$$consumption_{plank\ fish_i} = C_{max} \cdot SatFeeding_{plank\ fish_i} \cdot Temp_{limit} \cdot Bio_{pisc.fish}$$

$$SatFeeding_{plank\ fish_i} = Pref_{plank\ fish_i} \cdot \frac{Food_{plank\ fish_i}}{(Helping\ variable + FHalfSat_{plank\ fish_i})}$$

$$Helping\ variable = \sum_{i=1}^{f} Food_{plank\ fish_i} \cdot Pref_{plank\ fish_i}$$

$$Food_{plank\ fish_i} = Bio_{plank\ fish_i} - MinBio_{plank\ fish_i}$$

$$defecation = \sum_{i=1}^{f} defecation_{plank.fish_i}$$

$$defecation_{plank\ fish_i} = EgestionCoeff_{plank\ fish_i} \cdot consumption_{plank\ fish_i}$$

$$excretion = Excr \cdot respiration$$

$$respiration = StandardRespiration + DynamicAction$$

$$DynamicAction = Resp \cdot (consumption - defecation)$$

$$mortality = Mort \cdot Bio_{pisc.fish}$$

$$mortality = Mort \cdot Bio_{pisc.fish} + \exp\frac{(Temperature - T_{max})}{2} \cdot Bio_{pisc.fish}$$

$$Mort = f\ (Mort_0,\ toxicant\ concentration)$$

Chapter IV

An ecosystem modelling approach for deriving water quality criteria

Chapter IV

An ecosystem modelling approach for deriving water quality criteria

Abstract - Ecological effects of chemicals on ecosystems are the result of direct effects of the chemical, determined in single-species toxicity testing, and indirect effects due to ecological interactions between species. Current experimental methods to account for such interactions are expensive. Hence, mathematical models of ecosystems have been proposed as an alternative. The use of these models often requires extensive calibration, which hampers their use as a general tool in ecological effect assessments. Here we present a novel ecosystem modelling approach which assesses effects of chemicals on ecosystems by integrating single-species toxicity test results and ecological interactions, without the need for calibration on case-specific data. The methodology is validated by comparing predicted ecological effects of copper in a freshwater planktonic ecosystem with an experimental ecosystem data set. The two main effects reflected by this data set (a decrease of cladocerans and an increase of spring phytoplankton) which were unpredictable from single-species toxicity test results alone, were predicted accurately by the developed model. Effects on populations which don't interact directly with other populations, were predicted equally well by single-species toxicity test results as by the ecosystem model. The small amount of required data and the high predictive capacity can make this ecosystem modelling approach an efficient tool in water quality criteria derivation for chemicals.

redrafted from

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IV. 1. Introduction

The development of water quality criteria for chemicals should entail the assessment of potential ecological effects at the ecosystem level. Ecological effects are determined by (1) the direct effects of the chemical on single-species; and (2) ecological interactions between species (e.g., Chapman *et al*., 2003). Relatively straightforward single-species tests are used to determine the former, while experimental ecosystems have been used to account for the latter. However, experimental ecosystems are very demanding in terms of required resources. Therefore, most ecological effect assessments of chemicals have been based exclusively on single-species toxicity test results, i.e. without accounting for ecological interactions between species. In those cases, single-species toxicity test results are extrapolated using statistical models or pragmatic assessment factors to estimate a safe environmental concentration (EU, 2003). Water quality criteria resulting from such effect assessments may be inaccurate (Forbes and Calow, 2002) because of the great importance of ecological interactions in determining ecological effects (Fleeger *et al*., 2003). For this reason, ecosystem models have been proposed to assess ecological effects (e.g., Traas *et al*., 1998). However, these models are mostly calibrated to represent a specific ecosystem (e.g., Bartell *et al*., 1999), hence limiting their applicability in other systems. Moreover, parameters from ecosystem models are difficult to estimate (Loehle, 1997). From a mathematical point of view this is logical, since an ecosystem model consists of coupled equations with numerous feedback processes. One way to resolve this problem of limited parameter identifiability is to change the way in which ecosystem models are calibrated, i.e. by not relying on specific time-series data.

In this chapter, we present a novel ecosystem modelling approach to assess effects of chemicals on ecosystems based on (1) direct effects and (2) ecological interactions. The latter are represented by the ecosystem model equations in which single-species toxicity test results are incorporated to account for the former. The presented approach does not require calibration on specific time-series data. Instead, the model is parameterized using default values to qualitatively describe a number of very generic ecological concepts. This chapter consists of two parts. First, the modelling approach is presented emphasizing underlying concepts and innovations, rather than equations. A detailed overview of model equations is provided in chapter III. Second, predicted ecological effects of copper in a freshwater planktonic ecosystem are compared with observations from a unique experimental ecosystem data set (Schaeffers 2001).

IV. 2. Material and methods

IV. 2. 1. General concept of the ecosystem model

A dynamic ecosystem model was constructed in an object oriented framework. The model consists of a set of objects, where each object describes the growth of a population in terms of its total biomass using differential equations. By connecting different objects and defining the trophic links between them, a customized food web can be designed. The number of populations that can be modelled is unlimited and available objects are: phytoplankton, macrophytes, zooplankton, planktivorous and piscivorous fish. Additionally, the growth kinetics of these objects are differentiated by parameter tuning (slow growing populations vs. fast growing populations). A detailed overview of all model equations can be found in chapter III. A list of parameters, together with the values assigned to them in this chapter can be found in the appendix (XI.6.1).

IV. 2. 2. Population dynamics in the control

First, the model is used to simulate the population dynamics under control conditions, i.e. without toxicant addition. In contrast with other ecosystem modelling approaches (e.g., Traas *et al*., 2004a), no actual time series data is used to calibrate this ecosystem model. Instead, the model is parameterized so that it simulates a realistic succession of seasonal events. A synthesis of realistic planktonic events reported by different researchers working on a plethora of lakes is described in Sommer *et al*. (1986). These events are, (1) bloom of spring phytoplankton, (2) bloom of small zooplankton, resulting in a 'clear water phase', (3) a bloom of summer phytoplankton, followed by (4) a bloom of larger zooplankton, and (5) a small peak of fish, if present. To obtain this series of events, species are lumped into hypothetical populations based on their growth kinetics. The ecological interactions within the ecosystem studied are also defined following Sommer *et al*. (1986): large-bodied zooplankton graze on both spring and summer phytoplankton, while small-bodied zooplankton can only ingest spring phytoplankton. Phytoplankton blooming in spring is dominated by small-celled populations. In summer, large-celled phytoplankton tends to bloom, which can not be ingested by small zooplankton. Summer phytoplankton is often colony-forming or large-celled, which renders them unsuitable for ingestion by small zooplankton. Planktivorous fish are assumed to exclusively feed on large-bodied zooplankton (Sommer *et al.*, 1986). Piscivorous fish feed on planktivorous fish. These interactions are implemented using preference

factors (III.2.3.1) which vary between 0 and 1 and indicate the fraction in the diet consisting of the given food source. Note that although all these populations can be implemented in the model, also simpler systems can be constructed (e.g. without fish). Given these ecological constraints, default parameter values provided by USEPA (2002) are changed within a 10% range until this succession of seasonal events is predicted. Note that this is a qualitative calibration procedure solely relying on the model equations and generic ecological concepts.

IV. 2. 3. Population dynamics in different toxicant treatments

The population dynamics at a given toxicant concentration are predicted by changing growth rate-determining parameters of the populations in the ecosystem model, using logistic concentration-effect functions as toxic effect sub-models. These parameters are the mortality rate (for fish, zooplankton and macrophyte) and the photosynthesis rate (for phytoplankton and macrophytes). Single-species toxicity test results on fish and zooplankton mortality and phytoplankton and macrophyte growth rate are parameters used in these concentration-effect functions:

$$PS_{max} = \frac{PS_{max,0}}{1 + \left(\frac{tox}{EC_{50,PSmax}}\right)^{slope}} \quad \text{(eq IV.1)} \qquad Mort = \frac{1}{time} \cdot \ln\left(1 + \left(\frac{tox}{LC_{50}}\right)^{slope}\right) \quad \text{(eq IV.2)}$$

with:

$PS_{max,0}$ = maximum photosynthetic rate of phytoplankton in control conditions (d^{-1})

PS_{max} = maximum photosynthetic rate of phytoplankton at a toxicant concentration 'tox' (d^{-1})

$EC_{50,PSmax}$ = concentration at which 50% effect on PS_{max} is observed in a single-species toxicity test ($\mu g\ L^{-1}$)

LC_{50} = concentration at which 50% mortality is observed in a single-species toxicity test ($\mu g\ L^{-1}$)

slope = slope of the considered concentration-effect data obtained in a single-species toxicity test (-)

time = duration of the single-species toxicity test in which the LC_{50} was determined (d)

Mort = mortality rate of given zooplankton or fish at a toxicant concentration 'tox' (d^{-1})

If 'Mort' is smaller than the intrinsic mortality rate '$Mort_0$', '$Mort_0$' is used. As such, direct effects of a toxicant, characterized by single-species toxicity test results, are incorporated in the ecosystem model equations. The choice for logistic functions originates from the sigmoid pattern that single-species toxicity test results exhibit for most toxicants (Newman and Unger, 2003). The

use of other types of toxic effect sub-models is discussed in chapter V. Variability on single-species toxicity test results is propagated in the simulation results with a Monte Carlo approach (Cullen and Frey, 1999). The variability reflects possible differences between the 'true' ECx and the value distilled from literature. Hence, this variability would include inter-laboratory differences, measurement errors, interspecies variability, and estimation uncertainty. Latin hypercube sampling was performed with the number of shots determined by the rule of stabilization of variances (Cullen and Frey, 1999; Melching, 1995). As such, population dynamics at a concentration c are simulated n times, with "n" the number of shots.

IV. 2. 4. Modelling ecological effects

Ecological effects are quantified by comparing population dynamics of the exposed system with population dynamics at control. For each population, the average biomass is calculated at the control, as well as at the different toxicant concentrations, and this over the whole simulation period. This allows to calculate relative differences (RDs) of the average biomass of the populations at each toxicant concentration c:

$$RD_{\text{spring phytoplanton}} = \frac{X_{\text{spring phytoplanton,c}} - X_{\text{spring phytoplanton,control}}}{X_{\text{spring phytoplanton,control}}} \qquad \text{(eq IV. 3)}$$

with:

$X_{\text{spring phytoplankton,c}}$ = the average biomass in time of spring phytoplankton at concentration c (mg L^{-1})

$X_{\text{spring phytoplankton,control}}$ = the average biomass in time of spring phytoplankton at control (mg L^{-1}).

RD-equations for other populations are analogous to equation IV. 3.

IV. 2. 5. NOEC calculation

Because 20% is the minimum detectable difference for most population characteristics in the field (Suter II, 1993), RD-values of -0.2 or lower are considered as detectable decreases of biomass. Similarly, RD-values of 0.2 or higher are considered as detectable increases of biomass. Given the variability propagation discussed in the previous paragraph, n RDs are calculated per population and per toxicant concentration. The no observed effect concentration ($NOEC_{\alpha}$) for decrease of a population's biomass is defined as the highest concentration at which less than $100 \bullet (1- \alpha)$ % of the RD-values for this population were $\leq$ -0.2. This percentile is calculated by ranking the n RD-

values using the mean plotting position (Davison and Hinkley, 1997). Similarly, the $NOEC_{\alpha}$ for increase of a population, is defined as the largest concentration at which less than $100 \bullet (1 - \alpha)$ % of the RD-values for this population were ≥ 0.2. The ecosystem-$NOEC_{\alpha}$ is defined as the lowest $NOEC_{\alpha}$ of all populations. In this chapter, a default alpha value of 0.5 was taken. Note that this is equivalent with taking the median of the Monte-Carlo outputs. The effect of the chosen α-value on predictions is examined in chapters V and VI.

IV. 2. 6. Copper effects in aquatic microcosms

The developed methodology was used to predict population-NOECs for copper in a planktonic freshwater ecosystem (Fig VI.1A), for which a unique experimental ecosystem data set is available (Schaeffers, 2001). Indoor aquatic microcosms with a volume of about 1 m³, were permanently exposed to six levels of copper sulphate (5, 10, 20, 40, 80 and 160 µg Cu L^{-1}) while measuring biomass dynamics of various species. The biomass concentration data were lumped into two populations of large zooplankton (cladocerans and copepods), small zooplankton (rotifers), spring phytoplankton, summer phytoplankton, and one macrophyte. Preference factors of copepods for spring phytoplankton and summer phytoplankton are set to 0.5 to model that both food types are equally preferred by copepods (Fig IV.1A). The same holds for cladocerans. In contrast, the preference factors of rotifers for spring phytoplankton and summer phytoplankton are set to 1 and 0, respectively. As such, no summer phytoplankton is consumed by rotifers.

From the data set, RDs and NOECs were calculated using the same methodology as that used for the model predictions. In the remainder of this chapter, these are termed 'experimental' RDs and NOECs, because they are derived from the microcosm experiment.

Values of RD and NOEC for the six populations in the considered ecosystem were predicted with the ecosystem model and compared with the experimental RDs and NOECs. Single-species toxicity test results describing the effects of copper on aquatic biota were collected from literature (Table IV.1). Because of the known influence of water characteristics (e.g., pH, water hardness and dissolved organic carbon, DOC) on copper toxicity (e.g., Erickson *et al.*, 1996; De Schamphelaere and Janssen, 2002), all used single-species toxicity test results were normalized to the water characteristics of the microcosm study as further elaborated in chapter V. Because of the absence of adequate single-species toxicity test results, effects on the macrophyte mortality rate were taken from the calibration study in chapter IX. A slope value for concentration-effect curves of metals was taken from Smit *et al.* (2001). A 10% coefficient of variation on all single-species

toxicity test results were propagated by Monte-Carlo simulation. After 50-80 runs, variances on the output stabilized.

Table IV.1: Collected single-species toxicity test results after normalization to the water characteristics of the microcosm study. In the case of EC_{50}'s and LC_{50}'s, numbers represent the means of the normal distributions expressed as µg L^{-1}, characterizing their variability. Numbers between brackets represent the corresponding standard deviation, representing variability between BLM-predictions of the considered toxicity datum. Variability of Sm values was characterized by uniform distributions, the characteristics of which can be found in Smit *et al.* (2001). Test duration represents the reported duration of the acute mortality experiments. References of remaining toxicity data and of used models for normalization can be found in chapter V.
***In absence of experimental data, effects on macrophyte mortality were taken from another study examining copper effects on the same macrophyte (chapter IX), i.e. using an $EC_{50,mortality}$ and $EC_{10,mortality}$ of 105(12) and 58(4.2), respectively (standard deviations between brackets).**

	parameters of sub-models				
model population	**log(EC_{50}, photosynthesis) (µg L^{-1})**	**log(LC_{50}) (µg L^{-1})**	**log(EC_{50}, grazing rate) (µg L^{-1})**	**Sm (-)**	**acute test duration (days)**
$phytoplankton_{spring}$	1.76 (0.20)	-	-	1	-
$phytoplankton_{summer}$	1.76 (0.20)	-	-	1	-
macrophyte	1.76 (0.20)	*	-	1	*
rotifers	-	2.08 (0.30)	2.16 (0.30)	0.75 - 1.2	1
copepods	-	3.51 (0.30)	2.79 (0.30)	0.75 - 1.2	2
cladocerans	-	2.20 (0.30)	1.98 (0.30)	0.75 - 1.2	1

IV. 3. Results and discussion

IV. 3. 1. RD-predictions

In general, experimental and predicted RD-values are in fair agreement (Fig IV.1B-F). The drastic biomass decrease of cladocerans and $phytoplankton_{summer}$ at copper concentrations > 20 µg L^{-1} is accurately predicted by the model. To illustrate the necessity of including ecological interactions to predict ecological effects in this system, the direct effect of copper, as predicted by single-species toxicity test results alone, is also plotted (Fig IV.1B-F: triangular symbols). Clearly, at 40 and 80 µg L^{-1}, direct effects alone cannot explain the experimentally observed biomass decrease of cladocerans and $phytoplankton_{summer}$.

The predicted increase of $phytoplankton_{spring}$ biomass at copper concentrations of 40 and 80 µg L^{-1} is confirmed by the microcosm data, although the experimentally observed increase (up to 1000%) is much higher than the predicted increase (100 to 200%). Nevertheless, both predicted and experimentally observed increases are > 20% and as such indicate an observable effect. The direct effect alone erroneously indicates a decrease of $phytoplankton_{spring}$ biomass. Apparently, the

ecological interactions within this system result in an ecological effect which is opposite to the direct effect: the reduction of cladoceran biomass lowers the grazing pressure on phytoplankton in general, thus benefits the $phytoplankton_{spring}$. The same mechanism has also been observed by other authors in experimental ecosystems exposed to metal mixtures (Jak *et al*., 1996) and pesticides (Hanazato, 2001). The reason why $phytoplankton_{summer}$ does not benefit from this reduced grazing pressure, while $phytoplankton_{spring}$ does, may result from the competitive advantage for nutrients of the latter at elevated nutrient levels (Sommer *et al*., 1986). Indeed, the loss of (living) biomass resulting from exposure to copper, increases (dead) organic matter and nutrient concentrations.

At concentrations $\leq$ 10 µg L^{-1}, RDs of copepods are predicted correctly. At 40 µg L^{-1}, the ecosystem model predicts a large increase of copepods, while observations only indicate a small increase for this population at that concentration. Comparison of these ecosystem model predictions with the direct effects as predicted from single-species toxicity test results alone, indicates that the inclusion of ecological interactions did not improve the effect assessment for copepods. Yet, this inclusion did not impede the correct prediction of a biomass decrease at 160 µg L^{-1}.

For the macrophyte biomass, the decrease of biomass is slightly overestimated by the ecosystem model, especially at concentrations $\leq$ 80 µg L^{-1}. Direct effects did not differ too much from these ecosystem model predictions, indicating that ecological interactions had a limited influence on ecological effects on this population. This is logical since the macrophyte does not contribute to the feeding relationships within the food web (Fig IV.1A). The only ecological interaction in which the macrophyte takes part is the competition for nutrients with phytoplankton.

Predictions of rotifer RDs were different from experimental RDs (results not shown). This poor prediction performance can be explained by the very low rotifer densities (< 0.5 µg L^{-1}) in the microcosm experiment (Schaeffers 2001). Loss of a single organism thus has a serious impact on RD-values. It is therefore questionable whether the RD-values for rotifers, as derived from the microcosm data, give a reliable reflection of copper effects on this population. For the same reason, Schaeffers (2001) was not able either to calculate a reliable $NOEC_{rotifers}$. Hence, rotifer data and predictions were omitted for NOEC determination.

IV. 3. 2. NOEC predictions

Because predicted RDs were found to be fairly accurate for most populations, NOEC predictions showed good correspondence with experimental NOECs too. However, the low experimental RD

values at 5 and 10 µg L^{-1} result in an experimental $NOEC_{summer\ phytoplankton}$ < 5 µg L^{-1}. Given that at 20 µg L^{-1} biomass of that population returns to control levels, such a low NOEC can be questioned. The model suggests a $NOEC_{summer\ phytoplankton}$ of 20 µg L^{-1}.

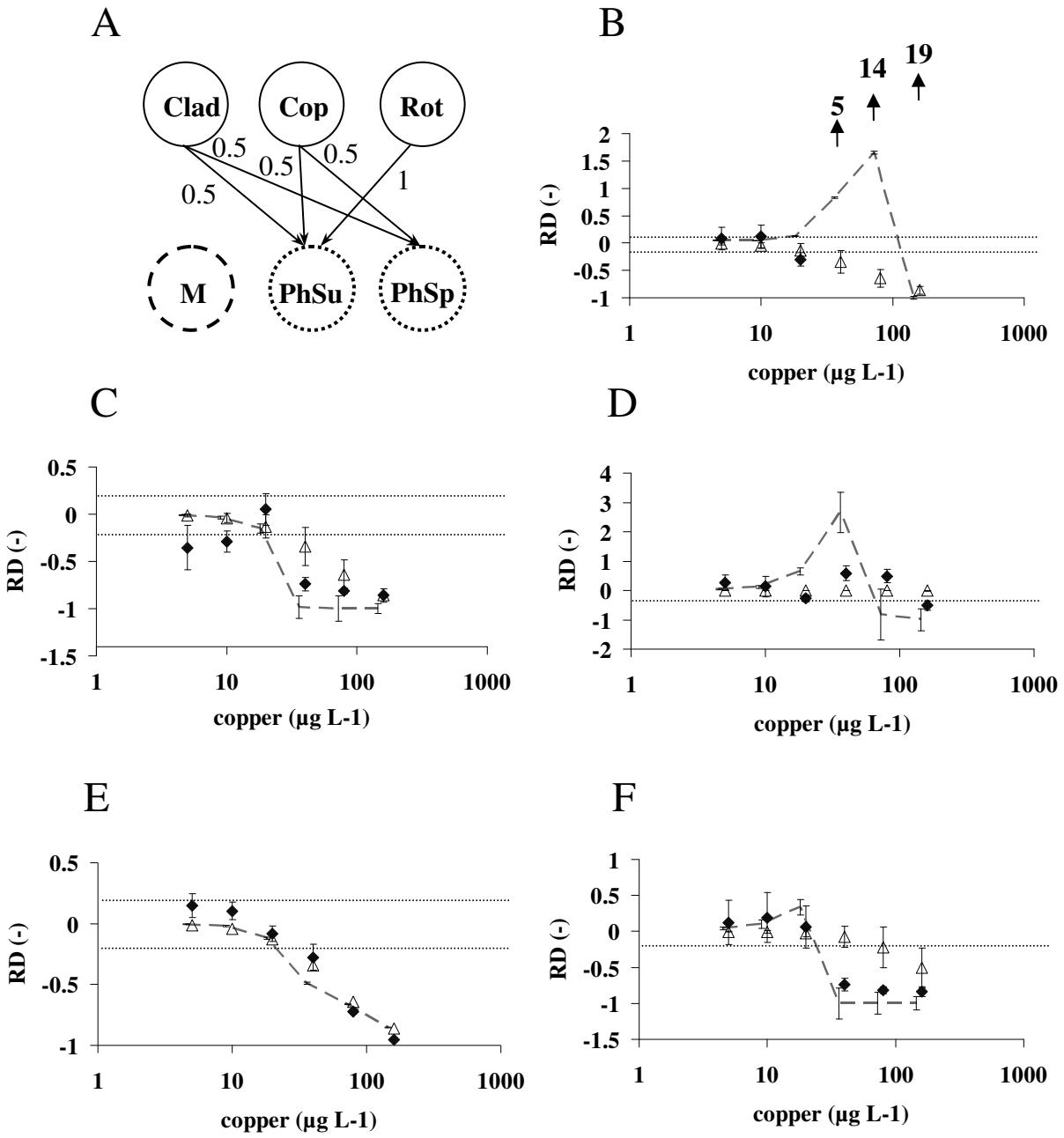

Figure IV.1: A: Food web of the considered ecosystem in which only direct interactions are presented, i.e. grazing; 'Rot' stands for small zooplankton, rotifers. Numbers represent values of preference factors. 1B-F: Biomass changes, relative to control (RD), as a function of copper concentration for the populations in the ecosystem: spring phytoplankton, PhSp (B); summer phytoplankton, PhSu (C); large zooplankton1, copepods, Cop (D); the macrophyte, M (E); large zooplankton2, cladocerans, Clad (F). RD values experimentally derived from the microcosm experiment and associated standard errors are indicated by ♦ and error bars, respectively. Dashed lines give predicted values. Dotted lines indicate RD = -0.2 and/or +0.2. The arrows in (B) point to experimental RD values that are larger than 2. Direct effects, as given by single-species toxicity test results are indicated by Δ.

In this work, population-NOECs were derived from the experimental microcosm data - termed 'experimental NOECs' - to allow comparison with predicted population-NOECs. Comparison of these experimental NOECs with those derived by Schaeffers (2001) shows a good agreement. Apparently, treating the microcosm data in two completely different ways results in the same NOEC, with the exception of the $NOEC_{macrophyte\ decrease}$. The fact that Schaeffers (2001) only used the macrophyte biomass concentration measured on the last day of the experiment for NOEC calculation, may have influenced the result for this population. The biomass of all the other populations was measured throughout the complete period of the experiment and subsequently used for NOEC calculation.

Generic ecological interactions were used to predict NOECs and RDs of the different populations. This was done to increase its robustness and applicability in other ecosystems for which less information is available (as in chapter VI). However, if the ecosystem model should be applied for a case-specific ecological effect assessment, ecological interactions should be more tailored to the specific ecosystem. An example of such a case-specific ecological effect assessment is the practical ecosystem study in chapter IX.

IV. 4. Conclusions

In this chapter, we developed a novel approach to predict ecological effects of chemicals in aquatic ecosystems. The approach is based on the ecosystem model presented in chapter III, generic ecological concepts, and single-species toxicity test results. As such, it can perform predictions, without the need for experimental ecosystem data. Ecosystem model predictions of ecological effects of copper in a freshwater ecosystem were remarkably accurate. For most populations, predictions of the difference of the average biomasses at different toxicant concentrations, relative to the control biomass (RD) were accurate, or at least indicated the same trend as the experimental microcosm data. The few inaccurate RD-predictions did not affect the accuracy of most population-NOEC predictions. These predictions were significantly better than predictions based on single-species toxicity test results alone. This again confirms the importance of accounting for ecological interactions when conducting ecological effect assessments.

It is concluded that single-species toxicity test results and very generic ecological concepts are sufficient to accurately predict ecological effects of copper in the system studied. Because of the ubiquity of single-species toxicity test results it is suggested that the approach presented here may contribute to an improved procedure to derive water quality criteria.

Chapter V

Comparison of different toxic effect sub-models in ecosystem modelling used for ecological effect assessments and water quality standard setting.

Chapter V

Comparison of different toxic effect sub-models in ecosystem modelling used for ecological effect assessments and water quality standard setting.

Abstract - Ecosystem models, combining a food web model with a toxic effect sub-model, have been proposed to incorporate ecological interactions in ecological effect assessments. Toxic effect sub-models in different studies tend to differ in (1) the used single species toxicity data, (2) the effects they consider, (3) the concentration-effect function used. In this chapter, we constructed four ecosystem models, each with a different toxic effect sub-model, and tested their capacity to predict biomass changes, and No Observed Effect Concentrations (NOECs) established in an experimental microcosm. For most populations, these predictions depended heavily on the type of ecosystem model. The ecosystem model with a toxic effect sub-model incorporating mortality effects using a logistic concentration-effect function made accurate predictions for most populations. Additional incorporation of sub-lethal effects did not result in better predictions. Ecosystem models using linear concentration-effect functions predict biomass decreases at concentrations which are 4 times lower than the observed NOECs.

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V. 1. Introduction

One of the major goals of ecotoxicology is the assessment of the effects of a chemical substance on the structure and function of ecosystems. Most of these assessments rely on the extrapolation of single-species effect data to higher level effects. These extrapolation methods are, however, based on largely unproven hypotheses (Versteeg *et al.,* 1999; Forbes and Calow, 2002). One of the most salient assumptions is that the sensitivity of a community can be represented by a set of independent species sensitivities obtained in single-species toxicity tests (Wagner and Løkke, 1991). This approach ignores ecological relationships between populations in communities (e.g., Sommer *et al.*, 1986; Preston and Snell 2001; Arhonditsis *et al.*, 2004). In experimental ecosystems and enclosures, toxic effects at the population- and community-level were found to be determined by (1) the inherent sensitivity of the species present, possibly altered by physical or chemical water characteristics; and (2) the ecological relationships between the species (Chapman *et al.,* 2003; Fleeger *et al.,* 2003). Hence, knowledge about these ecological interactions should be incorporated in ecological effect assessments in order to more accurately estimate ecosystem effects of chemicals.

It is well known that large scale experimental studies, i.e. mesocosm and field enclosure studies, are capable of accounting for such ecological relationships in effect assessments (Joern and Hoagland, 1996; Clements and Kiffney, 1994; Drenner *et al.*, 1993; Hoagland *et al.*, 1993). For instance, Shaw and Kennedy (1996) advocated their use as a higher tier of ecological effect assessment in the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA, US). However, given that this type of studies is very resource-demanding, they cannot be used as a routine practice in lower tiers. Especially in view of REACH (Registration, Evaluation and Authorisation of Chemicals; http://ecb.jrc.it/REACH/), EU-legislation aimed at assessing the risks of approximately 30,000 substances to human health and the environment, there is a clear need for alternative, less resource-demanding methodologies to extrapolate single-species effect data to ecosystem level-responses.

An obvious solution is the construction of ecosystem models. These models consist of (1) a food web structure to account for ecological interactions; (2) a structure for organic matter and nutrient cycling; and (3) toxic effect sub-models to account for toxicant effects. Although the food web structure of most existing ecosystem models is relatively similar (e.g. Bartell *et al*., 1999; Traas *et al*. 2004a), the design of their toxic effect sub-models exhibit more variation. In general, the latter can be grouped into two types: toxicokinetic and external concentration-effect functions. Toxicokinetic sub-models predict toxicity based on accumulated toxicant concentrations, which

are estimated with kinetic uptake and elimination parameters (Bartell *et al.*, 1988; Traas *et al.*, 1996 and 2004b). Although this type of sub-models appears to be more realistic, their application in effect assessments may increase uncertainty instead of reducing it, as the parameter values used in these sub-models tend to be rather uncertain (Hendriks, 1995a, b; Sijm and van der Linde, 1995). Other sub-models use external concentration-effect functions, established in single-species toxicity tests using organisms which are taxonomically and ecologically representative for the considered model populations. These external concentration-effect functions are used to define the change of (selected) growth rate-determining parameters of the populations at different exposure concentrations. The magnitude of these changes depends on the effect concentration (EC_x) of the considered population. The most frequently-used type of single-species effect data for zooplankton and vertebrates is the lethal concentration for x percent of the tested organisms (LC_x).

Which growth rate-determining parameters are a function of the toxicant concentration depends on the chosen approach. Traas *et al.* (1996) choose to solely change the mortality rates of the considered populations, while others choose to make all growth rate-determining parameters dependent on the toxicant concentration. The latter approach is termed as the "general stress syndrome (GSS)" and assumes that each physiological process is equally impacted by the toxicant (e.g., O'Neill *et al.*, 1982; Bartell *et al.*, 1988; DeAngelis *et al.*, 1989; Bartell *et al.*, 1992; Hanratty and Liber, 1996; Bartell *et al.*, 1999). As a result, all growth-rate determining parameters are a function of toxicant concentration. Typically used single-species toxicity data are LC_xs. As such, only mortality effects are truly incorporated in the toxic effect sub-model. A toxic effect sub-model which explicitly accounts for both lethal and sublethal effects on zooplankton and vertebrates was used by Traas *et al.* (2004a). These authors make the mortality rates of zooplankton a function of their LC_{50} or immobility-EC_{50}s, and make ingestion rates a function of immobility-EC_{50}s. Thereby, the assumption is made that increased immobility implies a decreased ingestion rate.

A further differentiation between the various toxic effect sub-models can be based on the type of concentration-effect function used. Reported functions include (1) probit (Bartell *et al.*, 1999); (2) linear (Hanratty and Liber, 1996; Naito *et al.*, 2003); or (3) logistic functions (Traas *et al.,* 2004a). Since for most toxicants, lab-derived concentration-effect data exhibit a sigmoidal pattern (Newman and Unger, 2003), a linear function does not represent the actual concentration-effect data. Naito *et al.* (2003) argued that linear concentration-effect functions tend to overestimate single-species effects resulting in over-predictions of ecosystem effects. However, this argument may not hold, because populations within an ecosystem may not respond proportionally to

increasing toxicant concentrations (Landis, 2002). Moreover, since the shape of a concentration-effect function may be indicative of the mode of action of a toxicant (van Wijk and Kraaij, 1994), the use of a linear function may also prohibit a correct estimation of population and ecosystem effects.

Until now, no studies have examined the importance of the above discussed options when using ecosystem models in ecological effect assessments and water quality standard setting. In this chapter, we constructed four ecosystem models which have identical food web structures, but different toxic effect sub-models. Their toxic effect sub-models differ in the type of effect considered and in the type of concentration-effect function they use. The potential use for ecological effects assessment of each of these four ecosystem models was evaluated. To this end, the accuracy in predicting population-level no observed effect concentrations (population-NOECs) of the four ecosystem models were tested through a comparison with population-NOECs experimentally derived in a previously conducted microcosm experiment with copper (Schaeffers, 2001). Subsequently, ecosystem-NOECs were derived using the four ecosystem models and these values were compared to the ecosystem-NOEC experimentally observed in the microcosm experiment. Because the process of water quality criteria setting seeks to determine the maximum chemical concentration which is not likely to result in adverse effects at the ecosystem level, the use of NOECs in the present study was deemed appropriate. As such, the four ecosystem models were evaluated for their potential use in water quality standard setting and ecological effect assessments.

V. 2. Material and methods

V. 2. 1. Description of the studied microcosm

All data used were obtained in a community level toxicity study with copper in aquatic oligotrophic microcosms (for details, see chapter IV or Schaeffers, 2001).

V. 2. 2. Ecosystem model

A dynamic ecosystem model was constructed in an object oriented framework. The model consists of a set of objects, and each object describes the growth of a model population in terms of its total biomass using differential equations. The differential equations on which these objects are based are described in detail in chapter III. The planktonic system used in the present study was composed of two phytoplankton objects (phytoplankton blooming in spring, and phytoplankton blooming in summer), one macrophyte object and three zooplankton objects (rotifers,

cladocerans, copepods). Fish were not present in the experimental system and were thus not included in the constructed models. The differentiation of phytoplankton based on their growth kinetics and the definition of their trophic links within the ecosystem model is supported by Sommer *et al.* (1986), as explained in chapter IV. The resulting customized food web was used for all four ecosystem models evaluated in this study (Fig IV.1A).

V. 2. 3. Toxic effect sub-models: type of effects included and type of function used

In the four ecosystem models, the toxic effect sub-models include toxicant effects on maximal photosynthesis rate of phytoplankton and macrophytes, and mortality effects on macrophytes. In the LOGC and LINC ecosystem models, mortality and sublethal toxicant effects are included for zooplankton. In the other two ecosystem models, LOG and LIN, only mortality effects are included for zooplankton. As sublethal effect criterion for zooplankton, the toxicant-induced effect on grazing rate is included as it is known to be affected by copper (e.g., Ferrando and Andreu, 1993). Next to the type of toxicant effect, also the type of function used to represent these effects, varies between the four ecosystem models. While LIN and LINC use linear concentration-effect functions, LOG and LOGC rely on logistic concentration-effect functions. The incorporation of test duration and coupling with the growth equations of the ecosystem model allow for simulation of time-varying exposure scenarios (Ashauer *et al.*, 2006). Characteristics of the toxic effect sub-models of the four ecosystem models are summarized in Table V.1.

V. 2. 4. Parameters of the toxic effect sub-models

Data on the effects of copper on aquatic biota were collected from literature (Table V.2). Because of the known influence of water characteristics (e.g., pH, water hardness and dissolved organic carbon) on copper toxicity (e.g., Erickson *et al*., 1996; De Schamphelaere and Janssen, 2002; De Schampelaere *et al.*, 2002), all toxicity data were normalized to the water characteristics of the microcosm study. LC_{50}s for cladocerans and rotifers were taken from Ferrando and Andreu (1993) and LC_{50}s for copepods were taken from Heijerick *et al.* (2001). These LC_{50}s were normalized to the water characteristics of the microcosm study using the acute Biotic Ligand Model (BLM) proposed by De Schampelaere *et al.* (2002). Normalization of ingestion rate -EC_{50}s for cladocerans and rotifers (Ferrando and Andreu, 1993) was done using the chronic BLM proposed by De Schampelaere and Janssen (2004), and De Schampelaere *et al.* (2006). EC_{50}s for copepod ingestion rate were estimated by applying the relation between acute and chronic toxicity data established by Brix *et al.* (2001) to the acute copper-LC_{50}s retrieved for copepods (Heijerick

et al., 2001). These ingestion rate - EC_{50}s were subsequently normalized to the water characteristics of the microcosm study using a chronic BLM (De Schampelaere and Janssen, 2004). The EC_{50}s for effects on photosynthesis rates of the phytoplankton and the macrophyte were calculated as the mean of three growth - EC_{50}s, predicted by three algal bioavailability models (De Schamphelaere *et al.*, 2003; 2006). In the absence of experimental data, effects on macrophyte mortality rate were taken from a calibration study examining copper effects on the same macrophyte (chapter IX). A slope value for concentration-effect curves of metals was taken from Smit *et al.* (2001) and assumed to be representative of the slope of concentration-effect functions for both mortality and sublethal effects. An overview of the used bioavailability-normalized toxicity data is presented in Table V.2.

Table V.1: Equations used in the toxic effect sub-models of the four ecosystem models, LIN, LINC, LOG, and LOGC, with parameter names as in appendix XI.4 and in abbreviation list. In the case of LOG and LOGC, $Mort_0$ is used when Mort < $Mort_0$.

Model	Photosynthesis effect	Mortality effect	Ingestion effect
LIN LINC	$PS_{max} = PS_{max,0} \cdot \left(1 - \frac{tox}{2 \cdot EC_{50,PSmax}}\right)$	$Mort = Mort_0 + \frac{\frac{\ln(2)}{time} - Mort_0}{LC_{50}} \cdot tox$	- $C_{max} = C_{max,0} \cdot \left(1 - \frac{tox}{2 \cdot EC_{50,Cmax}}\right)$
LOG LOGC	$PS_{max} = \frac{PS_{max,0}}{1 + \left(\frac{tox}{EC_{50,PSmax}}\right)^{slope}}$	$Mort = \frac{1}{time} \cdot \ln\left(1 + \left(\frac{tox}{LC_{50}}\right)^{slope}\right)$	- $C_{max} = \frac{C_{max,0}}{1 + \left(\frac{tox}{EC_{50,Cmax}}\right)^{slope}}$

Table V.2: Collected single-species toxicity test results after normalization to the water characteristics of the microcosm study. In the case of logEC_{50}'s and logLC_{50}'s, numbers represent the means of the normal distributions expressed as µg L^{-1}, characterizing their variability. Numbers between brackets represent the corresponding standard deviation, representing variability between BLM-predictions of the considered toxicity datum. Variability of Sm values was characterized by uniform distributions, the characteristics of which can be found in Smit *et al.* (2001). Test duration represents the reported duration of the acute mortality experiments. References of the remaining toxicity data and of used models for normalization can be found in the text. *In absence of experimental data, effects on macrophyte mortality were taken from another study examining copper effects on the same macrophyte (chapter IX), i.e. using an $EC_{50,mortality}$, $EC_{10,mortality}$ of 105(12), 58(4.2), respectively (standard deviations between brackets).

model population	log(EC_{50}, photosynthesis) (µg L^{-1})	log(LC_{50}) (µg L^{-1})	log(EC_{50}, grazing rate) (µg L^{-1})	Sm (-)	acute test duration (days)
$phytoplankton_{spring}$	1.76 (0.20)	-	-	1	-
$phytoplankton_{summer}$	1.76 (0.20)	-	-	1	-
macrophyte	1.76 (0.20)	*	-	1	*
rotifers	-	2.08 (0.30)	2.16 (0.30)	0.75 - 1.2	1
copepods	-	3.51 (0.30)	2.79 (0.30)	0.75 - 1.2	2
cladocerans	-	2.20 (0.30)	1.98 (0.30)	0.75 - 1.2	1

V. 2. 5. Relative differences: control vs. treatments population biomass

Initially, the dynamics of the unexposed customized ecosystem were simulated. All four ecosystem models were calibrated to obtain a plausible annual succession of seasonal events, as described in chapter IV and in Sommer *et al.* (1986). The ecosystem model was calibrated to obtain a realistic succession of seasonal events for this type of system. To obtain this succession of events, parameters of the different populations, e.g. mortality rate and ingestion rate of zooplankton, photosynthesis rate of phytoplankton and the macrophyte, and mortality rate of the macrophyte were calibrated. In a second phase, we simulated an exposure to copper of this customized ecosystem for a period identical to that used in the microcosm experiment (110 days). To compare a population's biomass status in the control situation with that in the different copper treatments, its biomass under both scenario's was averaged over the exposure period. Relative differences of a population's biomass between the control and the treatments were calculated, as described in chapter IV.

V. 2. 6. Comparison of experimental and predicted effects

To account for variability of each of the used single-species toxicity test results, the four ecosystem models were run in a Monte-Carlo setting. Characteristics of the statistical distributions describing this variability are given in Table V.2. Using latin hypersquare sampling, 100 simulations per concentration were run. The number of runs were determined using the stabilization of variances (Cullen and Frey, 1999). After 60 to 80 runs, standard deviations of all variables stabilized at all concentrations and the control. Each of these 100 simulations was compared with the control situation, yielding 100 RD values per model population and exposure concentration. For all four ecosystem models, predicted RD values for all populations as a function of copper were compared with experimental RD values obtained in the microcosm experiment. Derivation of experimental RD values was done using the raw microcosm data, applying the same methodology as that used for the model predictions.

V. 2. 7. Derivation of experimental and predicted population-NOECs

Because 20% is the minimum detectable difference in population characteristics in the field (Suter II, 1993), a RD-value of -0.2 or lower is considered as an observable decrease of a population and a value of 0.2 or higher as an observable increase of a population biomass. The $NOEC_{\alpha}$ for

decrease of a population's biomass was defined as the largest concentration at which less than 100 · (1 - α) % of the RD values for this population were smaller than -0.2. This percentile was calculated by ranking RD values and using the mean plotting position (Davison and Hinkley, 1997). Similarly, the $NOEC_{\alpha}$ for increase of a population, was defined as the largest concentration at which less than 100 · (1 - α) % of the RD values for this population were larger than 0.2. The influence of the α - level on the predicted NOECs was investigated for α between 0.01 and 0.5.
To allow a relevant model-data comparison, experimental population-NOECs were derived from the raw microcosm data using the same method as that used for the derivation of predicted population-NOECs, i.e. using the same 20% cut-off value for RD. The effect of the α - level on the experimental NOECs was investigated for α between 0.01 and 0.5. As such, also experimental population-NOECs will also change with changing α.
Note that the as such derived NOECs differ from single-species NOECs or EC_xs in that they incorporate ecological interactions, and as such take into account indirect chemical effects. Single-species toxicity test results alone can not account for such indirect effects.

V. 2. 8. Derivation of predicted and experimental ecosystem-NOECs

The ecosystem-NOEC was defined as the lowest population-NOEC. Exposure of the ecosystem to this NOEC will consequently not adversely affect the biomass of any of the populations of the modelled ecosystem: i.e. phytoplankton will not increase or decrease more than 20%, while zooplankton and the macrophyte will not decrease more than 20%. The predicted and experimental ecosystem-NOECs were derived based on predicted and experimental population-NOECs.

V. 2. 9. Comparison of the different toxic effect sub-models

Predicted population-NOECs were compared with experimental population-NOECs at α - levels of 0.01 to 0.5. The degree of agreement between predicted and experimental RD values, as well as between predicted and experimental population-NOECs, was used to assess the "predictive" capacity of the four ecosystem models. Their "protective" capacity was examined by comparing predicted ecosystem-NOECs and experimental ecosystem-NOECs. If an ecosystem model estimates the NOEC of the most sensitive population correctly, while completely misjudging effects on the other populations, its protective capacity would be adequate, although its predictive capacity would be low. That is, NOECs calculated by that ecosystem model are protective for the

whole ecosystem, yet fail to correctly predict most population-level effects. The predictive capacity of an ecosystem model can thus be interpreted as a measure for its usability in ecological effect assessments, where the main interest is on how toxicants affect populations. The protective capacity of an ecosystem model can be used to measure its applicability in water quality standard setting which aims at determining a maximum toxicant concentration which does not adversely affect the ecosystem.

V. 3. Results

V. 3. 1. Control vs. treatments population biomass: phytoplankton and macrophyte

The microcosm data show that the biomass of $phytoplankton_{spring}$ increases with increasing copper concentrations (Fig V.1A and A'). Up to 80 µg L^{-1}, all four ecosystem models predict an increase of $phytoplankton_{spring}$, although the experimentally observed increase (300 to 1000%) is larger than the predicted increase (100 to 200% for all four ecosystem models). At 160 µg L^{-1}, a complete collapse of $phytoplankton_{spring}$ is predicted, although observations indicate an increase of 2000%.

Experimental RD values for the $phytoplankton_{summer}$ decrease with increasing copper concentration, indicating a loss of biomass (Fig V.1B). Results from all four ecosystem models exhibit this decrease. Only at 20 µg L^{-1} the LOGC model predicts the RD values marginally better than the other three ecosystem models.

Decline of the macrophyte biomass with increasing copper concentrations, as predicted by all four ecosystem models, is confirmed by the microcosm observations (Fig V.1C).

V. 3. 2. Control vs. treatments population biomass: zooplankton

The microcosm data indicate that cladoceran biomass decreases drastically at concentrations ≥ 40 µg L^{-1} (Fig V.1D). At concentrations < 40 µg L^{-1}, experimentally observed biomass concentrations are maintained at the control level. This is predicted by the LOG model only. The LIN and LINC models severely overestimate effects on cladocerans at low concentrations.

The biphasic response of the copepods to the copper exposure (i.e. an increase followed by a decrease) is both reflected by the microcosm data and by the predictions of all four ecosystem models (Fig V.1E). Experimental RDs at 5 and 10 µg L^{-1} are more accurately predicted by LOG and LOGC than by LIN and LINC.

For rotifers, a disagreement between predicted and experimental RDs is observed (Fig V.1F). This can probably be explained by the very low rotifer densities (< 0.5 µg L^{-1}) in the microcosm experiment (Schaeffers 2001). Loss of a single organism will have a serious impact on their RD values. It is therefore questionable whether the RD values for rotifers, as derived from the microcosm data, give a reliable reflection of copper effects on this group. Hence, rotifer data and predictions were omitted for further analyses.

V. 3. 3. NOEC derivations

The number of predicted population-NOECs exceeding the corresponding experimental population-NOECs as a function of α is shown in Fig V.2. This number can be interpreted as a measure for underestimation of effects on populations. At $\alpha \neq 0.35$, LOG and LOGC underestimate effects on two to three populations, while LIN and LINC underestimate effects on only one population. A value of 0.35 appears to result in conservative NOEC estimates for all ecosystem models and will, as an example, be used to compare NOECs predicted with the four ecosystem models.

V. 3. 4. Population-NOECs: phytoplankton and macrophyte

Both LIN and LINC estimate a $NOEC_{spring\ phytoplankton}$ of 10 µg L^{-1}, while LOG and LOGC predict the $NOEC_{spring\ phytoplankton}$ of 20 µg L^{-1} accurately (experimental $NOEC_{spring\ phytoplankton}$ is 20 µg L^{-1}). Predicted values of $NOEC_{summer\ phytoplankton}$ (Fig V.3), differ most between LIN/LINC and LOG/LOGC models. The latter predict a $NOEC_{summer\ phytoplankton}$ of 20 µg L^{-1}, while the former models result in $NOEC_{summer\ phytoplankton}$ of 10 µg L^{-1}. The experimental $NOEC_{summer\ phytoplankton}$ is 20 µg L^{-1} (Fig V.3).
At 40 µg L^{-1}, all ecosystem models predict a significant decline in macrophyte biomass, (Fig V.3). All ecosystem models result in a $NOEC_{macrophyte}$ of 20 µg L^{-1}.

V. 3. 5. Population-NOECs: Zooplankton

The absence of effects on cladoceran biomass observed in the microcosms at concentrations of 5 to 20 µg L^{-1}, was only predicted by the LOG model (Fig V.3). The $NOEC_{cladocerans}$ derived with the LOG model was thus equal to the experimental value: 20 µg L^{-1}. LOGC already predicted an

effect at 20 µg L^{-1}, while according to both LIN and LINC effects are expected at the lowest treatment concentration (5 µg L^{-1}).

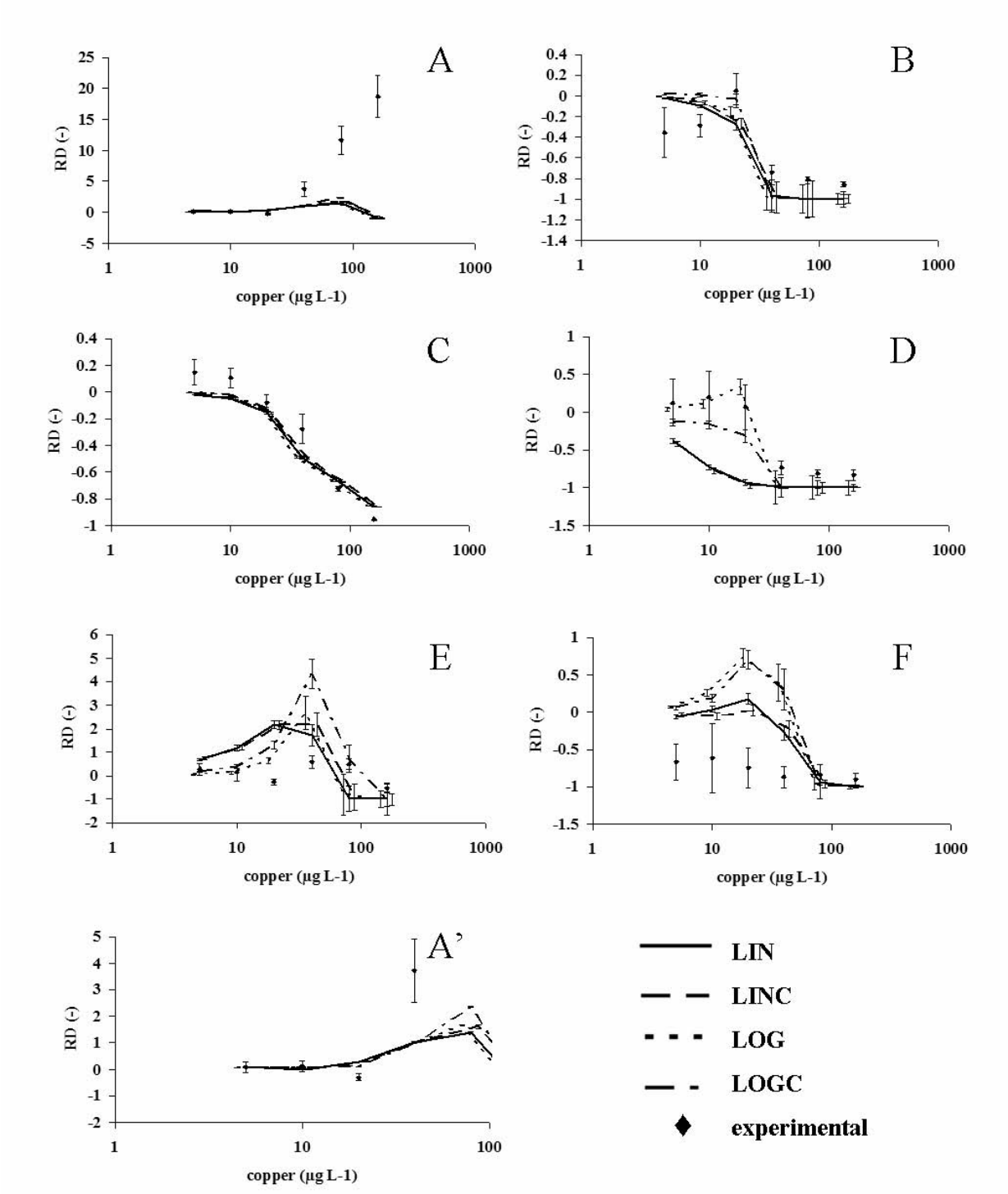

Figure V.1: Biomass changes, relative to a control condition, as a function of copper for the six populations in the ecosystem: spring phytoplankton (A); summer phytoplankton (B); the macrophyte (C); cladocerans (D); copepods (E); rotifers (F). Experimental relative differences (RD) and associated standard errors are indicated by ♦ and error bars, respectively. A more detailed graph is added for the spring phytoplankton biomass change as a function of copper concentrations between 5 and 40 µg L-1 (A'). Line codes are given in the legend.

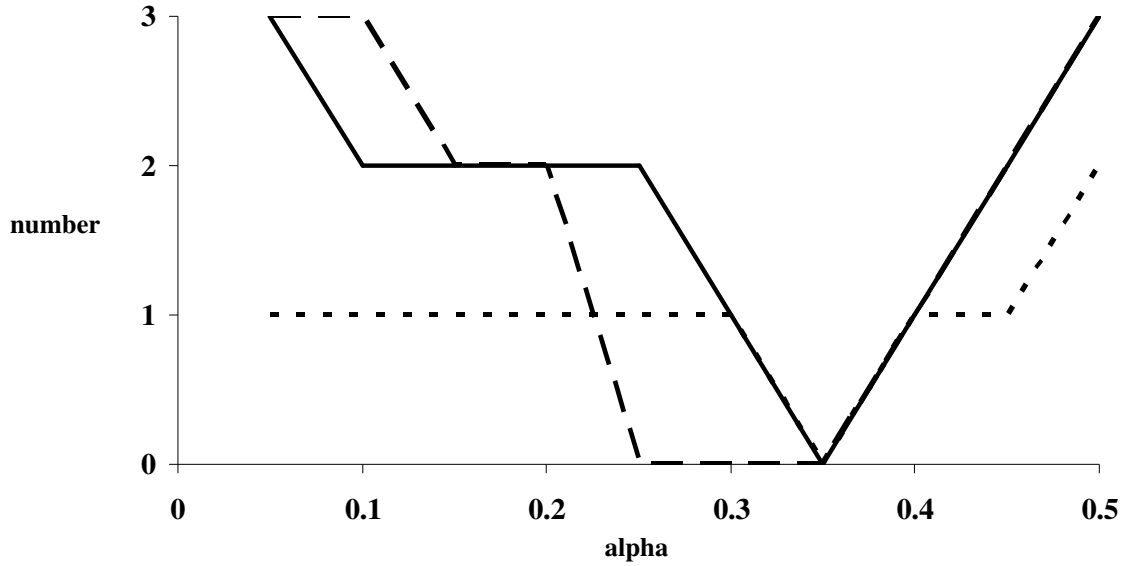

Figure V.2: The number of populations for which population-NOECs were predicted higher by the models than those observed in the microcosm study, for different alpha levels.

The experimental $NOEC_{copepods}$ is 80 µg L^{-1} (Fig V.3). At 80 µg L^{-1}, a decline of copepod biomass is estimated by LIN(C), resulting in a $NOEC_{copepods}$ prediction of 40 µg L^{-1}. Application of the other two ecosystem models yields a $NOEC_{copepods}$ equal to the experimental value.

V. 3. 6. Ecosystem-NOEC

As stated earlier, the ecosystem-NOEC is defined as the lowest population-NOEC. From the microcosm data, an experimental ecosystem-NOEC of 20 µg L^{-1} is derived. Since the population-NOECs vary depending on the ecosystem model applied, the ecosystem-NOEC also differs. Ecosystem-NOECs predicted by the different ecosystem models and that derived from the microcosm study are shown in Fig V.3. From this, it is clear that the LOG and LOGC models give better ecosystem-NOEC predictions, compared to the values derived with the LIN and LINC models. Yet, only the LOG model predicts the ecosystem-NOEC accurately (20 µg L^{-1}), while LOGC is a factor 2 conservative (10 µg L^{-1}).

Application of LIN and LINC resulted in an ecosystem-NOEC of < 5 µg L^{-1}, which is over 4 times lower than the experimental ecosystem-NOEC.

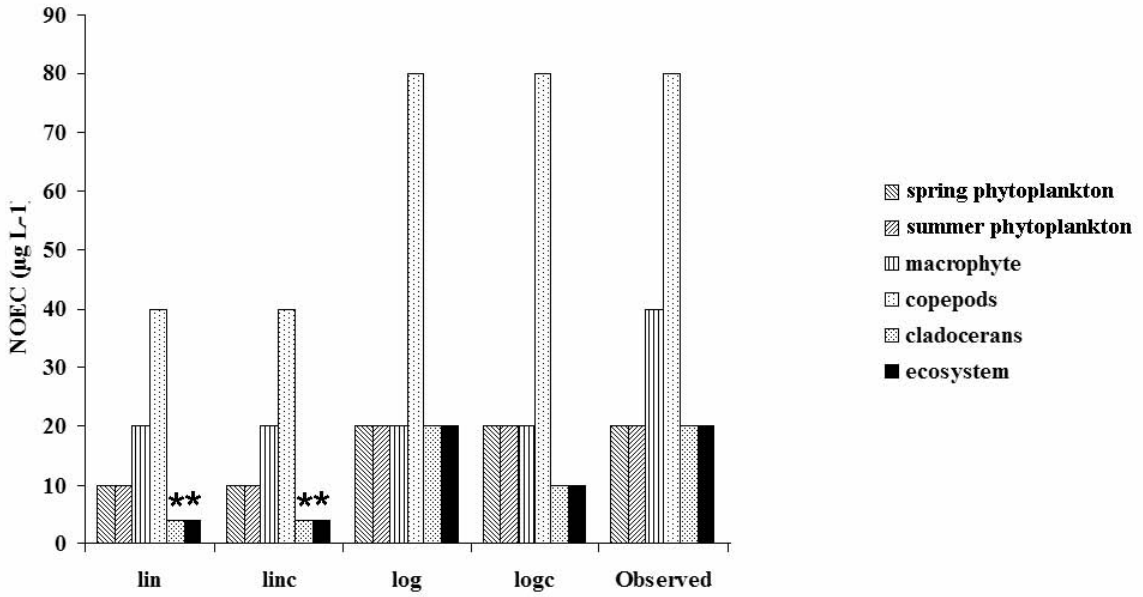

Figure V.3: Population-NOECs for spring phytoplankton, summer phytoplankton, the macrophyte, copepods, and cladocerans as predicted by the four models (lin, linc, log, and logc), observed in the microcosm experiment (DATA). Ecosystem-NOECs are represented by the black bars. Values of < 5 µg L-1 are plotted as 4 µg L-1 and indicated by *.

V. 4. Discussion

V. 4. 1. Control vs. treatments population biomass: phytoplankton and macrophyte

The increase of $phytoplankton_{spring}$ in experimental enclosures exposed to metals has also been observed by Jak *et al.* (1996). However, according to the concentration-effect functions (Table V.1), copper does not increase maximal photosynthesis rate (PS_{max}) of spring phytoplankton at these concentrations. For example, at 40 µg copper L^{-1}, the concentration-effect functions indicate a 30 to 40% decrease of PS_{max}, while at this concentration, a $phytoplankton_{spring}$ biomass increase of 150 to 250% is predicted. As such, a decline of zooplankton biomass and hence a reduced grazing pressure is proposed as an explanation for this phenomenon.

In contrast with the increase of $phytoplankton_{spring}$, $phytoplankton_{summer}$ biomass is found to decrease with increasing copper concentration. Yet, both $phytoplankton_{summer}$ and $phytoplankton_{spring}$ are grazed upon by zooplankton. Hence, they should both experience a reduction of grazing pressure. Moreover, PS_{max}-EC_{50}s of both populations are the same (Table V.2), indicating equal direct copper effects on PS_{max} of $phytoplankton_{summer}$ and

phytoplankton$_{spring}$. A possible explanation for the decrease of phytoplankton$_{summer}$ biomass is therefore the superiority of phytoplankton$_{spring}$ in competing for nutrients, as observed in other experimental studies (e.g., Havens *et al.*, 1994a and b).

V. 4. 2. Control vs. treatments population biomass: zooplankton

The severe overestimation of effects on cladoceran biomass by the LIN and LINC models (at low concentrations) may be explained as follows. At low concentrations the direct effect of copper on the cladoceran mortality rate is overestimated by a linear concentration-effect function. However, this does not necessarily imply an overestimated effect on cladoceran biomass within a food web, since the latter effect also depends on ecological interactions. Here, the competition with copepods for food will limit the biomass of the cladoceran population. Given their lower sensitivity, copepods will have a competitive advantage over the cladocerans, when exposed to copper, limiting cladoceran biomass even more. The combination of this food web effect with the overestimated direct effects on cladoceran mortality rate, results in an overestimation of the copper effect on cladoceran biomass.

V. 4. 3. NOEC derivations

The large influence of α on the predictive capacity of the four models originates from the small variability of the microcosm data, compared to that of the ecosystem model predictions. In general, coefficients of variation (CV's) of the ecosystem model predictions are a factor 5 to 7 larger than CV's of microcosm observations. The large variability of the ecosystem model predictions hampers the early detection of population effects at $\alpha = 0.05$ to 0.25, leading to severe underestimations of effects. Yet, applying α-levels of 0.4 to 0.5 on the microcosm data - which has a smaller variability - would result in experimental population-NOECs below 5 µg L^{-1}.

The different predictions of the population-NOECs by LIN, LINC and LOG, LOGC is probably due to the overestimation of cladoceran decrease by LIN and LINC. This overestimation results in an extremely reduced grazing pressure, and hence in a NOEC for increase of phytoplankton$_{spring}$ biomass which is too low.

The difference between model predictions (predicted NOEC$_{macrophyte}$ = 20 µg L^{-1}) and microcosm observations (experimental NOEC$_{macrophyte}$ = 40 µg L^{-1}) may be due to the use of a phytoplankton EC_{50} in the toxic effect sub-models of this macrophyte.

V. 4. 4. Ecosystem-NOEC

The rather conservative prediction of the ecosystem-NOEC by LIN and LINC is again due to the overestimation of effects on cladoceran biomass at lower concentrations ($NOEC_{cladocerans}$ of < 5 µg L^{-1}). Naito *et al.* (2003), who used the comprehensive aquatic systems model (CASM) equipped with a linear toxic effect sub-model (i.e. comparable with the LIN model in this chapter) predicted an ecosystem-NOEC approximately 20 times lower than the one measured in an artificial river system exposed to copper. However, if this factor 20 might be exclusively attributed to the use of a linear model is not sure. Naito *et al*. (2003) used the lake Suwa food web to predict copper effects in an artificial river system. As such, the ecosystem represented by their model was not representative for this artificial river system.

V. 4. 5. NOECs derived using other cut-off values

Although often cited, the 20% cut-off value used to derive NOECs in this dissertation is not definitive. Therefore, NOEC-derivations were *a posteriori* also performed using 10% and 30% cut-off values. Using a cut-off value of 30% resulted in the same experimental and predicted NOECs as when a 20% cut-off value was used. When a cut-off value of 10% is applied, only the experimental NOECs of the macrophyte and of $phytoplankton_{summer}$ are lower than those derived using 20% as a cut-off. The experimental NOEC for decrease of the macrophyte is 20 µg L^{-1} using the 10% cut-off. The experimental NOEC for decrease of $phytoplankton_{summer}$ is 5 µg L^{-1} using the 10% cut-off. This last NOEC is overpredicted by all four models. However, from Fig V. 1B, it can be seen that at 20 µg L^{-1}, experimentally observed biomass of $phytoplankton_{summer}$ returns to its control range. Hence, it can be questioned if the experimentally observed decrease at 10 µg L^{-1} is a copper effect, or results from data variability. For all other populations, the use of a 10% cut-off value resulted in the same conclusions regarding the predictive capacities of the four models as when a 20% cut-off value was used: the LIN and LINC models are conservative and the LOG model is most accurate in predicting NOECs.

V. 5. Conclusions

Based on the comparison of experimental data with predictions given in previous paragraphs, the largest differences in the predictive capacity of the ecosystem models are attributable to the different types of concentration-effect function. LOG and LOGC models gave more accurate predictions of population-NOECs and ecosystem-NOECs than LIN and LINC models, when using an α-level of 0.35. Apparently, implementation of the correct shape of concentration-effect functions is more important than the inclusion of sub-lethal grazing effects. Indeed, the latter resulted in only minor changes in biomass RD predictions. The extent to which these findings can be extrapolated to other ecosystems and toxicants will depend on the considered food web structure. In the food web used here, overestimation of direct effects on cladocerans by the LIN and LINC models resulted in inaccurate predictions of connected phytoplankton populations. In a more complex food web, one could expect two contrasting mechanisms. On one hand, as observed in this chapter, erroneous estimations of direct effects on one population could propagate to connected or competing populations. On the other hand, the influence of trophic interactions on biomass dynamics of the populations is assumed to be lower in more diverse, and hence more complex food webs (MacArthur, 1955). Which of these two phenomena will dominate is difficult to predict based on only the number of trophic links or 'connectance' within the food web.

This chapter has shown the high accuracy with which population- and ecosystem-NOECs for copper can be predicted by the LOG model. Moreover, this LOG model only requires a limited amount of standard single-species ecotoxicity data comparable to the type of information needed for ecosystem-NOEC determination using conventional extrapolation techniques. The quality of the toxicity data that are used is expected to influence the NOEC predictions, but this is also the case with conventional extrapolation techniques. In the LOG model, values for LC_{50} and PS_{max}-$EC_{50,\ photosynthesis}$ were combined with a slope value for metals taken from literature (Smit *et al.*, 2001). In contrast, application of the LOG(C) model would require additional single-species toxicity data on toxicant effects on invertebrate ingestion rates, i.e. information which is not always available in open literature.

Chapter VI

Validation of

an ecosystem modelling approach

as a tool for ecological effect assessments

Chapter VI

Validation of an ecosystem modelling approach as a tool for ecological effect assessments

Abstract - In ecotoxicology, derivation of a "safe" environmental concentration is usually achieved by the use of extrapolation factors or by statistical extrapolation from a set of single-species toxicity data. These approaches ignore ecological interactions between species in the field. An ecology-based alternative to this pragmatic approach can be ecosystem modelling, which can account for ecological interactions. However, it is largely unexplored how well the predictions of these models quantitatively agree with large-scale experimental studies. Therefore, we evaluated the capacity of a flexible ecosystem model to predict population and ecosystem-level no observed effect concentrations (NOECs) of 7 organic toxicants. These NOECs were compared with population and ecosystem -NOECs observed in 11 micro- and mesocosm studies. For each of the latter studies, the model was customized to account for the specific ecological interactions within these systems and combined with appropriate single-species toxicity data from literature. Population-NOEC predictions were accurate, or at least protective, for 60, and 86% of all considered model populations, respectively. For all 11 studies, a protective ecosystem-NOEC could be derived, being accurate in 7 cases, and conservative in 4 cases. In general, it can be stated that this type of models can serve as an ecology-based alternative to current extrapolation techniques in EEAs and water quality standard setting.

redrafted from

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VI. 1. Introduction

Ecological effect assessment aims at assessing or predicting potential effects of a chemical substance on the structure and function of ecosystems. These higher-level effects are usually estimated through extrapolation of single-species effect data. However, these approaches are based on largely unproven hypotheses and are therefore heavily criticized (Versteeg *et al.*, 1999; Forbes and Calow, 2002). One of the most crucial hypotheses is the representation of the community sensitivity as a set of independent species sensitivities (Wagner and Lokke, 1991). Possible ecological interactions between populations in communities (e.g. Sommer *et al.*, 1986; Preston and Snell, 2001) are thus ignored. Since effects on these ecological interactions combine with direct toxicant effects on populations to determine effects at the community- and ecosystem-level (Chapman *et al.*, 2003; Fleeger *et al.*, 2003), knowledge about such interactions should be incorporated in ecological effect assessments to more accurately estimate higher level effects of chemicals.

Large scale experimental studies, i.e. micro-, mesocosm and field enclosure studies, are capable of accounting for direct and indirect toxicant effects resulting from ecological interactions (e.g., Hoagland *et al.*, 1993; Clements and Kiffney, 1994; Joern and Hoagland, 1996). Unfortunately, these types of studies are very resource-demanding and can thus not be used for routine evaluation of chemical toxicity. Especially in view of REACH (Registration, Evaluation and Authorisation of Chemicals; http://ecb.jrc.it/REACH/), a EU-legislation aiming at environmental risk assessments for approximately 30,000 substances, there is a clear need for alternative, less resource-demanding methodologies to predict the effects of chemicals on ecosystem structure and function.

Mathematical models have been proposed as an alternative approach to incorporate ecological interactions in environmental effect assessments and water quality standard setting (e.g. Pastorok *et al.*, 2003). These models integrate toxic effect sub-models in ecosystem models to simulate effects of toxicants on ecosystems. Toxic effect sub-models vary in complexity and range from highly realistic toxicokinetic models (Bartell *et al.*, 1988; Traas *et al.*, 2004b) to rather simple concentration-effect functions (Bartell *et al.*, 1999; Traas *et al.*, 2004a). In terms of feasibility, use of the latter is preferable, since these sub-models only require a limited set of single-species toxicity test results. In contrast, toxicokinetic sub-models are often characterized by a large number of uncertain parameters (Sijm and Vanderlinde, 1995). In chapter V, it was demonstrated that population- and ecosystem-level no observed effect concentrations (NOECs) of copper in microcosms could be predicted accurately using an ecosystem model with logistic external

concentration-effect functions as toxic effect sub-models. Because of its object-oriented framework, the ecosystem model could be customized to reflect the ecological interactions within these microcosms. The toxicity data used were: lethal concentrations (LC_{50}) for invertebrates and fish, effect concentrations (EC_{50}) for phytoplankton and macrophyte growth rates, and published default slope values of concentration-effect functions (Smit *et al.*, 2001). Given these minimal data requirements, the idea of applying this type of model for assessing effects and setting water quality standards seems appealing. However, the use of this model as an ecology-based alternative to current statistical extrapolation approaches requires a validation of its predictive capacity for a wider range of toxicants and ecosystems. Naito *et al.* (2003) have performed such a validation by comparing model-predicted ecosystem-NOECs with those observed in different artificial ecosystems. However, these authors did not account for the specific ecological interactions in the considered ecosystems, but rather examined if one generic model-ecosystem could be used to predict effects in a range of large-scale experimental studies. Predicted population-level effects were only qualitatively compared with the observations.

This chapter presents a validation study, based on the quantitative comparison of predicted population- and ecosystem-NOECs with those observed in 11 experimental community-level studies (micro-, mesocosms and enclosure studies). In these studies, the effects of 7 different organic toxicants were examined. The use of the object oriented modelling framework allows for customizing the model to better reflect ecological interactions within the different experimental ecosystems. We examined whether this approach resulted in an adequate agreement between predictions and observations, both at the population-, and ecosystem-level.

VI. 2. Material and methods

VI. 2. 1. Ecosystem model

A dynamic ecosystem model was constructed consisting of a set of objects which describe the growth of model populations in terms of their biomass concentration using differential equations. The equations and conceptual framework of the model are given in Chapter III.

VI. 2. 2. Large-scale experimental studies

Pelagic, lentic community-level studies describing toxicant effects on the population's biomass and/or abundance were taken from literature. To include as much information on the effects of the

toxicant on population dynamics as possible, only studies conducted over at least one typical seasonal event were considered (Sommer *et al.*, 1986). As a compromise between data availability and experiment duration, a 40 day experiment duration was taken as a cut-off value for inclusion in this analysis. The selected studies represent a wide range of different ecosystems, i.e. from relatively simple planktonic systems to systems which include planktivorous and piscivorous fish (Table VI.1). Considered toxicants are: diflubenzuron, atrazine, linuron, esfenvalerate, metribuzin, azinphos-methyl, and fenthion. Considered studies are: Boyle *et al.* (1996; study 1), Hamilton *et al.* (1988; study 2), Webber *et al.* (1992; study 3), Fairchild *et al.* (1992; study 4), Brock *et al.* (2004; study 5), Sierszen and Lozano (1998; study 6), Hanazato and Kasai (1995; study 7), Tanner and Knuth (1995; study 8), Juttner *et al.* (1995; study 9), Denoyelles *et al.* (1982; study 10), Cuppen *et al.* (1997; study 11); van den Brink *et al.* (1997; study 11).

VI. 2. 3. Toxic effect sub-model

Given their high accuracy in predicting population- and ecosystem-NOECs in a previous exercise (chapter V), ecosystem models equipped with logistic concentration-effect functions as toxic effect sub-models were used. These sub-models describe direct effects on invertebrate and vertebrate mortality rate, and on the photosynthesis rate of phytoplankton and macrophytes. At every time step, the chemical concentration is read from the ecosystem model input file and used to calculate the values of those parameterts.

Macrophyte mortality rates were assumed to remain unaffected because no proof of effects of the considered chemicals on macrophyte mortality could be found in literature. Parameters of these sub-models are thus LC_{50}'s and photosynthesis-EC_{50}'s. Appropriate values for those parameters were collected from literature (Table VI.2). Other parameters of these sub-models are the slope values for the different toxicants and model populations, all of which can be found in Smit *et al.* (2001).

VI. 2. 4. Control and exposure simulations

To account for the species present in the experimental ecosystems, a different ecosystem model was constructed for each of the considered studies. Species were grouped into model populations, based on their single-species sensitivity for the considered chemical and their growth kinetics. Details of the grouping of species into model populations are given in the appendix (XI.7). Planktivorous fish were assumed to exclusively feed on large-bodied zooplankton (Werner and

Table VI.1: Overview of the large-scale studies considered in this chapter. Column 1 gives the ID-numbers assigned to the considered studies. Column 2 gives the α-level used to derive the NOEC-predictions. « n.a. » means that the quantity is not applicable. Columns 3 to 14 give the observed NOECs (µg L^{-1}, upper pannel) for the populations present in the experimental studies, and for the complete ecosystem (column 14). In the lower panel, columns 3 to 14 give predictions of population- and ecosystem-level NOECs by the proposed ecosystem modelling approach (µg L^{-1}). In these columns, 'plank. fish' stands for planktivorous fish, and 'pisc. fish' for piscivorous fish. 'Zoo' and 'Phyto' represent zooplankton and phytoplankton, respectively. An asterisk denotes that the observed NOEC of the considered population was statistically unreliable. Further details are given in the appendix (XI.7).

	n°	α	spring phyto1	spring phyto2	summer phyto1	summer phyto2	macrophyte	small zoo1	small zoo2	large zoo1	large zoo2	plank. fish	pisc. fish	ecosystem
data	1	n.a.	≥10		<10			<10		<10	<10	<10	≥10	<10
	2	n.a.	<100	≥100	<100			≥100		≥100				<100
	3	n.a.	0.18		0.18			≥0.69		0.18		≥0.69		0.18
	4	n.a.	0.25		0.25			≥1.71		≥1.71		0.67		0.25
	5	n.a.	*		56	18	≥180	18	≥180	≥180				18
	6	n.a.	≥20		≥20			≥20		≥20	4			4
	7	n.a.	≥200		<20			≥200		20	<20			<20
	8	n.a.	≥4		≥4			≥4		≥4	≥4	≥4		≥4
	9	n.a.	68	68	68			182		68				182
	10	n.a.	20		20			≥500		20		≥500	≥500	20
	11	n.a.	50		15	50	15	50		≥150	≥150			15
predictions	1	0.01-0.5	≥10		<10			≥10		≥10	<10	≥10	≥10	<10
	2	0.01-0.21	≥100	<100	≥100			≥100		<100				<100
		0.22-0.23	≥100	<100	≥100			<100		<100				<100
		0.24-0.5	<100	<100	<100			<100		<100				<100
	3	0.01-0.13	≥0.69		≥0.69			≥0.69		≥0.69		≥0.69		≥0.69
		0.14-0.19	≥0.69		≥0.69			0.18		≥0.69		≥0.69		0.18
		0.2-0.25	0.18		≥0.69			0.18		≥0.69		≥0.69		0.18
		0.26-0.39	0.18		0.18			0.18		≥0.69		≥0.69		0.18
		0.4-0.47	0.18		0.18			0.18		≥0.69		0.18		0.18
		0.48-0.5	0.18		0.18			0.18		0.18		0.18		0.18
	4	0.01-0.25	≥1.71		≥1.71			≥1.71		≥1.71		0.67		0.67
		0.26-0.47	0.25		0.69			≥1.71		≥1.71		0.67		0.25
		0.48-0.5	0.25		0.25			0.25		≥1.71		0.67		0.25
	5	0.01-0.5	5.6		1.8	1.8	≥180	56	≥180	≥180				1.8
	6	0.01-0.06	≥20		≥20			≥20		≥20	4			4
		0.07-0.5	≥20		≥20			≥20		≥20	1			1
	7	0.01-0.5	≥200		≥200			≥200		≥200	<20			<20
	8	0.01-0.5	≥4		≥4			≥4		≥4	≥4	≥4		≥4
	9	0.01-0.3	≥318	≥318	22			22		≥318				22
		0.31-0.38	≥318	68	22			22		≥318				22
		0.39-0.5	≥318	22	5			22		≥318				5
	10	0.01-0.5	20		20			≥500		20		≥500	≥500	20
	11	0.01-0.14	15		50	5	15	≥150		50	50			5
		0.15-0.5	5		0.5	50	15	≥150		15	15			0.5

Table VI.2: Overview of the mean of the used, log transformed toxicity data (µg L^{-1}) in the model to represent population sensitivities in the different studies. 'Phyto', 'zoo', 'plank. fish', and 'pisc. fish' stand for phytoplankton, zooplankton, planktivorous fish, and piscivorous fish, respectively. Phytoplankton and macrophyte values are growth-EC_{50}'s, the other values are LC_{50}'s. (-) indicates that no proof of effects of the considered toxicant at the tested concentration range was found. Hence, these populations were assumed not to be directly affected by the toxicant. A default of 10% was chosen arbitrarily as the standard deviation on these means for uncertainty propagation. In the case of planktivorous fish in study 8, a uniform distribution was chosen to represent uncertainty, of which the upper and lower limits are given. Slope values were derived from: $L(E)C_{50} \cdot L(E)C_5^{-1} = \exp(1.6449 \cdot Sm)$; and slope = $\ln(5 \cdot 95^{-1}) \cdot (\ln(L(E)C_5) - \ln(L(E)C_{50}))^{-1}$ (Smit *et al.*, 2001). Sm was characterized by a uniform distribution between 0.75 and 2, 0.45 and 0.7, 0.25 and 0.4 for phytoplankton, zooplankton, and fish, respectively (Smit *et al.*, 2001).

n°	$phyto_{spring1}$	$phyto_{spring2}$	$phyto_{summer1}$	$phyto_{summer2}$	macrophyte	zoo_{small1}	zoo_{small2}	zoo_{large1}	zoo_{large2}	plank. fish	pisc. fish	toxicant	references
1	-		-			1.48[a]		4[a]	0.71	4.95	4.95	diflubenzuron	Miura and Takahashi (1974); Julin and Sanders (1978); Hansen and Garton (1982); Mayer and Ellersieck (1986); Liber et al (1994); U.S.EPA (2000)
2	2.52	2.13	2.41			-		-				atrazine	Kallqvist and Romstad (1994); Kotrikla et al (1997); Carrasco and Sabater (1997); Tang et al (1997); Berard et al (1999b); Rojickova-Padrtova and Marsalek (1999); Benhræt al (1997); Okamura et al (2000)
3	-		-			-0.05[b]		-0.05[c]		0.28[c]		esfenvalerate	Fairchild et al (1992)
4	-		-			-0.05[b]		-0.05[c]		0.28[c]		esfenvalerate	Fairchild et al (1992)
5	1.01		1.01	2.74	1.32	-	-	-				metribuzin	Fairchild et al (1994 and 1998); U.S.EPA (2000)
6	-		-			4.93[b]		4.93	0.25			azinphos-methyl	Dortland (1980); Guzzella et al (1997); U.S.EPA (2000)
7	-		-			3.85		3.60	0.16			fenthion	Roux et al (1995); Kaur and Ansal (1996)
8	-		-			4.93[b]		4.93	0.25	0.68-0.74		azinphos-methyl	Dortland (1980); Guzzella et al (1997); U.S.EPA (2000)
9	2.52	2.13	2.41			-		-				atrazine	Kallqvist and Romstad (1994); Kotrikla et al (1997); Carrasco and Sabater (1997); Tang et al (1997); Berard et al (1999b); Rojickova-Padrtova and Marsalek (1999); Benhræt al (1997); Okamura et al (2000)
10	2.70		1.81			-		-		-	-	atrazine	Kallqvist and Romstad (1994); Kotrikla et al (1997); Carrasco and Sabater (1997); Tang et al (2000) 1997); Berard et al (1999b); Rojickova-Padrtova and Marsalek (1999); Benhræt al (1997); Okamura et al (2000)
11	1.14		1.59	1.59	0.40	-		-	-			linuron	U.S.EPA (2000)

[a] a field-observed value was used. [b] the LC_{50} value of the small zooplankton was assumed equal to that of the large zooplankton, because of lack of toxicity data. [c] the value was reported in the original paper describing that study.

Hall, 1974; Chang *et al.*, 2004). The other ecological interactions are as in chapter IV. The ecosystem models of every study were qualitatively calibrated to obtain a plausible succession of planktonic events as described above. To obtain this succession of events, growth related parameters of the different model populations were changed (appendix XI.6.2-XI.6.9). During this calibration, parameter values were constrained in several ways. Changes larger than 20% from default values proposed by USEPA (2000a) were not allowed. Maximum photosynthesis rates (i.e. PS_{max}) of spring phytoplankton were set higher than those of large phytoplankton (Knisely and Geller, 1986; Müller and Schlegel, 1999). Saturation constants K_P and K_N were set lower for summer phytoplankton species than for spring phytoplankton species to account for the competitive advantage summer phytoplankton species have to grow in low-nutrient conditions (Sommer *et al.*, 1982). Large zooplankton populations and fish have slower growth kinetics than small zooplankton populations, i.e. lower ingestion and mortality rates (Collins and Wlosinski, 1983; Leidy and Ploskey, 1980). The as such obtained dynamics are used as the control dynamics. We then simulated the exposure of the customized ecosystems to the same toxicant concentrations as those used in the respective experimental study. Starting date, exposure duration, and number of administrations in the simulations were identical to those reported in the respective large-scale experiments. Similarly, exposure concentrations were the same as those tested in the considered studies. To compare a model population's biomass in a control treatment with that at different exposure concentrations, its biomass concentration in both treatments was averaged during the exposure period. Relative differences of a model population's biomass between the control and other treatments were calculated as in chapter IV. As 20% has been suggested as the minimum detectable difference in population characteristics in the field (Suter II, 1993), a RD-value of -0.2 or lower is considered as an observable decrease of a population. Similarly, a RD-value of 0.2 or higher can be considered as an observable increase of a population biomass. In the context of ecological effect assessment, both increases and decreases of phytoplankton biomass are considered undesirable. For macrophytes, invertebrates, and fish, only biomass decreases are considered as undesirable.

VI. 2. 5. Derivation of predicted population-no observed effect concentrations (NOECs)

To account for variability (as in IV.2.3) of the used toxicity data (Table VI.2), the customized ecosystem models were run in a Monte-Carlo setting (Cullen and Frey, 1999). Using latin hypersquare sampling, 100 simulations per concentration were run. The number of shots (100) was determined by the rule of convergence (Melching, 1995). Each of these 100 simulations was

compared with its control simulation, i.e. with the control treatment, yielding 100 values of RD per model population and exposure concentration. From these RD values, one NOEC was derived per considered population. The highest exposure concentration at which less than 100 (1- α) % of the simulated RD values were smaller than -0.2 was defined as the $NOEC_{\alpha}$ for decrease. Similarly, the highest concentration at which less than 100 · (1- α) % of the simulated RD values were larger than 0.2 was defined as the $NOEC_{\alpha}$ for increase. The influence of the α-level was investigated for α-values between 0.01 and 0.5. All NOECs on a population level are termed 'population-NOEC' in the rest of this chapter.

Predicted population-NOECs were compared with those observed in the 11 experimental studies. Only NOECs describing effects on populations biomass or abundance were considered. Other reported NOECs (e.g., number of offspring for fish) were not included. Because NOECs were not always provided as such in the 11 considered studies, they had to be derived based on the reported results. A rationalization of the as such derived NOECs is provided in the appendix (XI.7).

In the "results and discussion"-section, predicted population-NOECs which are higher than, equal to, or lower than observed population-NOECs, will be termed 'underprotective', 'accurate', and 'conservative', respectively.

VI. 2. 6. Derivation of predicted ecosystem-NOECs

The ecosystem-NOEC was defined as the lowest population-NOEC. As such, it is assured that when exposing an ecosystem to a concentration equal to its NOEC, no model populations will be adversely affected. The used terminology (i.e.. 'accurate', 'conservative', and 'underprotective') for the description of predictions, relative to observations is the same as in the case of population-NOECs.

VI. 3. Results and Discussion

VI. 3. 1. Predicted population-NOECs

Predicted and observed NOECs for the different model populations at $0.01 \leq \alpha \leq 0.5$ are summarized in Table VI.1. For studies 1, 5, 7, 8 and 10, predicted population-NOECs were independent of α and were equal to the observed ones for 57, 50, 67, 100, and 100% of the model populations, respectively.

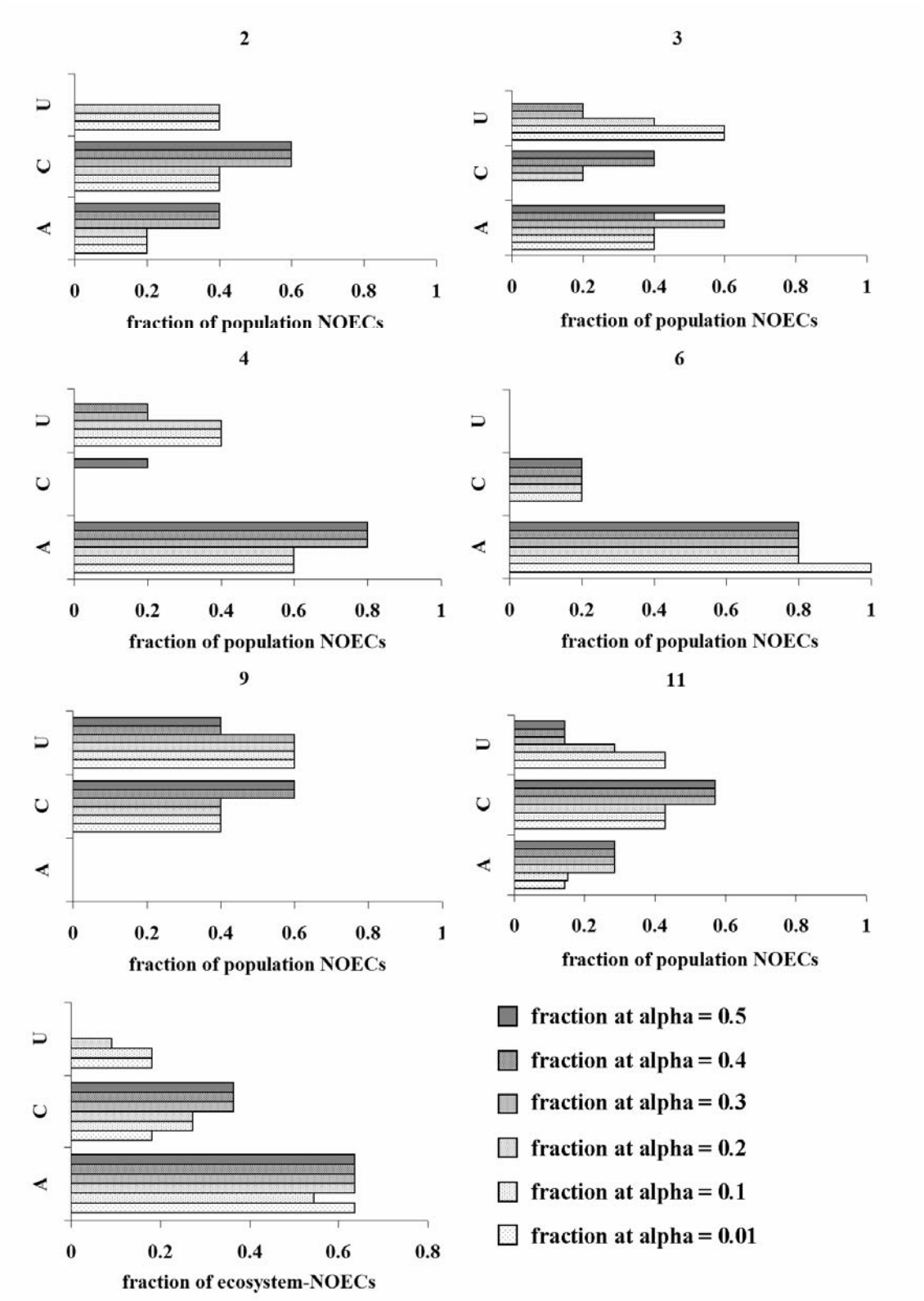

Figure VI.1: (upper 6 panels) Model performance in predicting population-level No Observed Effect Concentrations in 6 different studies (2, 3, 4, 6, 9, 11) at alpha-levels ranging from 0.01 to 0.5. Performance is expressed as the fraction of model populations for which the NOEC was predicted accurately (A), conservatively (C), or underprotective (U). Population-NOECs in the other 5 studies were independent of alpha and are given in the text.
(lower panel) Model performance in predicting ecosystem-level No Observed Effect Concentrations at alpha-levels ranging from 0.01 to 0.5. Performance is expressed as the fraction of accurate, conservative, and underprotective ecosystem-NOECs amongst the 11 studies.

When a population-NOEC is independent of α, this means that for this population, at all concentrations, the upper simulated 50% confidence region of RD did not encompass -0.2, nor did the lower simulated 50% confidence region encompass 0.2.

In the 6 remaining studies (2, 3, 4, 6, 9, and 11), predicted population-NOECs were up to a factor 7 lower at α = 0.5 than at α = 0.01. As such, the agreement with observed population-NOECs also varied with changing α for these 6 studies (Fig VI.1). For 2 out of these 6 studies (study 9: Juttner *et al.*, 1995; study 11: Cuppen *et al.*, 1997 and Van den Brinck *et al.*, 1997) the proportion of accurate population-NOEC predictions is never higher than 30%, regardless of α. Amongst the other 4 studies, the percentage of accurate predictions tends to increase with increasing α, while the percentage of underprotective predictions decreases. Study 6 is the only case in which applying a low α results in somewhat better population-NOEC predictions than applying a high α. At α = 0.01 and 0.1, model performance in predicting protective population-NOECs is low, as indicated by the high number of underprotective predictions for studies 3 and 9. At higher α-values, the percentage of accurate population-NOECs in studies 2, 3, 4, 6, 9, and 11 was only marginally higher, and even slightly lower in the case of study 6. However, the percentage of underprotective population-NOECs decreased at increasing α, resulting in a higher proportion of conservative population-NOECs. This conservatism can be regarded as the consequence of reducing the number of underprotective population-NOECs.

In Hanratty and Liber (1996), the use of an LC_{50} estimate for *Daphnia magna* of 4.5 µg L^{-1} to represent cladoceran sensitivity is claimed as the reason for the disagreement between predicted and observed population-level effects of diflubenzuron. In this chapter, the poor population-NOEC predictions by the model in studies 9 (Juttner *et al.*, 1995) and 11 (Cuppen *et al.*, 1997; Vandenbrinck *et al.*, 1997) did not seem to originate solely from the less representative toxicity data. For study 9, an EC_{50} of a green algae assemblage was used to represent the sensitivity of all phytoplankton not included in the diatom and Cryptophyceae model populations. In study 11, the sensitivity of the summer phytoplankton, was represented by a single EC_{50} of blue-green algae. However, a field-derived LC_{50} had to be used for small zooplankton in absence of laboratory-derived LC_{50}s in study 1, and in studies 3, 4, 6, and 8, the LC_{50} of small zooplankton was assumed equal to that of large zooplankton. Still, 57, 60, 80, 80, and 100% of population-NOECs were predicted accurately (at α = 0.5) in study 1, 3, 4, 6, and 8, respectively.

Next to the used single-species toxicity data, the aggregation of species into model populations within the model is equally important. An example of this can be found in Traas *et al* (2004b), where cladocerans and copepods are modelled as one population, despite their different sensitivity

for chlorpyrifos, the chemical evaluated. These authors acknowledge that this aggregation results in poor predictions of the effects on this aggregated model population.

Another possible source of disagreement between model predictions and observed NOECs may be type II errors, typically associated with observations from large-scale studies (Brett and Goldman, 1996). The apparent absence of observed effects on certain populations in an experimental setting may as such result from the high variability of the observations. Hamilton *et al.* (1988) report that in some large-scale experimental settings, reductions up to 50% are the smallest significant difference. This percentage will mostly depend on the used sampling techniques. For example, in study 3 (Webber *et al.*, 1992), passive fish trapping techniques characterized by a large variability were used. Based on these measurements, no significant effects on fish biomass were observed, i.e., the $NOEC_{fish\ biomass} \geq 0.69\ \mu g\ L^{-1}$ ($\geq$ the highest treatment). Whether adverse effects were truly absent or whether this was an artefact of the trapping method, is difficult to establish. The predicted NOEC for fish biomass reduction (0.18 µg L^{-1}) in study 3 should be considered in this context.

The main advantage of an ecosystem model approach versus currently used (statistical) extrapolation (e.g., the species sensitivity distribution, SSD) approaches is that the former can account for ecological interactions. These interactions can give rise to indirect toxicant effects which can not be predicted from single-species toxicity data alone, but which may be assessed through ecosystem model simulations. Indeed, in 8 out of the 11 cases in which populations were observed to experience indirect effects in the original study, these indirect effects were correctly predicted by our ecosystem model. In studies 1, 3 and 4, phytoplankton biomass was found to be higher in ponds treated with an insecticide (study 1: diflubenzuron; study 2 and 3: esfenvalerate) than in the control ponds, although the available data did not suggest that phytoplankton is directly stimulated by these chemicals. Boyle *et al.* (1996), Webber *et al.* (1992), and Fairchild *et al.* (1992) attribute these increases to decreased grazing activity of directly affected zooplankton. These increases were predicted correctly by the ecosystem model in all three studies (Table VI.1).

A reduction of small zooplankton (*Chydorus sphaericus, Lecane sp., Mytilana ventralis, Polyarthra remata*) in field enclosures treated with the photosynthesis-inhibiting herbicides metribuzin (study 5) was observed by Brock *et al*. (2004). Because metribuzin is a herbicide with a very specific mode of action, it is unlikely to have affected zooplankton in a direct way. Instead, an indirect effect, i.e. a reduction of its food source (spring phytoplankton), may explain this observation. The same mechanisms was suggested by Denoyelles *et al.* (1982; study 10), who observed a decrease of large zooplankton when exposed to the herbicide atrazine in experimental ponds. These authors demonstrated that this decrease was the result of a decrease in food

abundance, i.e. in phytoplankton density. These indirect effects were predicted by the ecosystem model in both study 5 and study 10.

Although the indirect effects observed in studies 9 and 11 were governed by similar mechanisms as those described in the previous paragraph, they were not predicted by our ecosystem model. However, in both studies model accuracy was generally low (Fig VI.1) as not only indirect but also direct toxicant effects were predicted inaccurately.

Of all population-NOEC predictions considered in this chapter, ≥ 55% were estimated accurately at all tested α-levels (0.01 to 0.5). Raising the α-level from 0.01 to 0.5 increased the proportion of accurate population-NOEC predictions to 60%. A concurrent increase of conservative predictions (from 15 to 26%) was observed and underprotective predictions were reduced from 29 to 14%. An increase in α can thus reduce the number of underprotective NOECs and increase the number of conservative NOECs bringing the percentage of protective population-NOECs on 86. Note that α = 0.5 corresponds to the median of the 100 RD values. This allows to rephrase our definition of NOEC for increase of a population to "the highest concentration at which the median of the RD values of that population is higher than -0.2." Similarly, the highest concentration at which the median of the RD values of that population is smaller than 0.2 is the NOEC for increase of that population.

The impact of the underprotective population-NOEC predictions on the resulting ecosystem-NOEC will depend on how these NOECs relate to those of the other model populations in the ecosystem. If the NOEC of the most sensitive population is overestimated, i.e. the effect is underestimated, the resulting ecosystem-NOEC will also be too high. Hence, it can not be used as a "safe" concentration for the considered ecosystem. Overestimation of a population-NOEC which is not the lowest observed in the study, will not impede an accurate estimation of the ecosystems' NOEC. Which of these two cases dominates in this validation exercise, will be discussed in the next paragraph.

VI. 3. 2. Predicted ecosystem-NOECs

The predicted ecosystem-NOECs of studies 1, 5, 7, 8, and 10 were independent of the α and were accurate for studies 1, 7, 8, and 10. For study 5, a conservative ecosystem-NOEC was predicted which was 10 times lower than the observed NOEC. Predictions of the other 6 ecosystem-NOECs varied with changing α-level. The percentage of accurate predictions increases with increasing α, and can reach 63% at α = 0.5. As for the predicted population-NOECs, the percentage of underprotective ecosystem-NOECs decreased with an increasing number of conservative

estimates at α = 0.5 (Fig VI.1). At α = 0.01, nearly 20% of the predicted ecosystem-NOECs were underprotective. At α = 0.5, the model predicted the same ecosystem-NOEC as the observed value for 7 of the 11 considered studies (i.e., in 63% of the studies). Predicted ecosystem-NOECs were never higher than the observed values at this α-level, i.e. they were never underprotective. This indicates that the 14% of underprotective population-NOEC predictions at α = 0.5, as derived in the previous paragraph, did not result in underprotective ecosystem-NOEC predictions. Hence, the model populations for which the predicted NOECs were too high were not the most sensitive populations in the considered studies. The NOECs of the most sensitive model populations were predicted accurately, or were conservative. This agrees with the finding that this type of ecosystem models predicts effects more accurately at low toxicant concentrations, i.e. the concentration range in which the most sensitive populations are affected, than at intermediate concentrations (Bartell *et al.*, 1992). Based on our simulations, or on literature, it is impossible to explain this phenomenon with a true causal relationship.

For studies 5, 6, 9 and 11, a conservative ecosystem-NOEC was predicted which was 4 to 30 times lower than the observed value. In a similar validation study with the comprehensive aquatic systems model (CASM), Naito *et al.* (2003) found that most of the predicted ecosystem-NOECs were a factor 100 lower than the observed ones. The lower factor found in the present study (10-20) may result from customizing the considered ecosystems, i.e. from the inclusion of the relevant model populations. The CASM model features one specific ecosystem, the Lake Suwa ecosystem. It was tested if this shallow lake ecosystem could be used as a model for other experimental systems. It is logical that the latter approach results in less accurate estimates than the methodology established in this thesis. Because of the importance of indirect effects, resulting from a combination of inherent sensitivities and ecological interactions, implementing the relevant populations is crucial, from an ecological point of view.

The incorporation of ecological interactions by applying the presented ecosystem model resulted in rather accurate predictions of ecological effects of chemicals, both on a population- and ecosystem-level. It should be recognized though that the proposed modelling approach can only increase ecological realism to a certain extent. Morphological and behavioural changes in zooplankton, altering their vulnerability to fish predation, and reduction of stress tolerance of populations in time are examples of insecticide effects which are not included in this modelling approach (for examples, see references in Hanazato, 2001). It should be clear that such phenomena can not be accounted for by the proposed modelling technique.

Although the current modelling technique does, technically spoken, allow incorporation of benthic populations, parameterization of such objects would be difficult. As demonstrated by

Vadeboncoeur and Vander Zanden (2002), the frequency of publication on primary producers, heterotrophic bacteria, and invertebrates was on average 10 times lower for benthic habitats than for pelagic habitats. As a consequence, the ecological relationships between benthic and pelagic populations are less well understood, let alone how human-induced stress may alter them (Lake *et al*., 2000).

VI. 4. Conclusions

No observed effect concentrations (NOECs) of 60% of all considered populations were predicted accurately in a total of 11 micro- and mesocosm studies by the developed ecosystem model. Only 14% and 26% of all population-NOECs were predicted too high or too low (underprotective or conservative), respectively. The predictive capacity of the ecosystem model was influenced by the α-level used to derive NOECs from raw model outputs. From this validation study, it becomes apparent that an α-level of 0.5 benefits the NOEC-predictions. At lower α-levels (e.g., 0.01), the amount of conservative NOEC-predictions was lower (15%), but the proportion of underprotective NOEC-predictions was higher (29%) than at $\alpha = 0.5$, which is equivalent with taking the median of the Monte-Carlo outputs. Compared to the use of $\alpha = 0.01$, using $\alpha = 0.5$ can reduce the amount of underprotective NOECs at the cost of a slightly higher amount of conservative NOECs. Predicted ecosystem-NOECs were never larger than the experimental NOECs at $\alpha = 0.5$, i.e. they were never underprotective. Because only single-species toxicity data are needed to successfully apply this modelling technique, it can serve as an ecology-based alternative for extrapolation approaches without any additional data needs.

Chapter VII

Do we have to incorporate ecological interactions in the sensitivity assessment of ecosystems?

Chapter VII

Do we have to incorporate ecological interactions in the sensitivity assessment of ecosystems? An examination of a theoretical assumption underlying species sensitivity distribution models.

Abstract - Species sensitivity distributions (SSDs) are statistical distributions which extrapolate single-species effect data to ecosystem effects. This SSD approach assumes that ecological interactions between populations do not influence the sensitivity assessment of ecosystems. The validity of this assumption in a simple freshwater lentic pelagic ecosystem was tested. For each of a 1000 hypothetical toxicants, a lognormal SSD was fitted to chronic single-species EC_{10}s of the species present. As such, these distributions did not account for ecological interactions and are therefore termed 'conventional SSDs' (cSSDs). Next, sensitivity distributions that did take into account ecological interactions were constructed (eco-SSD) for the same 1000 toxicants, using a validated ecosystem model. For 254 of the 1000 hypothetical toxicants, mean and/or variance of the cSSD were significantly higher than mean and/or variance of the eco-SSD, as such rejecting the general validity of the tested assumption. A classification tree approach indicated that especially toxicants which directly affect phytoplankton (i.e. herbicides) may have a higher mean for cSSD than for eco-SSD. Conversely, means of eco-SSD and cSSD are equal for toxicants directly affecting zooplankton and fish. For the 254 hypothetical toxicants for which the tested assumption was false, a predicted no effect concentration (PNEC) calculated with an application factor of 10 was on average a factor 10 lower than the corresponding ecosystem-NOEC calculated by the ecosystem model. If more assumptions underlying SSD models would be tested, the implications of applying the SSD approach for the protective capacity of resulting PNECs could be examined.

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De Laender F., De Schamphelaere, K.A.C., Vanrolleghem, P.A., Janssen, C.R. Do we have to incorporate ecological interactions in the sensitivity assessment of ecosystems? An examination of a theoretical assumption underlying species sensitivity distribution models. Environment International, accepted.

VII. 1. Introduction

Ecological effect assessments aim at evaluating or predicting the effects of a chemical substance on the structure and function of ecosystems. In environmental risk assessments, these "higher-level effects" are usually estimated by extrapolation of single-species effect data. Statistical models are used to perform such extrapolations and are known as 'species sensitivity distributions' (SSDs). A set of assumptions is associated with both the underlying theory and the application of SSDs (Table I.2). These assumptions can be divided into (1) T-assumptions, i.e. related to the theory underlying the SSD methodology, and (2) P-assumptions, i.e. related to the way the SSD methodology is applied in practice (Forbes and Calow, 2002). Several authors have examined these assumptions experimentally (e.g., Duboudin *et al.,* 2004a; Hose and van den Brink, 2004, Versteeg *et al.*, 1999). However, it has been more common to investigate the implications of a violation of an assumption for water quality standard derivation (e.g., Duboudin *et al.*, 2004a; Forbes *et al.*, 2001; Hose and van den Brink, 2004; Maltby *et al.*, 2005) than to test the validity of the assumption itself (e.g. Newman *et al.*, 2000; Selck *et al.,* 2002). Also, most efforts are skewed towards the testing of 'P-assumptions' (e.g., Kefford *et al.,* 2005; Maltby *et al.,* 2005; Duboudin *et al.,* 2004a; Forbes *et al.,* 2001; Hose and van den Brink, 2004). Studies on assumptions related to the theoretical background of SSDs, i.e. 'T-assumptions' are scarce. Until now only assumption T3, i.e. that ecosystem structure is equally or more sensitive than ecosystem function, has been tested (Selck *et al.,* 2002; Balczon and Pratt, 1994).

In this chapter, the assumption T1 will be tested, i.e. that ecological interactions between species do not influence the parameters of the sensitivity distribution. A conventional SSD is based on single-species toxicity test results (hereafter termed 'cSSD') and considers species as isolated entities without taking into account possible ecological interactions between populations. If ecological interactions between species do not influence the sensitivity distribution (i.e. if T1 is valid), a sensitivity distribution that does take into account ecological interactions should be the same as the cSSD, i.e. parameters describing both distributions should be the same.

In the present study we constructed cSSDs for 1000 hypothetical toxicants. Each cSSD was based on single-species toxicity test results of phytoplankton, zooplankton, and fish. In parallel, sensitivity distributions taking into account ecological interactions between species, here termed "eco-species sensitivity distributions" (eco-SSDs) were constructed for the same 1000 toxicants. Eco-SSDs were based on no observed effect concentrations for the populations present in the ecosystem, as such taking into account ecological interactions. These population-NOECs were calculated by the ecosystem modelling approach which was validated in chapter VI. A

comparison between the parameters of eco-SSDs and cSSDs was performed to test assumption T1. Statistical analyses were used to examine the relationship between validity of T1 and toxicant type.

VII. 2. Materials and Methods

VII. 2. 1. Considered ecosystem

The ecosystem for which hypothesis T1 was tested is a simple lentic pelagic system consisting of a population of one fish species, three zooplankton species (two are slow growing, one is fast growing) and two phytoplankton species (one is slow growing, one is fast growing). Slow growing populations tend to bloom in summer, while fast growing populations primarily bloom in spring and fall (Sommer *et al.*, 1986).

VII. 2. 2. Ecosystem model

A mechanistic dynamic ecosystem model was constructed using the object oriented framework elaborated in chapter III. The ecosystem used in the present study included two phytoplankton objects (spring phytoplankton and summer phytoplankton), three zooplankton objects (rotifers: fast growing; large cladocerans: slow growing; large copepods: slow growing), and one planktivorous fish object. Ecological interactions within the planktonic part of the ecosystem model were defined following Sommer *et al.* (1986). These authors state that large-bodied zooplankton (most copepods and cladocerans) graze on both small and large phytoplankton, while small-bodied zooplankton can only ingest small phytoplankton. Planktivorous fish preferred large-bodied over small-bodied zooplankton as food source (Werner and Hall, 1974; Chang *et al.*, 2004). The resulting customized food web is shown in Fig VII.1.

The ecosystem model was calibrated to obtain a realistic succession of seasonal events for this type of system, as described in chapter IV and in Sommer *et al*. (1986). Parameter values resulting in population dynamics representing those events are given in the appendix (XI.6.10).The toxic effect sub-models embedded in the ecosystem model, consist of logistic concentration-effect functions describing the effects of the toxicants on the parameters of the ecosystem model. Modelling the dynamics of an exposed ecosystem is performed by adjusting these parameters according to the concentration-effect functions and the exposure concentration. Parameters in the ecosystem model that vary as a function of toxicant concentration are (1) the

mortality rate of zooplankton and fish, and (2) the photosynthesis rate of phytoplankton. An overview of the equations and the values assigned to their parameters is given in Table VII.1.

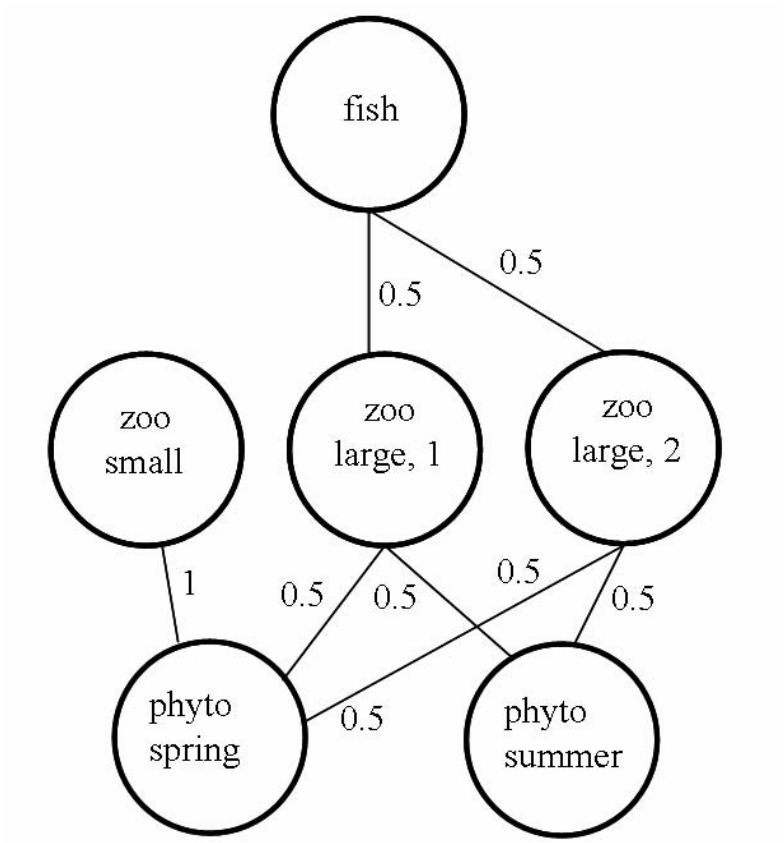

Figure VII.1: Food web diagram of the considered ecosystem. Nodes represent the populations present and lines represent trophic links between them. The preference of a population for feeding on a connected population is given by the preference factor alongside the connection. Zooplankton and phytoplankton are coded by "zoo" and "phyto". "Small" and "large" indicate dimensions of zooplankton organisms. "Spring" and "summer" indicate when the considered phytoplankton population blooms.

Table VII.1: Equations used in the toxic effect sub-models of the applied ecosystem model, with parameter names as in appendix XI.4 and in the list of abbreviations. time = duration of toxicity assay (d), set to two days for all zooplankton and fish. Values for LCR (6.1 for zooplankton and 9.5 for fish) were found in Lange *et al.* (1998). Values for slope (1.8 for all populations) were found in Smit *et al.* (2001). EC_{10} values were randomized (see methodology).

Phytoplankton: effect on photosynthesis	**Zooplankton and fish: effect on mortality**
$PS_{max} = \frac{PS_{max,0}}{1 + \left(\frac{tox}{EC_{50,PSmax}}\right)^{slope}}$	$Mort = \frac{1}{time} \cdot \ln\left(1 + \left(\frac{tox}{LC_{50}}\right)^{slope}\right)$
$EC_{50,PSmax} = \exp\left(\ln\left(EC_{10,PSmax}\right) - \frac{1}{slope} \cdot \ln\left(\frac{1}{9}\right)\right)$	$\frac{LC_{50}}{EC_{10}} = LCR$

VII. 2. 3. cSSD vs. eco-SSD for one hypothetical toxicant

Assume that for a toxicant tx_1, all chronic EC_{10}s of all possible aquatic species, are represented by a lognormal species sensitivity distribution SSD_1 with a mean μ_1 and a standard deviation σ_1:

$SSD_1 \sim (\mu_1, \sigma_1)$

As for any toxicant, the parameters of SSD_1 are not known, as it is impossible to subject each and every species to toxicity testing. Instead, these parameters have to be estimated experimentally by testing the sensitivity of only a small fraction of all possible species. It was thus assumed that for tx_1, chronic EC_{10}s had been experimentally derived for standard test species which are representative for the populations in the considered ecosystem. As too little is known about the sensitivity of standard test species relative to that of untested species, 6 EC_{10}s were sampled randomly from SSD_1. To estimate the parameters of the "true" SSD_1, a conventional species sensitivity distribution ($cSSD_1$) was fitted to this set of 6 EC_{10}s:

$cSSD_1 \sim (\hat{\mu}_1, \hat{\sigma}_1)$ with:

$E[\hat{\mu}_1] = \mu_1$

$E[\hat{\sigma}_1] = \sigma_1$

Next, the same 6 EC_{10}s were used in the toxic effect sub-models of the 6 populations in the ecosystem model (Table VII.1). With the as such parameterized ecosystem model, the dynamics of these populations at different exposure concentrations of tx_1 were predicted. Exposure concentrations ranged from the 1st to the 95th percentile of SSD_1. The exposure period was from late spring to late summer, i.e. comparable to many large-scale studies. To compare the biomass status of a population in the unexposed (control) situation with its status at the different exposure concentrations, relative differences (RDs) were calculated, as demonstrated in chapter IV. Because 20% is the minimum detectable difference for most population characteristics in the field (Suter II, 1993), RD-values of -0.2 or lower were considered as detectable decreases of biomass. Similarly, RD-values of 0.2 or higher were considered as detectable increases of biomass. In the context of ecological effect assessments, both increases and decreases of phytoplankton biomass were considered undesirable: the former because of an increased eutrophication risk, the latter because of a loss of primary production, a key process in pelagic aquatic ecosystems. For fish and zooplankton, biomass decreases were considered as undesirable. The no observed effect concentration (NOEC) of a population, hereafter termed 'population-NOEC', was defined as the

highest concentration at which no observable undesired effects was predicted for that population. Note that these population-NOECs were determined using an ecosystem model, as such incorporating ecological interactions in this NOEC. A cumulative plot of those 6 population-NOECs was defined as the eco-species sensitivity distribution for tx_1 (eco-SSD_1):

eco-SSD_1~ ($\mu_{1,eco}$, $\sigma_{1,eco}$)

Using these definitions, the hypothesis T1 was rephrased as:

$$\mu_1 = \mu_{1,eco} \wedge \sigma_1 = \sigma_{1,eco}$$

Consequently, the validity of T1 was tested for tx_1 using two-sided t and F-tests and a p-level of 0.05.

VII. 2. 4. Extension to 1000 hypothetical toxicants

The methodology described in the previous paragraph was followed for toxicants tx_i from tx_1 to tx_{1000}. SSD_1 to SSD_{1000} differed in mean but had the same standard deviation ($\sigma_1 = \sigma_2 = \ldots = \sigma_i = \ldots = \sigma_{1000} = 1$). A standard deviation of one order of magnitude is representative for SSDs of many chemicals (e.g. examples in Duboudin *et al.*, 2004b). The means of the 1000 toxicants were sampled from a lognormal distribution with mean -0.43 and standard deviation 0.92. These variability settings were calculated from Gonzalez-Doncel *et al.* (2006) from means and standard deviations of NOEC values of fish (n = 343), crustaceans (n = 414), and algae (n = 186) for all toxicants included in different toxicity databases.

VII. 2. 5. Comparing 'safe concentrations' derived from cSSD with ecosystem-NOECs derived from the ecosystem model.

We tested if 'safe concentrations' derived from a cSSD, i.e. not accounting for ecological interactions, were different from their corresponding ecosystem-NOECs, i.e. accounting for ecological interactions.

A predicted no effect concentration (PNEC) based on the cSSD was established by means of two frequently used methods: (1) using the lowest of the 6 chronic single-species EC_{10}s (which

represent three trophic levels), divided by an application factor of 10 (AF-PNEC). and (2) the left side 50% confidence limit of the hazardous concentration for five percent of the species (HC_5-PNEC), as in Wagner and Lokke (1991). Note that the AF-PNEC was derived based on EC_{10} data, in absence of single-species NOEC data, as proposed by the TGD (EU, 2003). The ecosystem-NOEC was defined as the lowest population-NOEC in the eco-SSD: when exposed to this ecosystem-NOEC, no population will experience an observable biomass decrease, according to ecosystem model predictions.

VII. 2. 6. Relationship between toxicant type and validity of T1

Here, we examined whether the validity of T1 is related to the type of toxicant. Toxicant type was arbitrarily defined here on the basis of the relative sensitivity of the considered species for the toxicant. In this context, the relative sensitivity is defined by the following two quantities:

$r_{PZ} = \log (EC_{10,phytoplankton}) - \log (EC_{10,zooplankton})$
$r_{ZF} = \log (EC_{10,zooplankton}) - \log(EC_{10,fish})$

with $\log(EC_{10,phytoplankton})$ and $\log(EC_{10,zooplankton})$ equal to the logarithm of the geometric mean of the EC_{10} values of the two phytoplankton and three zooplankton species, respectively. These quantities are an indication of which species are directly targeted by the toxicant. For example, a toxicant with a value of -2 for r_{PZ} ($EC_{10,phytoplankton}$ is two orders of magnitude smaller than $EC_{10,zooplankton}$) directly targets phytoplankton; e.g. a herbicide. We examined if the validity or violation of T1 was related to toxicant type, i.e. to r_{PZ} and r_{ZF}. This was performed using two associated statistical approaches: discriminant analysis and classification trees.
A stepwise discriminant function analyses (Jennrich, 1977) was used to determine which variable (r_{PZ} and r_{ZF}) discriminates best between two or more naturally occurring groups. In a first analysis, these variables were r_{PZ} and r_{ZF} and the two groups were toxicants tx_i for which the means of cSSD and eco-SSD are equal ($\hat{\mu}_i = \mu_{eco,i}$; group 0) and those for which the means of cSSD and eco-SSD differ ($\hat{\mu}_i \neq \mu_{eco,i}$; group 1). In a second analysis, the variables were again r_{PZ} and r_{ZF}, but now, the two groups were toxicants tx_i for which the standard deviations of cSSD and eco-SSD are equal ($\hat{\sigma}_i = \sigma_{i,eco}$; group 2) and those for which the standard deviations of cSSD and eco-SSD differ ($\hat{\sigma}_i \neq \sigma_{i,eco}$; group 3). Table VII.2 lists these groups. Partial lambda values were calculated for r_{PZ} and r_{ZF} to indicate the discriminating power of these two variables. A partial

lambda value of 0 indicates a perfect discriminative power, and 1 indicates no discriminative power at all.

Next, a classification tree based on r_{PZ} and r_{ZF} was build in order to classify toxicants into groups 0 and 1, using the CART-style (Breiman *et al.,* 1984) exhaustive search for univariate splits (Statsoft, Tulsa OK). The same was done to classify toxicants into groups 2 and 3. These trees were constructed using two-third of the 1000 toxicants (training-set). Split conditions were calibrated to maximize the amount of correctly classified training-set toxicants. Afterwards, the remaining one-third (test-set) of the 1000 toxicants, i.e. not used in the tree development, was used as a cross validation of these split conditions. The results of this cross-validation reflect the predictive capacity of the constructed trees. Note that the ratio of group 0 toxicants vs. group 1 toxicants was equal between training-set and test-set, as demanded by the classification tree-methodology. This was also the case for the ratio of group 2 toxicants vs. group 3 toxicants. Prior probabilities were estimated from the simulated data, and misclassification costs were equal for all classes. As goodness-of-fit, a Gini measure was selected, as proposed by Breiman *et al,* (1984). The stopping rule was set as 'pruning on misclassification error' with parameters 'standard error' and 'n' equal to 1 and 5, respectively.

Table VII.2: The characteristics of the four groups used for statistical analysis of toxicant type vs. validity of T1.

group	characteristic
0	$\hat{\mu}_i = \mu_{eco}$
1	$\hat{\mu}_i \neq \mu_{eco}$
2	$\hat{\sigma}_i = \sigma_{eco}$
3	$\hat{\sigma}_i \neq \sigma_{eco}$

VII. 3. Results and Discussion

VII. 3. 1. Mean and variance of cSSD and eco-SSD

For 254 of the 1000 toxicants, the mean and/or variance of the eco-SSD were significantly different from those of the corresponding cSSD. In 190 cases, the mean of the cSSD was significantly different from that of the eco-SSD. In 94 cases, the variance of the cSSD was found to be significantly different from that of the eco-SSD. In 30 cases, both mean and variance of the cSSD were found to be significantly different from those of the eco-SSD. In an *a posteriori* re-analysis, one-sided t and F-testing revealed that all significant differences indicated higher means

and standard deviations for cSSD than for eco-SSD, i.e.: $\hat{\mu}_i > \mu_{eco,i}$ and $\hat{\sigma}_i > \sigma_{i,eco}$. Therefore, in the results of the discriminant analysis and decision tree approach, groups 0 and 1 were redefined as toxicants for which $\hat{\mu}_i = \mu_{eco,i}$ and those for which $\hat{\mu}_i > \mu_{eco,i}$, respectively. Similarly, groups 2 and 3 were redefined as toxicants for which $\hat{\sigma}_i = \sigma_{i,eco}$ and those for which $\hat{\sigma}_i > \sigma_{i,eco}$, respectively. The difference between $\hat{\mu}_i$ and $\mu_{eco,i}$ was on average 0.6 log-units and the difference between $\hat{\sigma}_i$ and $\sigma_{i,eco}$ was on average a factor of 3. Power analysis (Statistica software, Statsoft, Tulsa, Ok) of the t and F-tests with $\alpha = 0.05$ and N=6 revealed that the statistical power of detecting such differences was about 0.8.

The reason that for none of the 1000 toxicants $\hat{\sigma}_i$ was found to be lower than $\sigma_{i,eco}$ has to be sought in the inclusion of ecological interactions in the eco-SSD. Indirect effects caused by ecological interactions make the sensitivity of the considered populations interdependent. Fleeger *et al.* (2003) cite 47 experimental large-scale studies in which effects on one or more pelagic populations indirectly affect other pelagic populations, and hence make sensitivities of the species present interdependent. For example, Hamilton *et al.* (1988) found that a reduction of the abundance of phytoplankton species by the herbicide atrazine resulted in a parallel decrease of ecologically related zooplankton species, although the latter were not directly affected by the toxicant at the tested concentrations. Van Donk *et al.* (1995) noticed an increase of phytoplankton because of reduced zooplankton grazing pressure after application of the insecticide chlorpyrifos. In the context of the present study, this should be interpreted as follows: in a cSSD for "a herbicide", the EC_{10}s of zooplankton species are located in the higher percentiles, as those are not directly targeted by the toxicant. In contrast, in an eco-SSD for a herbicide, the population-NOEC of the zooplankton is located close to the population-NOECs of their food (phytoplankton). The same reasoning can be followed in the case of an insecticide, where ecological interactions will bring the population-NOEC of phytoplankton populations close to the population-NOECs of the ecologically related zooplankton populations. These shifts in sensitivity explain the lower variance of eco-SSDs compared to the cSSDs.

VII. 3. 2. Comparing 'safe concentrations' derived from cSSD with ecosystem-NOECs derived from the ecosystem model

PNECs derived with an application factor (AF-PNECs) were, on average, 10 times lower than the corresponding ecosystem-NOECs. For 769 of the 1000 considered toxicants, HC_5-PNECs were found to be, on average, a factor 3 lower than the corresponding ecosystem-NOECs. For 95 of the

190 toxicants for which only the $\hat{\mu}_i > \mu_{eco,i}$, the HC_5 was larger than the ecosystem-NOEC. For all toxicants for which only $\hat{\sigma}_i > \sigma_{i,eco}$, the HC_5 was found to be smaller than the ecosystem-NOEC. For 28 of the 30 toxicants for which both $\hat{\mu}_i > \mu_{eco,i}$ and $\hat{\sigma}_i > \sigma_{i,eco}$, the HC_5 was larger than the ecosystem-NOEC.

In a comparison of HC_5s derived from SSDs with experimentally derived ecosystem-NOECs, Versteeg *et al.* (1999) found the former to be consistently lower than the latter, a finding which is also observed by Hose *et al.* (2003) and Selck *et al.* (2002). However, in a comparison of HC_5s with ecosystem-NOECs for 6 insecticides, Maltby *et al.* (2005) found the latter to be lower than the former for continuous exposure to lindane and fenvalerate.

In summary, literature indicates that, although cases exist in which the HC_5 is higher than an experimentally derived ecosystem-NOEC, these cases are scarce. The probability that this will occur is probably lower than what the results in this chapter suggest. A reason for this might be that in the cited studies, cSSDs were constructed using more species than those present in the experimental ecosystem study. For example, Selck *et al.* (2005) included single-species fish EC_xs to construct cSSDs for LAS and TBT. A subsequent comparison with NOEC data obtained in ecosystem-level studies without fish revealed a highly protective HC_5s. For that reason, Posthuma *et al.* (2002) have suggested to carefully consider the composition of the ecosystem to be protected when constructing a cSSD. In our work, EC_{10}s in the cSSD were assumed to be representative for the sensitivity of the species in the considered ecosystem model. As such, it was possible to test T1, and exclude possible effects of species composition of the cSSD.

VII. 3. 3. For which toxicants is T1 valid?- discriminant analysis approach

When r_{PZ} and r_{ZF} values of the 1000 considered toxicants are plotted (Fig VII.2), it appears that the power to discriminate between group 0 toxicants (i.e. for which $\hat{\mu}_i = \mu_{eco,i}$) and group 1 toxicants (i.e. for which $\hat{\mu}_i > \mu_{eco,i}$) is larger for r_{PZ} than for r_{ZF}. Group 1 toxicants are primarily located left from $r_{PZ} = 0$, while group 0 toxicants are located slightly more to the right of $r_{PZ} = 0$. Indeed, the partial lambda values of r_{ZF} (0.89) and r_{PZ} (0.68 (< 0.89)) indicate that r_{PZ} has more power to discriminate between both groups of toxicants than r_{ZF}. This means that one can *a priori* classify a toxicant in group 0 or 1, based on the r_{PZ} value of that toxicant. Since the r_{PZ} value is simply log ($EC_{10,phytoplankton}$) - log ($EC_{10,zooplankton}$), two toxicity test results ($EC_{10,phytoplankton}$ and $EC_{10,zooplankton}$) are sufficient to classify a toxicant in group 0 or group 1.

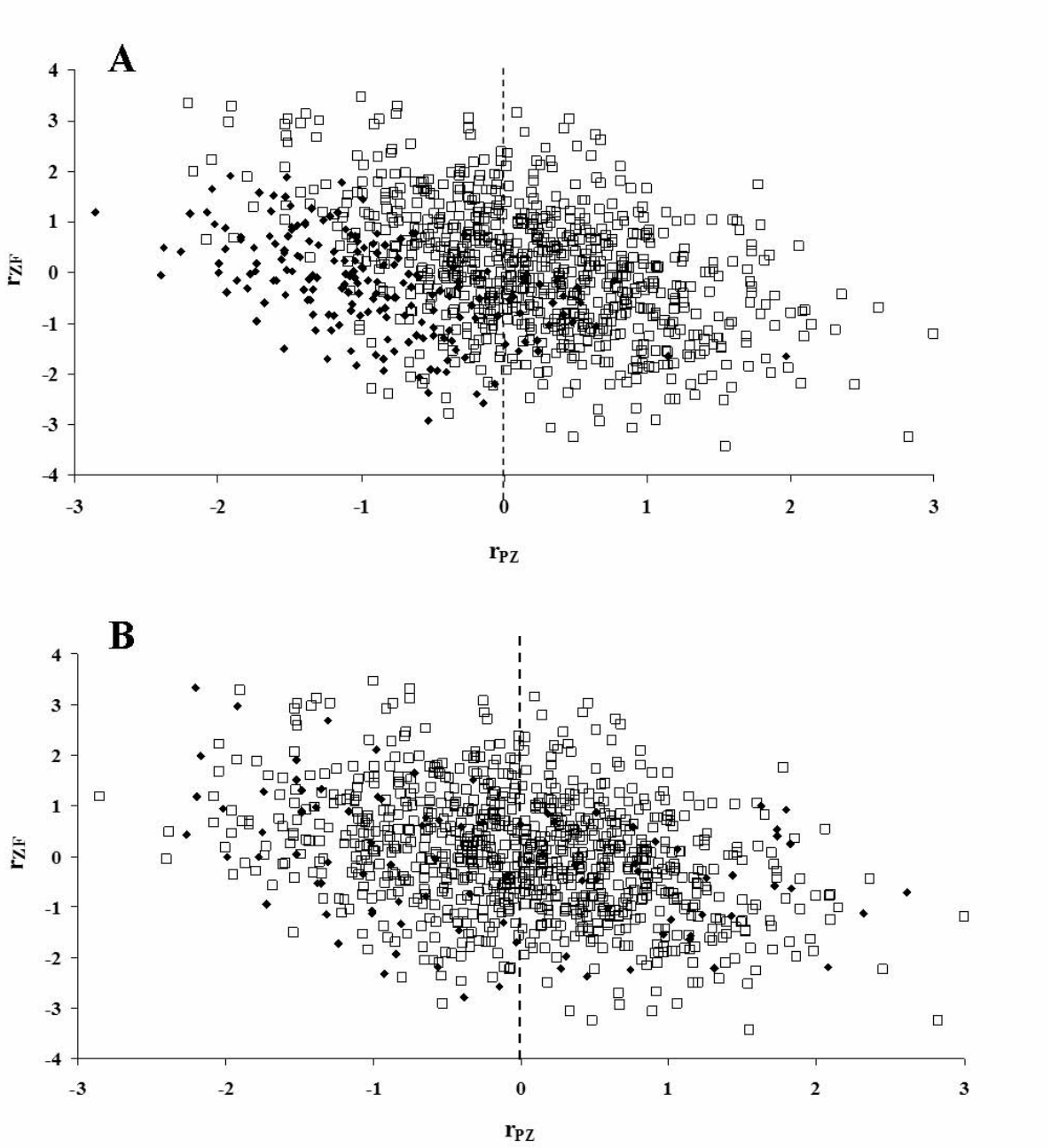

Figure VII.2: A: Scatterplot of the 1000 considered toxicants based on their r_{PZ} and r_{ZF} value. A black diamond indicates that $\hat{\mu}_i > \mu_{eco,i}$ for that toxicant. A white square indicates that $\hat{\mu}_i = \mu_{eco,i}$ for that toxicant. B: Scatterplot of the 1000 considered toxicants as a function of their r_{PZ} and r_{ZF} value. A black diamond indicates that $\hat{\sigma}_i > \sigma_{eco,i}$ for that toxicant. A white square indicates that $\hat{\sigma}_i = \sigma_{eco,i}$ for that toxicant. A dashed line indicates $r_{PZ} = 0$ in both plots.

In contrast, r_{PZ} and r_{ZF} have no power at all to discriminate between group 2 (i.e. for which $\hat{\sigma}_i = \sigma_{i,eco}$) and group 3 toxicants (i.e. for which $\hat{\sigma}_i > \sigma_{i,eco}$), as reflected by partial lambda values of 0.99 (≈ 1) for both r_{PZ} and r_{ZF}.

VII. 3. 4. For which toxicants is T1 valid?- classification tree approach

The classifying capacity of r_{PZ} and r_{ZF} using classification trees is reflected by the number of correctly classified toxicants within the training-set (Fig VII.3). The comparison of correctly and erroneously classified training-set toxicants within an end node, reflects the probability of a correct classification. As such, toxicants classified in end nodes marked with an asterisk (Fig VII.3), have a probability of ≥ 90% of being classified correctly. The importance of r_{PZ} in distinguishing group 0 from group 1 toxicants, as suggested by the discriminant analysis, is confirmed by this classification tree, where r_{PZ} determines the first split, and hence has the most influence on the resulting classification of a toxicant.

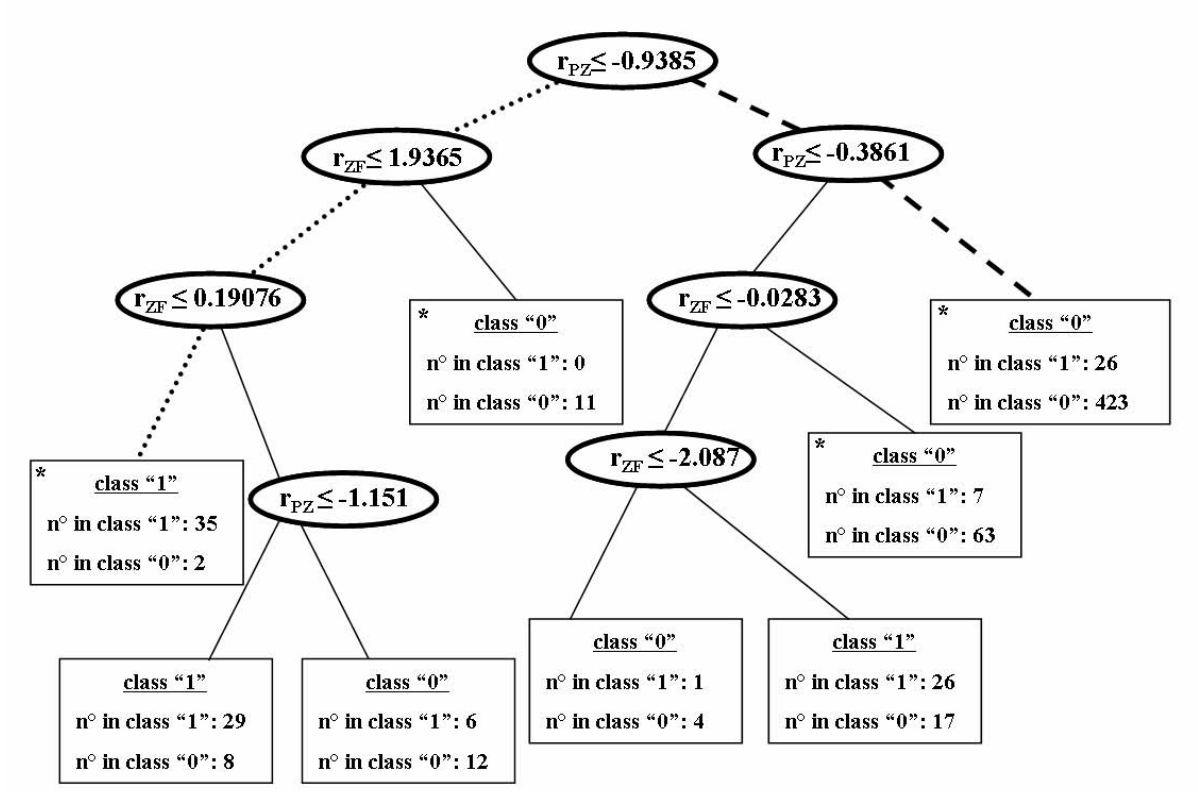

Figure VII. 3: Classification tree predicting if μ_i > $\mu_{eco,i}$ (coded class "1") or if μ_i = $\mu_{eco,i}$ (coded class "0") based on combinations of r_{PZ} and r_{ZF}. If a split condition (ellips) is fulfilled, this results in a continuation to the left branch. The tree is followed until an end node is reached (box). This end node gives the resulting classification (underlined). The number of training-set toxicants which were correctly and erroneously classified using these split conditions are also given in these end nodes. Toxicants classified in end nodes marked with an asterisk, have a probability of ≥ 90% of being classified correctly. The dashed line indicates the pathway for a toxicant for which r_{PZ} = 2. The dotted line indicates the pathway for a toxicant for which r_{PZ} = -2 and r_{ZF} = 0 .

The subsequent cross-validation of this classification tree indicates that the tree also has some predictive power for the test-set toxicants. Within the test-set, 63% of the group 1 toxicants were classified correctly by the tree. Also, 93 % of the group 0 toxicants within the test-set were

classified correctly by the tree. Note that these test-set toxicants were not used in the construction of the classification tree.

Van den Brink *et al*. (2006) found that the HC_{50}s of chronic invertebrate-SSDs for herbicides are on average two orders of magnitude higher than those of chronic phytoplankton-SSDs for herbicides, i.e. that $r_{PZ} = -2$ for many herbicides. In the same study, the difference between invertebrate and fish-HC_{50}s was found to be < 1 order of magnitude, i.e. corresponding to $r_{ZF} = 0$. Hence, it can be safely hypothesised that toxicants primarily targeting phytoplankton have $r_{PZ} = -2$ (EC_{10}s of zooplankton are two orders of magnitude higher than those of phytoplankton), and $r_{ZF} = 0$ (EC_{10}s of zooplankton and fish are equal). From the classification tree, it becomes apparent that those toxicants may have $\hat{\mu}_i > \mu_{eco,i}$, as indicated by the dotted line in Fig VII.3. Thus, for these toxicants, T1 is not valid. The mean ecosystem sensitivity for herbicides, given by $\mu_{eco,i}$, may not be reflected by the mean of the cSSD. Hence, the applications of the cSSD approach for herbicides may lead to inaccuracies caused by differences in distribution parameters. Conversely, toxicants primarily targeting zooplankton and fish (e.g. $r_{PZ} = 2$ and $r_{ZF} = 0$), would have $\hat{\mu}_i = \mu_{eco,i}$, as indicated by the dashed line in Fig VII.3. This suggests that, for these toxicants, the mean of eco-SSD and cSSD are comparable. An explanation for the different results obtained for both toxicant types may be found in the number of populations experiencing food web-mediated indirect effects. Toxicants primarily targeting phytoplankton, can give rise to a reduction of zooplankton resulting from a decrease in available phytoplankton biomass. A reduction in fish biomass can be observed as a second-order indirect effect. Because a cSSD approach would categorize the phytoplankton as the trophic level being mostly affected by the toxicant, it ignores possible (indirect) effects on two trophic levels. Conversely, in case of toxicants targeting zooplankton and fish, a cSSD approach categorizes both zooplankton and fish as being affected, thereby only ignoring possible (indirect) effects on one trophic level, i.e. on phytoplankton. These considerations seem to justify earlier suggestions to only incorporate organisms from sensitive trophic levels in the cSSD (e.g. Posthuma *et al.*, 2002). However, while these earlier suggestions have mainly been based on statistical considerations (i.e. the violation of the assumption of (log)normality of SSDs that include both sensitive and insensitive species), our present simulation study seems to justify these suggestions from an ecological point of view. Indeed, incorporating species in an SSD which are not directly targeted by the toxicant (e.g. zooplankton in the case of herbicides), reflects the erroneous idea that those species are also not affected in an ecosystem context. Consequently, the mean of such a cSSD will be higher than a cSSD only consisting of sensitive species. Schmitt-Jansen and Altenburger (2005) have shown that the mean of a cSSD for

a herbicide containing only phytoplankton species (i.e. sensitive for the herbicide) agreed well with the mean sensitivity of those species within an ecosystem.

A similar classification tree approach for σ did not result in any classifying nor predictive power, because of the limited fraction of toxicants in group 3. The difference between standard deviations of cSSD and eco-SSD does not necessarily make the eco-SSD more conservative than the cSSD. The lower percentiles of the cSSD will still be lower than the lower percentiles of the eco-SSD (Fig VII.4B). In contrast, the opposite may hold when the mean of the eco-SSD is lower than the mean of the cSSD (Fig VII.4A). However, this will depend on the chosen percentile of a cSSD (i.e. what "y" is in "HC_y") to derive a PNEC. When both mean and standard deviation are lower for eco-SSD than for cSSD (Fig 4C), it is difficult to *a priori* predict how this will influence the protective capacity of a cSSD. Yet, the different locations of cSSD and eco-SSD, as indicated by the difference between $\hat{\mu}_i$ and $\mu_{i,eco}$ should primarily be regarded as an indication of the violation of T1 for a substantial amount (25%) of toxicants. Although the possible implications of this violation for the protective capacity of a cSSD give valuable insights, underlying assumptions of the SSD approach are many (Table I.2). Hence, the protective capacity of this approach will depend on the validity of all of these assumptions, and not only on the validity of the assumption examined here.

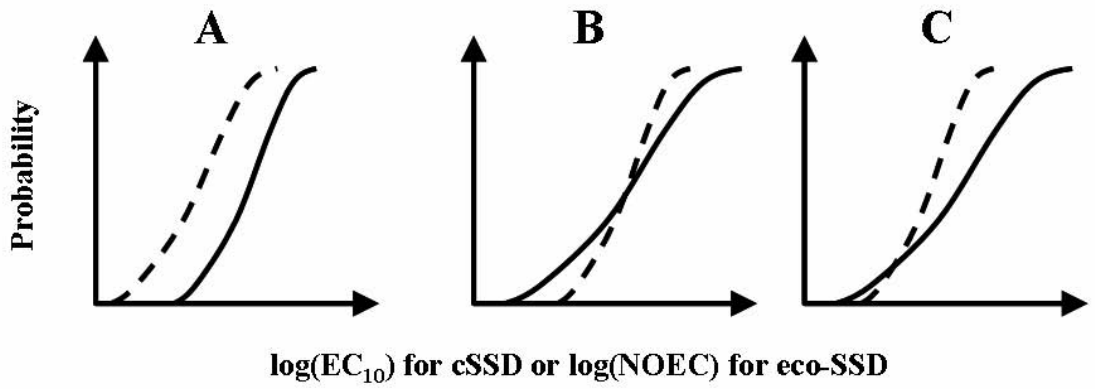

Figure VII.4: Visualisation of possible differences between parameters of cSSD (bold line) and eco-SSD (dashed line): A: $\hat{\mu}_i > \mu_{eco,i}$; B: $\hat{\sigma}_i > \sigma_{eco,i}$; C: $\hat{\mu}_i > \mu_{eco,i} \wedge \hat{\sigma}_i > \sigma_{eco,i}$.

VII. 4. Conclusions

For 254 of the 1000 toxicants, the mean and/or variance of the eco-SSD were significantly lower than those of the corresponding cSSD. In 190 cases, the mean of the cSSD was significantly lower than that of the eco-SSD. These 190 cases predominantly represented toxicants directly targeting phytoplankton (i.e. $EC_{10,fish} \approx EC_{10,zooplankton} >> EC_{10,phytoplankton}$). In contrast, for toxicants directly targeting zooplankton and fish ($EC_{10,fish} \approx EC_{10,zooplankton} << EC_{10,phytoplankton}$) the mean of the eco-SSD tends to be equal to that of a cSSD. In 94 cases, the variance of the eco-SSD was found to be significantly lower than that of the cSSD. In 30 cases, both mean and variance of the eco-SSD were found to be significantly lower than those of the cSSD. Hence, it can be concluded that, depending on toxicant type, sensitivity distributions may have a lower mean when ecological interactions are accounted for than when they are constructed in a conventional way, i.e. without ecological interactions. The tested assumption T1 is thus not valid for all toxicants.

Chapter VIII

Is ecosystem structure the target of concern in ecological effect assessments?

Chapter VIII

Is ecosystem structure the target of concern in ecological effect assessments? An examination of a theoretical assumption underlying species sensitivity distributions.

Abstract - Species sensitivity distributions are statistical distributions used to derive environmentally "safe" concentrations of chemicals. Associated with the underlying theory and the practical application are a set of inadequately tested assumptions. One of these assumptions is that ecosystem structure is as or more sensitive than ecosystem function, i.e. that structure is the target of concern. In this chapter, we tested this assumption for a simple freshwater ecosystem exposed to different toxicants. Using an ecosystem model we calculated no observed effect concentrations for ecosystem structure (ecosystem structure-NOECs) and function (ecosystem function-NOECs) for each of 1000 hypothetical toxicants. For 979 of these toxicants, the ecosystem structure-NOEC was lower than or equal to the ecosystem function-NOEC, indicating that the tested assumption can be considered valid. For 239 of these 979 toxicants, both NOECs were equal. For half of the 1000 toxicants, structure of lower trophic levels (i.e. phytoplankton) appears to be more sensitive than structure of higher trophic levels (i.e. fish). As such, ecosystem structure-NOECs are primarily determined by the sensitivity of the structure of lower trophic levels. In contrast, ecosystem functions associated with higher trophic levels (e.g., total ingestion by fish) are more sensitive than functions associated with lower trophic levels (e.g., total photosynthesis by phytoplankton) for 749 toxicants. Top-down regulation of ecosystem structure and cascading of effects from lower trophic level ecosystem functions to higher trophic level ecosystem functions are suggested as possible explanations for these two contrasting findings.

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De Laender F., De Schamphelaere, K.A.C., Vanrolleghem, P.A., Janssen, C.R. Is ecosystem structure the target of concern in ecological effect assessments? An examination of a theoretical assumption underlying species sensitivity distributions. Environmental Science and Technology, submitted.

VIII. 1. Introduction

In ecological effect assessments, higher-level effects are usually estimated by extrapolation of single-species toxicity test results. If sufficient single-species toxicity test results are available, statistical models, termed 'species sensitivity distributions' (SSDs) are used to perform this extrapolation. A set of assumptions is associated with both the underlying theory ('T-assumptions') and the application of SSDs ('P-assumptions') (Table I.2), as discussed in detail by Forbes and Calow (2002). Several authors have examined these assumptions experimentally (e.g., Duboudin *et al.,* 2004a; Hose and van den Brink, 2004, Versteeg *et al.*, 1999). However, these efforts have been focused on testing the P-assumptions (e.g., Keffort *et al.,* 2005; Maltby *et al.,* 2005; Duboudin *et al.,* 2004a; Forbes *et al.,* 2001; Hose and van den Brink 2004), rather than on testing the T-assumptions (Selck *et al.,* 2002; Balczon and Pratt, 1994). Yet, the underlying theory is of fundamental importance for the SSD concept. While the way in which SSDs are applied can be customized according to the specific effect assessment, the underlying T-assumptions cannot as they are an inherent part of the SSD concept. Indeed, proper application of a methodology may still result in incorrect evaluation of ecological effects if the theory underlying the methodology is invalid.

Crucial to the endurance of ecosystems is the maintenance of ecosystem functions, as reflected by the stability concept (e.g. Steiner *et al.,* 2005). Ecological stability is referred to as the ability of a community to (1) maintain ecosystem functions (resistance) when exposed to a stressor, and (2) recover to control levels of functioning after disappearance of the stressor (resilience) (Mac Gillivray *et al.*, 1995). As such, an effect on ecosystem functions may indicate a loss of stability, possibly threatening ecosystem endurance.

Although ecosystem function is generally considered less sensitive than ecosystem structure, theoretical ecology indicates that the opposite may also hold. On the one hand, ecosystem functions may be less sensitive than ecosystem structure because species performing an ecosystem function may be replaced by less sensitive species capable of maintaining the same function (i.e., functional redundancy) (Pratt and Cairns, 1996; van Leeuwen *et al.*, 1996). This was experimentally confirmed by Selck *et al.* (2002) for tributyltin (TBT) and linear alkylbenzene sulfonates (LAS). On the other hand, environmental contamination may act as a selective force against populations of sensitive species, resulting in the loss of these species and possible cascading effects on ecosystem function (Lawler *et al*., 2002). Although necessary (Chapman *et al.*, 2003), an examination using a general hypothesis testing framework has not been performed.

In this chapter, the assumption that ecosystem structure is less sensitive than ecosystem function (T3, Table I.2) was tested in a simple freshwater ecosystem exposed to different toxicants. ‘‘Ecosystem function’’ is defined *sensu* Duffy (2002) and Schlapfer and Schmid (1999), i.e. transfers of energy, quantified by biomass. Examples are total primary production, secondary production, aggregate consumption, community respiration, and nutrient uptake. In this chapter, we studied the sensitivity of the photosynthesis of phytoplankton, the ingestion by zooplankton, and the ingestion by fish. An ecosystem model was used to predict the no observed effect concentrations (NOECs) for those three functions in an ecosystem exposed to 1000 hypothetical toxicants. With the same model, also NOECs for changes in ecosystem structure, expressed as biomass, were calculated. This allowed to compare ecosystem function-NOECs with corresponding ecosystem structure-NOECs for each of the 1000 considered toxicants.

VIII. 2. Materials and Methods

VIII. 2. 1. Ecosystem type

The ecosystem for which hypothesis T3 was tested, is the same as the one for which hypothesis T1 was tested, i.e. a lentic pelagic freshwater system, consisting of populations of one fish species, three zooplankton species and two phytoplankton species, as shown in Fig VII.1. The ecosystem functions studied were total photosynthesis of phytoplankton ($PS_{phyto,tot}$; mg L^{-1} d^{-1}), total ingestion by zooplankton ($I_{zoo,tot}$; mg L^{-1} d^{-1}) and ingestion by the one fish population (I_{fish}; mg L^{-1} d^{-1}):

$$PS_{phyto,tot} = PS_{phyto,summer} + PS_{phyto,spring} \quad \text{(eqs 1)}$$
$$I_{zoo,tot} = I_{zoo,large} + I_{zoo,small}$$

with “large” and “small” indicating large, slow-growing and small, fast-growing populations, respectively, and “summer” and “spring” indicating populations blooming in summer and spring, respectively.

The choice to express ecosystem functions as fluxes of biomass was made because these are intuitively sensible, practical measures of energy assimilation (Johnson *et al*., 1996). Also, field studies tend to use some measure of biomass fluxes as the ecosystem function response variable (Johnson *et al.*, 1996).

VIII. 2. 2. Ecosystem model

The ecosystem model used in this chapter, and its embedded toxic effect sub-models are identical to those used in chapter VII. Again, the ecosystem model was calibrated to obtain a succession of seasonal events for this type of system, as described in chapter IV and in Sommer *et al.* (1986).

VIII. 2. 3. Structural vs. functional sensitivity for one hypothetical toxicant

Assume that for a toxicant tx_1, all chronic single-species EC_{10}s of all possible aquatic species, are represented by a lognormal species sensitivity distribution SSD_1:

$SSD_1 \sim (\mu_1, \sigma_1)$

From SSD_1, six EC_{10}s were randomly sampled to represent the single-species sensitivity of the 6 considered populations. These 6 EC_{10}s were used in the toxic effect sub-models of the 6 populations in the ecosystem model. This allowed for the simulation of the dynamics of these populations at different exposure concentrations of tx_1. Exposure concentrations ranged from the 1^{st} to the 95^{th} percentile range of SSD_1. The exposure period was taken from late spring to late summer, which is comparable to many large-scale studies.

Changes in ecosystem structure were quantified by changes in biomass status of the populations. To compare the biomass status of a population in the unexposed (control) situation with its status at the different exposure concentrations, relative differences (RDs) were calculated, as in chapter IV. Again, a 20% cut-off value was used as the minimum detectable difference (Suter II, 1993). RD-values of -0.2 or lower were considered as detectable decreases of biomass. Similarly, RD-values of 0.2 or higher were considered as detectable increases of biomass. In the context of ecological effect assessments, both increases and decreases of phytoplankton biomass were considered undesirable. For fish and zooplankton, biomass decreases were considered as undesirable. The NOEC of a population, hereafter termed 'population-NOEC', was defined as the highest concentration at which no observable undesired effect was predicted for that population. The NOEC of the ecosystem structure, hereafter termed 'ecosystem structure-NOEC', was defined as the lowest population-NOECs. Note that the ecosystem structure-NOEC bears exactly the same meaning as 'ecosystem-NOEC' in the previous chapters.

Similarly, the rate of an ecosystem function "f" in the unexposed (control) situation was compared with its rate at the different exposure concentrations by calculating relative differences.

Also for these ecosystem functions, RD-values of -0.2 or lower were considered as detectable decreases of ecosystem function rate. The highest concentration at which no detectable decrease of 20% or more on a considered ecosystem function occurred was defined as the ecosystem function-NOEC, allowing to rephrase hypothesis T3 as:

ecosystem structure-NOEC ≤ ecosystem function-NOEC

VIII. 2. 4. Extension to 1000 hypothetical toxicants

The methodology described in the previous paragraph was followed for toxicants tx_1 to tx_{1000}. SSD_1 to SSD_{1000} differed in mean but, for reasons of feasibility, had the same default standard deviation ($\sigma_1 = \sigma_2 = \ldots = \sigma_{1000} = 1$). A standard deviation of one order of magnitude is representative for SSDs of many chemicals (e.g. examples in Duboudin *et al.*, 2004b). The means of the 1000 toxicants were sampled from a lognormal distribution with mean -0.43 and standard deviation 0.92. These variability settings were calculated from Gonzalez-Doncel *et al.* (2006) from means and standard deviations of NOEC values of fish (n = 343), crustaceans (n = 414), and algae (n = 186) for all toxicants included in different toxicity databases.
In the next phase, we examined whether the type of toxicant could predict if ecosystem structure-NOEC was smaller than, or equal to the ecosystem function-NOEC. Toxicant type was arbitrarily defined here on the basis of relative sensitivities of the considered species to the toxicant. Relative sensitivities were defined by the following two quantities:

$$r_{PZ} = \log (EC_{10,phytoplankton}) - \log (EC_{10,zooplankton}) \quad \text{(eqs 2)}$$
$$r_{ZF} = \log (EC_{10,zooplankton}) - \log(EC_{10,fish})$$

with $\log(EC_{10,phytoplankton})$ and $\log(EC_{10,zooplankton})$ equal to the logarithm of the geometric mean of the EC_{10} values of the two phytoplankton and three zooplankton species, respectively (chapter VII). A stepwize discriminant function analyses (Jennrich, 1977) was used to determine which variable (r_{PZ} and r_{ZF}) discriminates best between toxicants for which *ecosystem structure-NOEC* ≤ *ecosystem function-NOEC* and those for which *ecosystem structure-NOEC* > *ecosystem function-NOEC*. Partial lambda values were calculated for r_{PZ} and r_{ZF}, with a value of 0 indicating a perfect discriminative power, and 1 no discriminative power at all.

VIII. 3. Results and Discussion

VIII. 3. 1. Structural vs. functional sensitivity for hypothetical toxicants

For 979 of the 1000 toxicants, the ecosystem structure-NOEC was lower than or equal to the corresponding ecosystem function-NOEC (Fig VIII.1). As such, the tested assumption T3 appears to hold for the functions studied in this simple ecosystem. However, among these 979 toxicants, 239 had an ecosystem structure-NOEC equal to the corresponding ecosystem function-NOEC. Thus, for the latter toxicants a protection of structure is not necessarily a more conservative approach for the protection of ecosystem functions, but rather an accurate one. Based on the relationship of ecosystem resistance and resilience with ecosystem functions (Mac Gillivray *et al.*, 1995), protection of structure seems crucial when this ecosystem is exposed to these 239 toxicants. Unfortunately, toxicant type could hardly distinguish toxicants for which ecosystem function-NOEC equals ecosystem structure-NOEC. A discriminant analysis showed limited power for r_{ZF}, as indicated by a partial lambda value of 0.86. The partial lambda value of r_{PZ} was 1, indicating no discriminative power at all for this variable. As such, determining *a priori* if ecosystem structure and function NOEC are equal, based on toxicant type alone, was not possible.

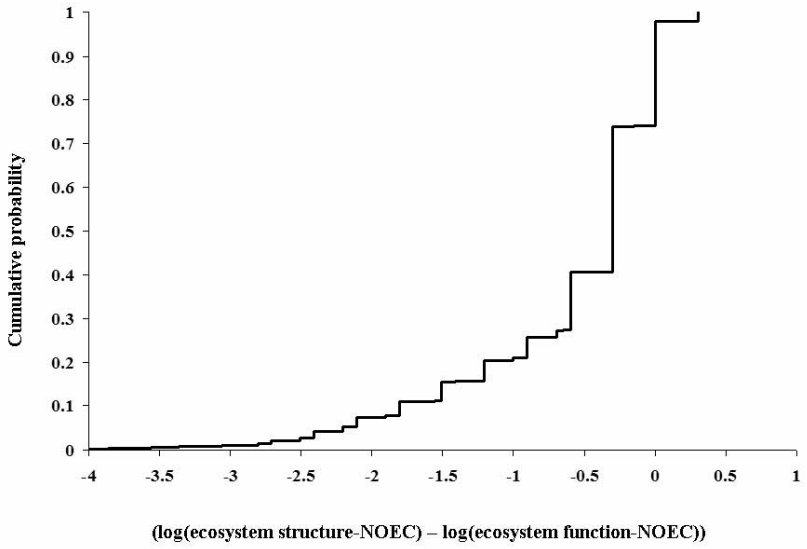

Figure VIII.1: Cumulative probability distribution of the difference (log(ecosystem structure-NOEC) – log(ecosystem function-NOEC)). Negative values indicate toxicants for which the ecosystem structure-NOEC was lower than the ecosystem function-NOEC. Values equal to "0" indicate toxicants for which the ecosystem structure-NOEC was equal to ecosystem function-NOEC.

VIII. 3. 2. Which populations determine the ecosystem structure-NOEC?

For 467 of the 1000 toxicants, the most sensitive population, i.e. the one with the lowest population-NOEC, was a phytoplankton population. For 216 toxicants, this was a zooplankton population, while for only 64 toxicants this was the fish population. For the remaining 253 toxicants, populations from different trophic levels had the lowest population-NOEC. These calculations suggest that in the system studied, population-NOECs increase with increasing trophic level, regardless of the toxicant considered. Because it is defined as the lowest population-NOEC, the ecosystem structure-NOEC is determined by phytoplankton for 467 of the 1000 toxicants. In contrast, fish seem to play a role in the determination of the ecosystem structure-NOEC for only 64 of the 1000 toxicants. Because these findings are independent of the toxicant type considered, they only result from the ecological interactions included in the ecosystem model. As stated before, ecological interactions will lead to indirect effects on populations initially not targeted by the toxicant. A number of authors use the food web concept to explain how these indirect effects may occur (e.g. Relyea and Hoverman, 2006; Chapman *et al.*, 2003; Fleeger *et al.,* 2003; Preston and Snell, 2001). However, an extensive enumeration of possible indirect effects was not pursued here. Instead, the increase of population-NOECs with increasing trophic level was generally understood as an indication of dominant top-down regulation in this food web. Apparently, a change in a population's biomass resulting from direct toxicant effects will affect the biomass of connected populations at lower trophic levels (i.e. indirect toxicant effect) more than it affects the biomass of connected populations at higher trophic levels. This finding agrees with indirect effects of toxicants observed in micro- and mesocosm studies (e.g., Releya and Hoverman, 2006, Kneib, 1991; Posey and Ambrose, 1994; Menge, 1995; Brett and Goldman, 1996; Hay, 1997; Havens, 1995). Indeed, these authors have found that top-down regulated indirect effects are more frequently observed than bottom-up regulated indirect effects in experimental ecosystems exposed to toxicant stress.

VIII. 3. 3. Which functions determine the ecosystem function-NOEC?

For 749 toxicants, the ecosystem function with the lowest NOEC was I_{fish}, as such determining the ecosystem function-NOEC. This is confirmed by cumulatively plotting the NOECs of the three studied ecosystem functions (Fig VIII.2). A mechanistic explanation for this is that I_{fish} is the only function maintained by one single population (fish). In contrast, $I_{zoo,tot}$ and $PS_{phyto,tot}$ can be maintained by three and two populations, respectively. As such, the functional roles of these

populations are redundant with respect to $I_{zoo,tot}$ and $PS_{phyto,tot}$, making those two ecosystem functions less sensitive.

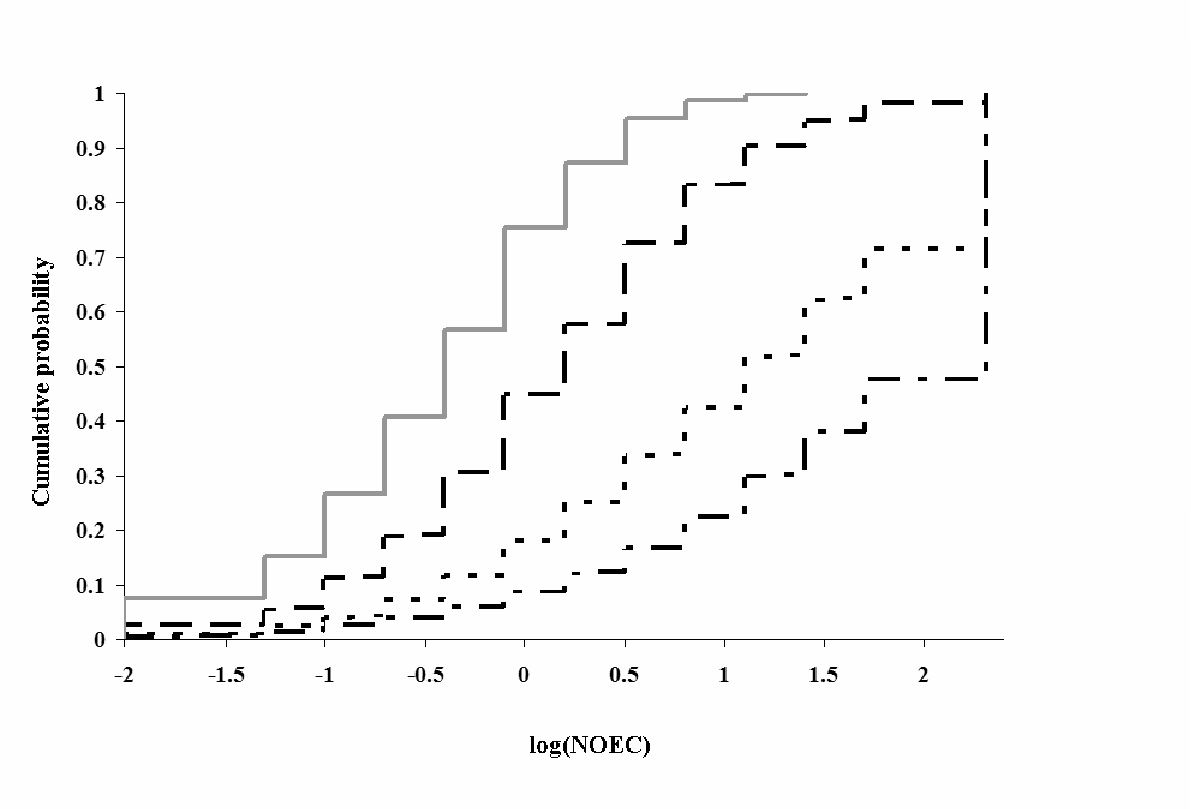

Figure VIII.2: Cumulative probability distribution of: grey: log(ecosystem structure-NOECs); dashed: log(I_{fish}-NOEC); dotted: log($I_{zoo,tot}$-NOEC); dotted and dashed: log($I_{PSPhyto,tot}$-NOEC).

However, the relative sensitivity of ecosystem functions is not entirely explained by the number of populations maintaining it. The NOECs of $I_{zoo,tot}$, a function maintained by three populations (zoo_{small} and $zoo_{large,1}$ and $zoo_{large,2}$), appear to be lower than those of PS_{tot}, a function maintained by only two populations ($phytoplankton_{small}$ and $phytoplankton_{large}$, Fig VIII.2). This suggests that ecosystem functions maintained by populations at higher trophic levels have a lower NOEC. At this point, we need to underline that ecosystem functions were defined as transfer rates of energy which are quantified by biomass, as is usually done (e.g., Duffy, 2002; Schläpfer and Schmid, 1999). Transfer rates associated with higher trophic levels are lower because of metabolic energy losses (Odum, 1971). Use of this concept reformulates the ecosystem functions studied as follows:

$$PS_{phyto,tot} = \eta_{Light \rightarrow PSphyto,tot} \cdot Light \qquad \text{(eqs3)}$$

$$I_{zoo,tot} = \eta_{PSphyto,tot \rightarrow Izoo,tot} \cdot PS_{phyto,tot} = \eta_{PSphyto,tot \rightarrow Izoo,tot} \cdot \eta_{Light \rightarrow PSphyto,tot} \cdot Light$$

$$I_{fish} = \eta_{Izoo,tot \rightarrow Ifish} \cdot I_{zoo,tot} = \eta_{Izoo,tot \rightarrow Ifish} \cdot \eta_{PSphyto,tot \rightarrow Izoo,tot} \cdot \eta_{Light \rightarrow PSphyto,tot} \cdot Light$$

with η representing the efficiency coefficient (<1) indicating energy (biomass) transfer efficiency between two transfers (functions). Names of ecosystem functions are as in equations 1. Toxicant effects on these ecosystem functions can be represented as follows:

$$PS'_{phyto,tot} = (1 - E_{PSphyto,tot}) \cdot \eta_{Light \rightarrow PSphyto,tot} \cdot Light \qquad \text{(eqs4)}$$

$$I'_{zoo,tot} = (1 - E_{Izoo,tot}) \cdot \eta_{PSphyto,tot \rightarrow Izoo,tot} \cdot PS'_{phyto,tot} = (1 - E_{PSphyto,tot}) \cdot (1 - E_{Izoo,tot}) \cdot \eta_{Light \rightarrow PSphyto,tot} \cdot \eta_{PSphyto,tot \rightarrow Izoo,tot} \cdot Light$$

$$I'_{fish} = (1 - E_{Ifish}) \cdot \eta_{Izoo,tot \rightarrow Ifish} \cdot I'_{zoo,tot} = (1 - E_{PSphyto,tot}) \cdot (1 - E_{Izoo,tot}) \cdot (1 - E_{Ifish}) \cdot \eta_{Light \rightarrow PSphyto,tot} \cdot \eta_{PSphyto,tot \rightarrow Izoo,tot} \cdot \eta_{Izoo,tot \rightarrow Ifish} \cdot Light$$

with E representing the direct effect of a toxicant on the ecosystem function indicated in subscript, and affected ecosystem functions indicated by a quotation mark (‘). It can be readily calculated that exposing the considered ecosystem to a toxicant not directly affecting I_{fish} (i.e. $E_{Ifish} \approx 0$) may still result in an observable effect on I_{fish}. Assume that when exposing the ecosystem to a concentration c of this toxicant, $E_{PSphyto,tot}$ and $E_{Izoo,tot}$ are both 0.2, and that $E_{Ifish} \approx 0$. Consequently, $(1 - E_{PSphyto,tot}) \cdot (1 - E_{Izoo,tot}) \cdot (1 - E_{Ifish})$ will be 0.64, indicating a 36% effect on I_{fish}, even though I_{fish} was not directly affected (i.e. $E_{Ifish} \approx 0$).

As such, the trend of the relationship between NOEC and trophic level was found to be opposite for ecosystem structure (NOEC (fish) > NOEC (zooplankton) > NOEC (phytoplankton)) and ecosystem function (NOEC ($PS_{phyto,tot}$) > NOEC ($I_{zoo,tot}$) > NOEC (I_{fish})). Since these trends are independent of toxicant type, explanations for these trends were sought in the ecological interactions within the system studied (see current and previous section). This confirms the importance of ecological interactions for the resulting ecological effects of toxicants. Apparently, these ecological interactions have resulted in the ecosystem structure to be almost consistently as or more sensitive than ecosystem function in the ecosystem studied. Whether this will be the case in other systems will depend on the food web’s configuration and its constituents. In particular, the results obtained here should not be extrapolated to ecosystems with a higher diversity than the system studied here. The possible presence of one or more keystone species (Mills *et al*., 1993; Menge *et al*., 1994), will likely make certain ecosystem functions more sensitive than suggested here. However, whether a higher diversity necessarily results in the presence of keystone species, i.e. in less functional redundancy, is still under debate in ecological literature (Hooper *et al.,* 2005). Once a better insight is gained in these issues, more complex experiments can be designed to elucidate the relation between the sensitivity of ecosystem structure and function in ecosystems with a higher diversity.

VIII. 4. Conclusions

For 979 of 1000 hypothetical toxicants, the ecosystem structure-NOEC was lower than or equal to the ecosystem function-NOEC, indicating that ecosystem structure is as or more sensivite than ecosystem function for those toxicants. Hence, the tested assumption T3 was found to be valid for the tested ecosystem. For 239 of these 979 toxicants, both NOECs were equal. For half of the 1000 toxicants, structure of lower trophic levels (i.e. phytoplankton) appears to be more sensitive than structure of higher trophic levels (i.e. fish). As such, ecosystem structure-NOECs are primarily determined by the sensitivity of the structure of lower trophic levels. In contrast, ecosystem functions associated with higher trophic levels (e.g., total ingestion by fish) are more sensitive than functions associated with lower trophic levels (e.g., total photosynthesis by phytoplankton) for 749 toxicants. Top-down regulation of ecosystem structure and cascading effects on lower trophic level functions to higher trophic level ecosystem functions are discussed as possible explanations for these two contrasting findings.

Chapter IX

Ecological significance of different SSD percentiles for copper in a simple ecosystem

Chapter IX

Ecological significance of different SSD percentiles for copper in a simple ecosystem

Abstract - Species sensitivity distributions (SSDs) are statistical distributions of single-species toxicity test results. It is assumed that a percentile y of this SSD is hazardous for y% of the species within an ecosystem (hazardous concentration for y% of the species, 'HC_y'). To elucidate the ecological significance of such a percentile, we used an ecosystem model to estimate effects of different HC_ys of copper on ecosystem structure (biomass) and function (photosynthesis by phytoplankton, $PS_{all\ phytoplankton}$; and ingestion by zooplankton, $I_{all\ zooplankton}$) in a planktonic ecosystem. Zooplankton biomass and the associated ecosystem function rate ($I_{all\ zooplankton}$) remained unaffected when exposed to concentrations $\leq HC_{30}$ of an SSD based on EC_{20}s. Phytoplankton biomass and $PS_{all\ phytoplankton}$ increased at concentrations > HC_5 or HC_{30} of an SSD based on EC_{20}s or EC_{10}s, respectively. Thus, exposing the ecosystem studied to a HC_y does not necessarily result in ecological effects on y% of the species.

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De Laender F., De Schamphelaere, K.A.C., Vanrolleghem, P.A., Janssen, C.R. Ecological significance of different SSD percentiles for copper in a simple ecosystem. Ecotoxicology and Environmental Safety, submitted.

IX. 1. Introduction

Ecological effect assessments aim at predicting the effects of a chemical substance on the structure and function of ecosystems. These ecological effects are usually estimated by extrapolation of single-species effect data. Such extrapolations can be done using statistical distributions of single-species toxicity test results, i.e. 'species sensitivity distributions' (SSDs). This approach assumes that exposing an ecosystem to a concentration corresponding with a chosen (low) percentile y of this SSD does not result in adverse effects to (100 - y) % of the species present. Hence, this concentration is termed the hazardous concentration for y % of the species (HC_y), i.e. (100-y) % of the species present is assumed to remain unaffected.

In most applications y equals 5, i.e. the fifth percentile is chosen as the low percentile (EU, 2003), hence assuming that 95 % of the species present will not be affected. However, the science-based reasons for selecting the fifth percentile instead of any other percentile are unclear. Indeed, in the peer-reviewed literature, no paper was found that demonstrates the superiority of the fifth percentile over another percentile in protecting ecosystems against adverse ecological effects. The choice for a certain percentile is often referred to as an ethical/philosophical choice, thereby classifying this as a regulatory issue (Posthuma *et al.,* 2002; Forbes and Calow, 2002). This statement implies that exposing an ecosystem to a HC_y will actually put y % of the species at risk in natural ecosystems, i.e. that y is a good measure of true ecological effects. It has been shown that this is almost never the case (Kefford *et al.*, 2005; Versteeg *et al.*, 1999). Therefore, the question remains which ecological effects actually occur when exposing an ecosystem to an HC_y. Addressing this question may allow policy makers to choose a percentile (i.e. a value for y) based on the occurrence of real ecological phenomena.

Although the experimental determination of the ecological effects of HC_5s (i.e. y = 5) has been performed in the past (e.g., Maltby *et al.,* 2005; Selck *et al.,* 2002), estimation of ecological effects of other HC_ys (e.g. y = 10, 20, …) is rare. One exception is the work by Versteeg *et al.,* (1999) who, for a plethora of chemicals, examined which percentile of an SSD corresponds with an experimentally derived ecosystem-no observed effect concentration (NOEC). For most chemicals, this comparison suggests that HC_ys other than the HC_5 may still be lower than the ecosystem-NOEC, i.e. not resulting in adverse ecological effects.

In this chapter, we modelled ecological effects of HC_ys of copper with y = 5, 10, 15, 20, 25, and 30 in a specific planktonic ecosystem. Effects on ecosystem structure and on ecosystem function were simulated. These effects were calculated with a newly developed ecosystem model which was calibrated with data obtained from a microcosm experiment with copper (Schaeffers, 2001).

Ecosystem structure was characterized by the biomass of phytoplankton and zooplankton ($Bio_{all\ phytoplankton}$ and $Bio_{all\ zooplankton}$), while considered ecosystem functions were (1) overall ingestion by all zooplankton populations ($I_{all\ zooplankton}$), and (2) overall photosynthesis by all phytoplankton populations ($PS_{all\ phytoplankton}$).

IX. 2. Material and methods

IX. 2. 1. Description of the microcosm study: experimental data

In this study, data obtained in an aquatic oligotrophic microcosm exposed to copper (Schaeffers, 2001) were used. This study included the following community elements: diatoms, green algae, cladocerans, copepods, and the macrophyte *Elodea densa* and is described further in chapter IV and in Schaeffers (2001).

IX. 2. 2. Description of the constructed ecosystem model

To model the behaviour of the model ecosystem discussed above, the dynamic ecosystem model described in chapters III and IV was used. Included objects were 2 phytoplankton populations (diatoms and green algae), 2 zooplankton populations (cladocerans and copepods), and one macrophyte. As this chapter focuses on the simulation of a case-specific ecosystem, a higher resolution of the ecological interactions was pursued. Because they are filter-feeders, the cladocerans present were modelled as having equal feeding preferences for both phytoplankton types and detritus. Copepods were modelled as feeding primarily on green algae and detritus, and less on diatoms. The reason for this is that 50% of the diatoms present are large pennales, which are too large to be ingested by the copepods, which primarily consist of naupliae (Schaeffers, 2001). To simulate dynamics of the copper-exposed populations, differential equations are combined with logistic equations as toxic effect sub-models (Bruce and Versteeg, 1992; Leverberg, 1944; Marquardt, 1963) describing direct copper effects on traits of the separate populations. These traits are: maximum grazing and mortality rates of the two different zooplankton populations (Ferrando and Andreu 1993), mortality rate of the macrophyte (Mal *et al.,* 2002) and maximum photosynthesis rates of the two phytoplankton populations (Fernandes and Henriques, 1991) and the macrophyte (Mal *et al.,* 2002). Parameters of the logistic equations are the control value, the EC_{50} and EC_{10}.

IX. 2. 3. Model calibration

In the previous chapters, a methodology to predict ecological effects based on an ecosystem model was developed (chapters III to V), validated (chapter VI), and applied in theoretical exercises (chapters VII and VIII). A characteristic of this methodology was the absence of a formal calibration on experimental data. However, in this chapter, knowledge about the relationship between single-species toxicity and ecosystem effects in this particular ecosystem was pursued. Therefore, a more formal calibration was performed here.

As stated by Loehle (1997), calibrating a model solely with a goodness-of-fit statistic to one time series is of limited use in ecosystem modelling. An observed outcome of an ecosystem is only partially the result of the processes incorporated in the model and is prone to randomness, a feature shared by all biological processes. Therefore, it is preferable to calibrate the ecosystem model to a range of possible system observations, i.e. to test whether we can distinguish the model predictions from real system observations. A test criterion described by Loehle (1997) was used. Replications of the time series at a given treatment concentration were used to calculate the upper and lower limit of possible observations of the system when exposed to a treatment concentration. These are defined as the mean of the data series plus and minus one standard deviation, respectively. If predictions fall within these limits, a testing variable (T) is said to be 1; i.e. biologically not distinguishable from the experimental observation. In the other case, T will have a value < 1, depending on the broadness of the region of the observations which is the region between their upper and lower limit. Predictions further away from this region and the narrowness of the region will both lower the T value. Parameters were thus altered until an optimal (maximum) T value was reached.

The calibration criterion consists of two parts which compare the observed and simulated trends (IX.2.3.1) and observed and simulated temporal variability (IX.2.3.2) of the biomass dynamics, respectively. For a given simulation, the two criteria are evaluated and only if both are satisfactory, the simulation is approved. Otherwise, the simulation is rejected as are the corresponding parameter values. If T = 1, simulations are said to be undistinguishable from the observations and as such an adequate fit is obtained. In this chapter, T = 0.7 was set as a lower limit for simulation approval.

IX. 2. 3. 1. Trend

$$T_{trend} = 1 / (t_{max} - t_{min}) \cdot \Sigma_{tmin}^{tmax} \exp((s_{Bio,obs,i} - |Bio_{obs,i}-Bio_{pred,i}|)/s_{Bio,obs,i})) \cdot (\Delta t_i)$$

with:

t_{max} and t_{min} = last and first time instant of observations

$Bio_{obs,i}$ = mean of observed biomass at time i

$Bio_{pred,i}$ = predicted biomass at time i

$s_{Bio,obs,i}$ = standard deviation of observed biomass at time i

Δt_i = time interval between two observations

This is a straightforward implementation of the criterion as described by Loehle (1997) where simulated biomass of a model population is compared with their observed biomass at days t_i. T is calculated for each model population and subsequently an average T value is calculated for the complete community.

IX. 2. 3. 2. Variability

$T_{var} = \exp(s_{s,obs} - |s_{obs}-s_{pred}|)/s_{s,obs})$

with:

s_{obs} = observed standard deviation of biomass in time

s_{pred} = predicted standard deviation of biomass in time

$s_{s,obs}$ = standard deviation of observed standard deviation of biomass in time

This is a customized implementation of the criterion as described by Loehle (1997) to compare simulated temporal variability with the observed one. Temporal variability is represented by the standard deviation in time. The standard deviation in time of the simulated biomass concentration of a model population is compared with their observed standard deviation in time. T is calculated for each model population and subsequently an average T value is calculated for the complete community.

The parameters used for calibration are listed in the appendix (XI.6.11). Note that apart from this list, also the parameters of the logistic equations, describing the sensitivity of the different populations, were calibrated using the microcosm data.

IX. 2. 4. Derivation of HC_ys

The EC_{10}s which were derived from calibration with the microcosm data (see previous paragraph) were used to construct an SSD of the model ecosystem for copper. A lognormal distribution was

fitted to the EC_{10}s based on the most sensitive trait of each population (EU, 2003): maximum ingestion rates of copepods and cladocerans, maximum photosynthesis rates of all phytoplankton populations and the macrophyte. The same was done with the EC_{50}s which were derived by calibration. Using the calibrated EC_{10}s and EC_{50}s and the logistic equations, also EC_{20}s, EC_{30}s, and EC_{40}s of the most sensitive traits were calculated. As such, 5 different SSDs were constructed, based on EC_{10}s, EC_{20}s, EC_{30}s, EC_{40}s, and EC_{50}s, respectively. For each of these 5 distributions, the median of the y^{th} percentiles was derived with y = 5, 10, 15, 20, 25, 30, using the method of Wagner and Lokke (1991). This resulted in 30 percentiles, all representing a certain copper concentration.

IX. 2. 5. Ecosystem structure and function

The ecosystem structure and function were evaluated at these 30 copper concentrations and compared to the control values. Ecosystem structure was characterized here by the average overall biomass of phytoplankton ($Bio_{all\ phytoplankton}$) and zooplankton ($Bio_{all\ zooplankton}$) during the experiment (110 days), calculated as:

$$Bio_{all\ phytoplankton} = \frac{1}{110} \cdot \sum_{t=0}^{110} \left(Bio_{green\ algae,t} + Bio_{diatoms,t} \right)$$

$$Bio_{all\ zooplankton} = \frac{1}{110} \cdot \sum_{t=0}^{110} \left(Bio_{cladocerans,t} + Bio_{copepods,t} \right)$$

with $Bio_{green\ algae,t}$, $Bio_{diatoms,t}$, $Bio_{cladocerans,t}$, and $Bio_{copepods,t}$ the biomass on day t of green algae, diatoms, cladocerans, and copepods, respectively. The effect of a copper concentration c on these average biomasses was calculated using relative differences between control biomass and biomass at the different exposure concentrations. Because 20% is the minimum detectable difference for most population characteristics in the field (Suter II, 1993), RD-values of -0.2 or lower were considered as detectable biomass decreases. Similarly, RD-values of 0.2 or higher were considered as detectable biomass increases.

Ecosystem function was defined *sensu* Duffy (2002) and Schlapfer and Schmid (1999), i.e. transfers of energy. Mean daily total invertebrate ingestion rate ($I_{all\ zooplankton}$) of all zooplankton populations and mean daily total photosynthesis rate ($PS_{all\ phytoplankton}$) of all phytoplankton populations were selected as ecosystem functions and calculated as:

$$I_{all\ zooplankton} = \frac{1}{110} \cdot \sum_{t=0}^{110} \left(I_{copepod,t} + I_{cladoceran,t} \right)$$

$$PS_{\text{all phytoplankton}} = \frac{1}{110} \cdot \sum_{t=0}^{110} \left(PS_{\text{diatom},t} + PS_{\text{green algae},t} \right)$$

with $PS_{\text{green algae},t}$, $PS_{\text{diatom},t}$ the photosynthesis rate on day t of green algae and diatoms, respectively, and $I_{\text{cladoceran},t}$, and $I_{\text{copepod},t}$ the ingestion rate of cladocerans and copepods, respectively. As with ecosystem structure, the effect of a copper concentration c on these ecosystem function rates was calculated using relative differences (RD). RD-values of -0.2 or lower, and 0.2 or higher, were considered as detectable decreases and increases of ecosystem function rate, respectively (Suter II, 1993).

Uncertainty in the estimated values of EC_{10}s and EC_{50}s was propagated through the ecosystem model using a Monte-Carlo approach (50 simulations per copper concentration c, and 50 simulations for the control) as described in Cullen and Frey (1999). The number of simulations was determined using the convergence rule (Melching, 1995; Cullen and Frey, 1999). Per copper concentration c, all 50 simulations were compared with all 50 control simulations to calculate a total of 2500 RD-values ($50 \cdot 50$) per function ($I_{\text{all zooplankton}}$ and $PS_{\text{all phytoplankton}}$) and structure characteristic ($Bio_{\text{all phytoplankton}}$ and $Bio_{\text{all zooplankton}}$). From this range of RD values, the probability that RD is larger than 0.2, or smaller than -0.2 at a certain copper concentration c can be derived non-parametrically. These probabilities were derived for $Bio_{\text{all phytoplankton}}$, $PS_{\text{all phytoplankton}}$, $Bio_{\text{all zooplankton}}$, $I_{\text{all zooplankton}}$ at all 30 copper concentrations.

IX. 3. Results and Discussion

IX. 3. 1. Model Calibration

After calibration, the model was capable of describing the main trends observed in the microcosm reasonably well. The main trends, both reflected in microcosm data and model predictions, are (1) the decrease of cladocerans at concentrations higher than 20 µg L^{-1}; and (2) an increase of green algae at most exposure concentrations (Fig IX.1 and Schaeffers, 2001). Values of EC_{10} and EC_{50} resulting from this calibration (Table IX.1) suggest that phytoplankton and cladocerans are the most sensitive populations in the considered system. The uncertainty of the calibrated EC_xs was characterized by normal distributions (Table IX.1).

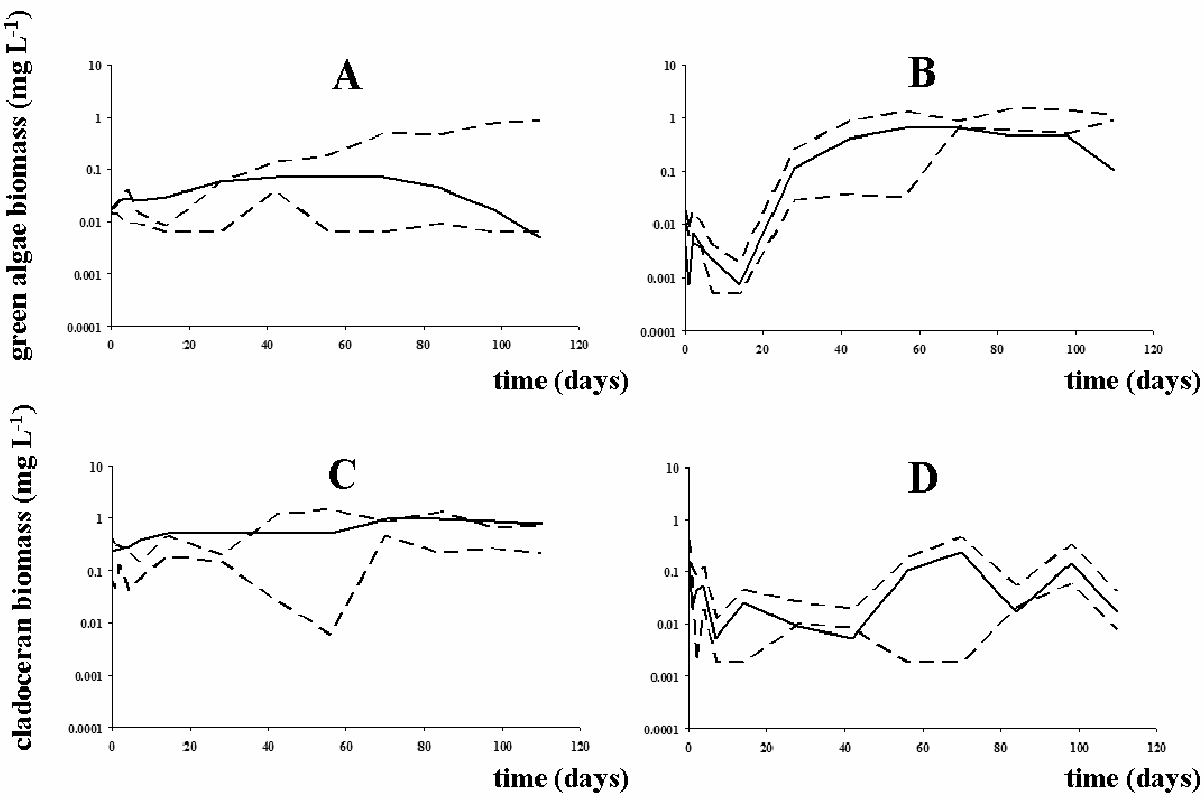

Figure IX.1: Biomass dynamics of green algae at control (A) and at 80 µg L^{-1} copper (B): upper and lower limit of microcosm data (dashed line) and simulation (bold line). Biomass dynamics of cladocerans at control (C) and at 40 µg L^{-1} copper (D). Upper and lower limit of microcosm data (dashed line) and simulation (bold line)

IX. 3. 2. SSD construction

Lower 50% confidence limits of the HC_ys (with y = 5 to 30) of SSDs based on EC_xs (with x = 10 to 50) ranged from 2 (for (x,y) = (10, 5)) to 80 µg L^{-1} (for (x,y) = (50, 30)) (Fig IX.2).

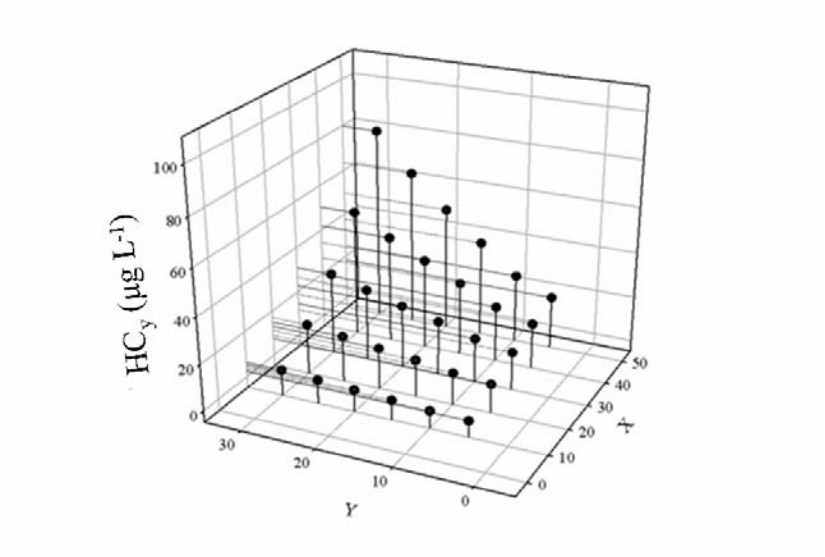

Figure IX.2: Copper concentrations corresponding with y^{th} percentiles (with y = 5 to 30) of SSDs based on EC_xs (with x = 10 to 50).

Table IX.1: Values of toxic effect sub-model parameters, after calibration on microcosm data. EC_x stands for the concentration of copper resulting in x % effect. Calibrated values of other parameters are given in appendix (XI.6.11). Numbers between brackets represent standard deviations of the calibrated values.

parameter	value μ(g L^{-1})	description
EC_{10} clad_mort	120 (11)	EC_{10} for cladoceran mortality rate
EC_{10} clad_Cmax	8.5 (0.29)	EC_{10} for cladoceran ingestion rate
EC_{10} cop_mort	270 (15)	EC_{10} for copepod mortality rate
EC_{10} cop_Cmax	10 (2.6)	EC_{10} for copepod ingestion rate
EC_{10} mort_macro	58 (4.2)	EC_{10} for macrophyte mortality rate
EC_{10} PSmax_macro	5.5 (0.45)	EC_{10} for macrophyte photosynthesis rate
EC_{10} PSmax_dia	2.9 (0.45)	EC_{10} for diatom photosynthesis rate
EC_{10} PSmax_greens	2.5 (0.2)	EC_{10} for green algae photosynthesis rate
EC_{50} clad_mort	400 (11)	EC_{50} for cladoceran mortality rate
EC_{50} clad_Cmax	53 (1.4)	EC_{50} for cladoceran ingestion rate
EC_{50} cop_mort	400 (15)	EC_{50} for copepod mortality rate
EC_{50} cop_Cmax	374 (54)	EC_{50} for copepod ingestion rate
EC_{50} mort_macro	105 (12)	EC_{50} for macrophyte mortality rate
EC_{50} PSmax_macro	48 (1.0)	EC_{50} for macrophyte photosynthesis rate
EC_{50} PSmax_dia	26 (1.9)	EC_{50} for diatom photosynthesis rate
EC_{50} PSmax_greens	104 (3.0)	EC_{50} for green algae photosynthesis rate

IX. 3. 3. Ecological effects at different HC_ys

At most (x,y) combinations, the probability that zooplankton biomass decreases differs from the probability that $I_{all\ zooplankton}$ decreases (Fig IX.3A). These probabilities are only affected by the chosen percentile y at $x \geq 20$, where a higher y value implies a higher probability of decrease, especially for $Bio_{all\ zooplankton}$. At x = 10, the probabilities of a decrease in zooplankton biomass and function are < 5%, regardless of the value of y. At x = 20, the probability of a $Bio_{all\ zooplankton}$ decrease is about 10%, while for $I_{all\ zooplankton}$ this probability is still < 5%. Within the zooplankton, the probability of a decrease of cladoceran biomass is 10% at $x \leq 20$ (Fig IX.3C). Copepods, in contrast, have a limited or zero probability of decreasing at those x values. At x > 20, the decrease of zooplankton biomass becomes more probable than the decrease of $I_{all\ zooplankton}$ (Fig IX.3A). At x > 30 and y > 20, the probabilities that zooplankton biomass and $I_{all\ zooplankton}$ decreases are > 80%.

At all (x,y) combinations, an increase of phytoplankton biomass is equally probable as an increase of the phytoplankton function $PS_{all\ phytoplankton}$ (Fig IX.3B). Only at x = 10, this probability is influenced by the chosen percentile y, where a higher y value results in a higher probability. At x > 20, the probability of phytoplankton biomass and $PS_{all\ phytoplankton}$ increase are 100%, regardless of the value of y chosen. Within the phytoplankton, green algae are likely to increase (Fig IX.3D),

especially at x > 10 (probability > 80%), while diatoms are not (probability < 10% in all except two cases).

In summary, it is highly unlikely that the planktons structural characteristics ($Bio_{all\ zooplankton}$ and $Bio_{all\ phytoplankton}$) or functions ($I_{all\ zooplankton}$ and $PS_{all\ phytoplankton}$) will experience significant effects when exposed to copper concentrations corresponding to the fifth percentile of an SSD based on EC_{10}s, i.e. (x, y) = (10, 5).

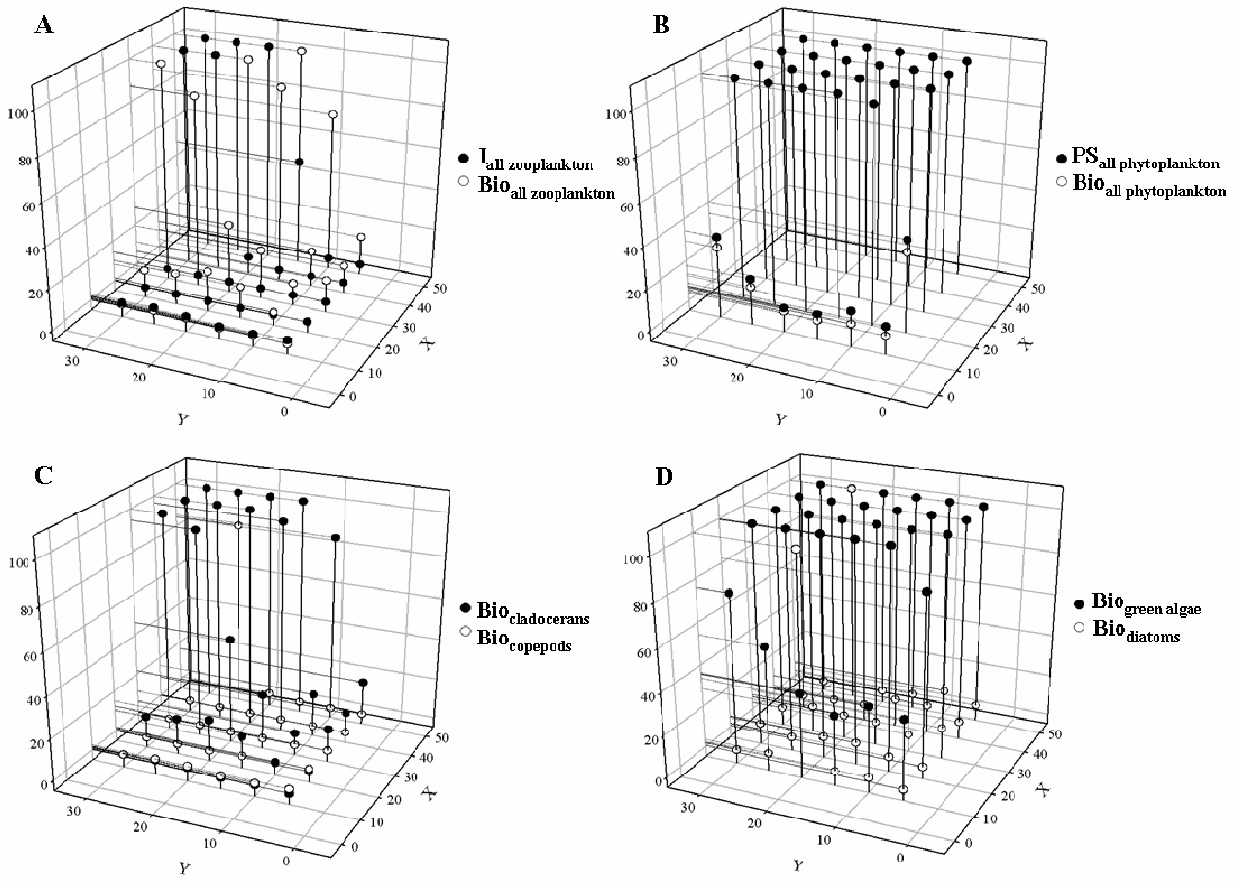

Figure IX.3: Probability of ecological effects of the copper concentrations corresponding with y^{th} percentiles (with y = 5 to 30) of SSDs based on EC_xs (with x = 10 to 50) on structure and functions of a planktonic ecosystem. A: 20% decrease of zooplankton function ($I_{all\ zooplankton}$) and structure ($Bio_{all\ zooplankton}$) . B: 20% increase of phytoplankton function ($PS_{all\ phytoplankton}$) and structure ($Bio_{all\ phytoplankton}$). C: 20% decrease of copepod ($Bio_{copepods}$) and cladoceran ($Bio_{cladocerans}$) biomass. D: 20% increase of diatom ($Bio_{diatoms}$) and green algae ($Bio_{green\ algae}$) biomass.

IX. 4. Discussion

IX. 4. 1. Calibration

The EC_{10}s and EC_{50}s which were derived from ecosystem model calibration are in fair agreement with laboratory-derived experimental EC_xs reported in literature. The EC_xs of cladocerans, as derived from ecosystem model calibration, were found to be lower than those of copepods, a finding supported by experimental data reviewed by Brix *et al*. (2001). The lower EC_{50} of diatoms, compared to the EC_{50} of green algae (Table IX.1), is less well documented in literature. Since different diatom species and strains are known to exhibit different sensitivities to metals in general (e.g., Sanders and Cibik, 1988), it is difficult to compare the diatom EC_xs derived here with those obtained in other chapters. Moreover, most chapters deal with the sensitivity of marine diatoms, or freshwater diatoms from lotic systems. Nevertheless, the limited information available indicates that diatoms may be fairly sensitive to metals. For example, Burton *et al*. (1987) found that number of species and diatom population abundance were good indicators of metal impacts and concentrations in a Montana stream. More specifically, in metal-stressed periphyton-communities, diatoms have been found to decrease drastically, or may even be absent, while green algae tend to dominate (Patrick, 1978; Rushforth *et al.*, 1981; Genter and Lehman, 2000).

IX. 4. 2. Ecological effects of different HC_ys

When exposing the model ecosystem to copper concentrations corresponding to the y^{th} percentiles from SSDs, effects on phytoplankton structure (i.e. biomass) were found to be equal to effects on phytoplankton function (i.e. $PS_{\text{all phytoplankton}}$). However, at some combinations of x an y, effects on zooplankton biomass were higher than effects on $I_{\text{all zooplankton}}$ (Fig IX.3A). The fact that, for the same (x,y) combination, zooplankton biomass is more likely to be affected than zooplankton function ($I_{\text{all zooplankton}}$) is most probably related to the differential sensitivity of copepods and cladocerans to copper. Indeed, the EC_xs of the copepods' maximal ingestion rate and mortality rate are in general higher than those of the cladocerans (Table IX.1). As such, the function $I_{\text{all zooplankton}}$ is maintained (by copepods) while zooplankton biomass is affected, which confirms findings by Yan *et al.* (2004). Also Slijkerman *et al.* (2004) found that effects on zooplankton structure occur at lower concentrations than effects on ingestion by zooplankton in the case of the fungicide carbendazim.

The increase in both phytoplankton biomass and $PS_{all\ phytoplankton}$ is due to the highly probable increase of green algae biomass (Fig IX.3D) at x > 10. Diatom biomass, in contrast, is unlikely to increase (Fig IX.3D) and does therefore not contribute to this increase of phytoplankton biomass or $PS_{all\ phytoplankton}$. The similar probabilities of phytoplankton biomass decrease and $PS_{all\ phytoplankton}$ decrease (Fig IX.3B) originate from the dominance of green algae in the exposed system: the increase in $PS_{all\ phytoplankton}$ is a reflection of increasing green algae biomass.

Probabilities of ecological effects of other substances in other ecosystems have been predicted by the same ecosystem model and compared with observed effects in micro- and mesocosms (chapter VI). The highest concentration at which the probability of an effect on the biomass of a population was less than 50% corresponded with that population's experimentally observed no observed effect concentration (NOEC). Applying this 50% probability on the results obtained here reveals that zooplankton biomass ($Bio_{all\ zooplankton}$) and function ($I_{all\ zooplankton}$) are unaffected when the system is exposed to the 10^{th} percentile of an SSD based on EC_{40}s, to the 20^{th} percentile of an SSD based on EC_{30}s, or to the 30^{th} percentile of an SSD based on EC_{10}s or EC_{20}s. However, if also phytoplankton biomass ($Bio_{all\ phytoplankton}$) and function ($PS_{all\ phytoplankton}$) is to be kept within their control range, the system can only be exposed to the fifth percentile of an SSD based on EC_{20}s, or the 30^{th} percentile of an SSD based on EC_{10}s.

In this chapter, we did not want to make statements about whether a 95% protection of all species in an ecosystem is acceptable from a philosophical or regulatory point of view. Here, the HC_y was purely regarded as a statistical characteristic of the SSD. The goal of this work was to relate this characteristic with the probability of ecological effects occurring in a planktonic ecosystem. In general, exposing this ecosystem to the fifth percentile of an SSD based on EC_{10}s (i.e. x, y = 10, 5) does not result in effects on ecosystem structure ($Bio_{all\ zooplankton}$ and $Bio_{all\ phytoplankton}$), nor does it impede ecosystem function ($I_{all\ zooplankton}$ and $PS_{all\ phytoplankton}$). This agrees with previous efforts (Versteeg *et al.*, 1999; Maltby *et al.*, 2005; Selck *et al.,* 2002; van den Brink *et al.,* 2006), although in this study the species in the SSDs were the same as those in the considered system, which was not the case in those other cited studies.

It is unclear if the relationships between x, y, and probability of ecological effects derived here (Figs IX.3A to 3D) can be extrapolated to other ecosystems and/or toxicants. As stated before, the use of the same species in the SSD construction as those present in the system studied, will influence the results obtained. For organics, it has been shown that species composition of the SSD determines its shape and capacity to protect ecosystem structure (e.g., Maltby *et al.*, 2005, Versteeg *et al.* 1999). Hence, inclusion of other, less representative species, is likely to change HC_y values. For example, the inclusion of EC_xs of fish in these SSDs will enlarge their standard

deviations. Hence, the resulting HC_ys will shift to lower values, because of the symmetry of the lognormal distribution (Posthuma *et al.*, 2002). As a result, there is good reason to presume that probabilities of effect on structure and function will be lower for the same HC_ys in that case. Also, the closed nature of the modelled ecosystem, hampers recolonization (Yount and Niemi, 1990), i.e. nearby populations can not replace affected populations to maintain ecosystem functions. Recovery of ecosystem function is therefore limited, hence potentially overestimating the ecological effects. This is not only related to the design of the microcosm experiment, but also to the nature of the mechanistic ecosystem model used here. Classic mechanistic models implicitly assume that ecosystem processes are entirely reversible. By doing this, they ignore the directional behaviour (known as succession) of ecosystems (Ulanowicz and Abarca Arenas, 1997). As such, it is impossible to predict longer-term behaviour of stressed systems using the presented ecosystem model.

IX. 5. Conclusion

In this chapter, ecological effects of exposing a specific planktonic ecosystem to different HC_ys were derived based on a calibrated ecosystem model. After calibration, the ecosystem model was capable of describing the main ecological effects observed in an experimental microcosm reasonably well. Where comparable, the resulting values of ecosystem model parameters, i.e. EC_{10}s and EC_{50}s, are in fair agreement with literature.

Model predictions revealed that the main ecological effects can be explained by (1) the increase of green algae biomass and (2) the decrease of cladoceran biomass. The increase of green algae biomass was reflected in a proportional increase of phytoplankton photosynthesis. The decrease of cladoceran biomass (Fig IX.3C), did not result in a proportional decrease of $I_{all\ zooplankton}$ (Fig IX.3.A). Apparently, the relative insensitive copepods were able to maintain this function at its control level at those concentrations.

Based on the results of chapter VI (i.e. the use of a 50% probability), it was demonstrated that the system can be exposed to copper concentrations ≤ the fifth percentile of an SSD based on EC_{20}s, or the 30^{th} percentile of an SSD based on EC_{10}s, without causing adverse effects on the functional and structural characteristics of the system. However, if this result can be extrapolated to other ecosystems and/or toxicants is unclear, since species composition of the SSD and the spatial and temporal scale of the system studied may affect the outcome of this type of simulations.

Chapter X

General conclusions

X. 1. Introduction

In this chapter, the conclusions obtained in this work are summarized following the structure of this dissertation (cf. chapter I). Each paragraph reflects the conclusions drawn in one (or more) chapters and is preceded by the question it seeks to address. Suggestions for further research and, where possible, links between different chapters are highlighted. A short paragraph briefly outlining the general scope of this research is given hereunder.

X. 2. Scope

After entering the aquatic environment, micropollutants have the potential of being widely dispersed in space and time. Based on their chemical and toxicological properties, their presence may pose problems for organisms and ecosystems. Current knowledge indicates that such problems are complex, because they result from an interplay between various factors. Conducting studies with artificial ecosystems, so-called micro- and mesocosm studies, to examine ecological effects of toxicant stress is useful, but logistic and financial issues hamper the frequent use of these approaches. Facing the scarcity of ecosystem-level effect data, potential ecological effects are usually predicted based on results of single-species toxicity tests conducted in the laboratory. This is achieved through the use of extrapolation techniques, such as species sensitivity distributions (SSDs), which do not have a sound scientific basis and mainly rely on unproven assumptions.

X. 3. The relationship between single-species toxicity and ecosystem effects

→ Chapter II: what is meant with 'ecosystem effects' and how has their relation with single-species toxicity test results been examined until now?

The majority of single-species toxicity test results available in the aquatic part of the USEPA database ECOTOX report acute effects of chemicals on the mobility or survival of **animals**, i.e. information related to the abundance of organisms. Roughly half of the micro- and mesocosm studies in open literature refer to 'ecosystem effects' as effects on abundance of one or more animal populations within an ecosystem. As they are mostly based on abundance or biomass, effects observed in ecosystems and in single-species toxicity assays are intrinsically highly comparable. Results from such comparisons indicate that for most species, the effect concentration (EC_x) observed in a **micro- or mesocosm study** is **within a factor 2** of the effect concentration (EC_x) observed in a **single-species toxicity test**. However, this conclusion partly originates from the **focus on effects on invertebrates in ecosystem studies with insecticides**. If similar exercises would be performed with phytoplankton species and herbicides, this conclusion may change, depending on the relative sensitivities of the interacting populations. Although they are perfectly suited for such exercises, **ecosystem models** are only rarely used.

X. 4. Ecosystem model development and validation

→ Chapter III and IV: Can an ecosystem model accurately predict ecosystem effects if it is not calibrated on observed population dynamics?

In this thesis, a novel approach to predict ecological effects of chemicals in aquatic ecosystems was developed. **The approach is based on ecosystem modelling, generic ecological concepts, and single-species toxicity test results.** The dynamic ecosystem models consist of (1) a bioenergetic foodweb model; (2) a model for nutrient and detritus cycling; and (3) toxic effect sub-models using single-species toxicity test results as input. As such, the developed ecosystem model can perform predictions without the need for calibration on experimental ecosystem data. In chapter IV, ecosystem model predictions of biomass changes of populations in an experimental microcosm exposed to copper **proved to be accurate, or at least indicated the same trend** as the experimental data. The fact that these model predictions were significantly better than those

based on single-species toxicity test results alone, indicates that ecological interactions have to be accounted for when conducting ecological effect assessments.

→ Chapter V: Which toxic effect sub-model should be incorporated in the developed ecosystem model to increase the accuracy of the predictions?

The predictions of population- and ecosystem-no observed effect concentrations (NOECs) performed in chapter V benefit from the use of **logistic functions** as toxic effect sub-models, compared to the use of linear functions. Effects on the following endpoints were included: **(1) photosynthesis rates of phytoplankton and macrophytes, and (2) mortality rates of animals and the macrophyte**. Ecosystem models equipped with this type of toxic effect sub-models predicted nearly all population- and ecosystem-NOECs accurately. The inclusion of sub-lethal effects to zooplankton in the toxic effect sub-models had little or no influence on the NOEC-predictions of the ecosystem models. Apparently, the implementation of the logistic shape that most concentration-response data exhibit, is more important than including sub-lethal effects in toxic effect sub-models. Ecosystem models equipped with l**inear toxic effect sub-models** resulted in lower predicted values of the $NOEC_{cladocerans}$ (at least a factor 4 lower). This resulted in inaccurate predictions of connected population densities. Moreover, because cladocerans are amongst the most sensitive populations for copper in the microcosm studied, they determine the ecosystem-NOEC. An underestimation of $NOEC_{cladocerans}$ results in an underestimation of the ecosystem-NOEC.

→ Chapter VI: Can the developed ecosystem model be used in ecological effect assessments?

Ecosystem models equipped with the logistic toxic effect sub-models made accurate predictions of the effects of toxicants other than copper on populations and other ecosystems. In the validation exercise performed in this thesis (chapter VI), a different model was constructed for each ecosystem considered to assure the inclusion of the relevant populations present in the experimental studies. By comparing the results from this validation with those obtained by other authors, the superior accuracy of the developed model predictions was demonstrated. **No observed effect concentrations (NOECs) of 60% of all considered populations were predicted accurately in a total of 11 micro- and mesocosm studies**. Only 14% and 26% of all population-NOEC predictions were too high or too low (underprotective or conservative), respectively. The predictive capacity of the ecosystem model was influenced by the α-level used

to derive NOECs from raw model outputs. From this validation study, it becomes apparent that an α-level of 0.5 benefits the accuracy of the NOEC-predictions. This corresponds to taking the median of the ecosystem model outputs. At lower α-levels (e.g., 0.01), the number of conservative NOEC-predictions was lower (15%), but the number of underprotective NOEC-predictions was higher (29%) than at α = 0.5. Compared to the use of α = 0.01, application of α = 0.5 can reduce the number of underprotective NOECs at the cost of a slightly higher amount of conservative NOECs. **Predicted ecosystem-NOECs** were never larger than the experimental NOECs at α = 0.5, i.e. they **were never underprotective**. Because only single-species toxicity data are needed to successfully apply this modelling approach, it can serve as an ecology-based alternative for extrapolation approaches without any additional data needs.

→ suggestion for further research

The premise of including the relevant populations in an ecosystem model might pose problems from a practical point of view. Indeed, determining the configuration (i.e. species present and interactions between them) of a new (i.e. non-studied) ecosystem is a difficult task. In the validation study (chapter VI), the relevant populations were *a priori* known because they were given in the corresponding papers of on the considered micro- and mesocosm studies. However, in practice, this will rarely be the case. Therefore, it seems sensible to predict NOECs for a variety of ecosystems. Because of the feasibility of the modelling approach discussed here in terms of its limited data requirements, the number of simulations is only constrained by the time needed for interpretation of the results.

X. 5. Theoretical model applications

→ Chapters VII and VIII: Which assumptions associated with current approaches for ecological effect assessments are valid and for which chemicals?

The theoretical ecosystem studies in this dissertation indeed suggest that the assumptions on which species sensitivity distributions (SSDs) are based can be valid for one toxicant type, while they are invalid for another toxicant type. This result was obtained by comparing SSDs consisting of single-species EC_{10}s (i.e. not accounting for ecological interactions) with SSDs consisting of population-NOECs predicted by an ecosystem model (i.e accounting for ecological interactions).

In chapter VII, assumption T1 (Table I.2), i.e. that ecological interactions between populations do not influence the sensitivity distribution, has been found to be **valid for toxicants directly targeting zooplankton and fish** ($EC_{10,fish} \approx EC_{10,zooplankton} << EC_{10,phytoplankton}$). Sensitivity distributions of toxicants targeting phytoplankton (i.e. $EC_{10,fish} \approx EC_{10,zooplankton} >> EC_{10,phytoplankton}$) have a lower mean when ecological interactions are accounted for than when they are constructed in a conventional way, i.e. without ecological interactions. This makes assumption T1 invalid for this type of toxicants. Apparently, ecological interactions only have a limited influence on SSDs for toxicants targeting zooplankton and fish. This conclusion agrees with what was found in chapter II: effect concentrations (EC_xs) for invertebrates exposed to insecticides (i.e. toxicants targeting zooplankton) were similar (Fig II.4) in experimental ecosystems (accounting for ecological interactions) and in single-species toxicity tests (not accounting for ecological interactions).

→ suggestion for further research

The disproportionate interest in the effects of insecticides on higher trophic levels reflected in literature may result in incorrect conclusions concerning the relationship between single-species toxicity test results and ecological effects. Therefore, it seems there is a need for more studies on the effect of herbicides on phytoplankton, not only on an ecosystem level, but also in single-species tests which are performed in parallel.

A second theoretical ecosystem study (chapter VIII) tested the validity of assumption T3 (Table I.2), i.e. that ecosystem structure is as or more sensitive than ecosystem functions. **Ecosystem structure**, expressed as biomass of the considered populations, was **as or more sensitive than the ecosystem functions** total photosynthesis by phytoplankton, total ingestion by zooplankton, and total ingestion by fish for 979 of 1000 tested hypothetical toxicants. However, for 239 of these 979 toxicants, the considered ecosystem had an ecosystem structure-NOEC (='ecosystem-NOEC' in other chapters) equal to the ecosystem function-NOEC, suggesting that protecting ecosystem structure is **not necessarily** a **conservative** approach for ecosystem function, but rather an **accurate** one. For nearly half of the 1000 toxicants, the population with the lowest NOEC was a phytoplankton population. This means that in nearly 50% of the cases, the ecosystem-NOEC is determined by phytoplankton. In contrast, for only 7 % of the toxicants, the population-NOEC of fish determined the ecosystem structure-NOEC. This is a remarkable result, considering that the

1000 toxicants represent many different toxicant types (and not only toxicants targeting phytoplankton). The **dominance of top-down control of biomass** can be suggested as an explanation of these observations. In contrast, across toxicants, NOECs of ecosystem functions associated with higher trophic levels (e.g. ingestion by fish) tended to be lower than NOECs of functions associated with lower trophic levels (e.g., total photosynthesis): $NOEC_{photosynthesis\ by\ phytoplankton} > NOEC_{ingestion\ by\ zooplankton} > NOEC_{ingestion\ by\ fish}$. As such, it was mostly the $NOEC_{ingestion\ by\ fish}$ which determined the ecosystem function-NOEC. The **decreasing efficiency of energy transfer rates with increasing trophic level** can be proposed as a possible explanation of these observations.

→ suggestion for further research

After having tested assumption T1 and T3 (Table I.2), it seems logical to also subject T2 to testing: "all species in an ecosystem are equally important in conserving its structure and function." However, answering this question with the models developed in this thesis is difficult because of the limited number of distinct ecological roles covered within these models: photosynthesis by phytoplankton, ingestion by zooplankton, and ingestion by fish. This modest level of detail can be used for examining assumptions T1 and T3, and for predicting ecological effects in micro- and mesocosms, because these exercises are concerned with predicting aggregate measures (e.g., total ingestion by zooplankton, ecosystem-NOECs). In contrast, testing assumption T2 requires an ecosystem model reflecting a system with a higher functional diversity preferably containing one or more species with a unique trait. A possible drawback of such a task is the remaining uncertainty in the relationship between biodiversity and ecosystem functioning (BEF). More specifically, it is unclear if increased diversity implies (1) a higher occurrence of 'keystone species', i.e. species with very specific and crucial roles; or (2) a higher functional redundancy. The former would indicate that T2 is invalid, while the latter would imply the opposite. Because of the increasing affinity of ecotoxicology with stress ecology, the collaboration of BEF-research with ecotoxicological studies (such as the testing of T2) seems a logical future perspective. In line with the evolvement of ecotoxicology towards stress ecology, efforts could be aimed at different sources of stress, rather than at toxicant exposure alone (e.g. eutrophication of aquatic ecosystems).

X. 6. Practical model application

→ Chapter IX: What is the significance of different HC_ys in a planktonic ecosystem?

After having tested the validity of some theoretical SSD-assumptions, the ecosystem model was used to reveal the significance of different SSD-percentiles for copper in a planktonic microcosm for which an experimental data set was available. Calibration of the model on this data set allowed to simulate ecological effects at concentrations which were initially not tested in the experimental microcosm study. Effects of HC_ys (y = 5, 10, 15, 20, 25, 30) for copper on ecosystem structure and function were simulated (Figs IX.3A-D). Results confirmed that the significance of the **HC_y is not "the hazardous concentration for y% of the species within an ecosystem"**, as already suggested by other authors. Also, the reason why y is set at 5 in most applications is unclear: copper concentrations ≤ **HC_5 of an SSD based on EC_{20}s, or HC_{30} of an SSD based on EC_{10}s** did not cause adverse effects on the functional and structural characteristics of the ecosystem. This indicates that **choosing y = 5** to protect the considered ecosystem from copper toxicity is a **rather conservative** approach and is dependent on the single-species toxicity data used to construct the SSD

X. 7. OVERALL CONCLUSION: ecosystem models can predict "safe" environmental concentrations and can improve ecological effect assessments

1. The type of ecosystem model constructed in this dissertation can serve as an ecology-based method to accurately predict ecological effects, provided that (1) the relevant populations are included in the model; and (2) a logistic toxic effect sub-model is used to integrate mortality effects of animals. The use of ecosystem models in ecological effect assessments benefits from the limited data needs of such models. The amount of standard single-species toxicity test results needed to follow such an approach is comparable to the amount of data needed by conventional extrapolation techniques.

2. Apart from a predictive role, the models developed here can also support current extrapolation approaches. The validity of the assumptions underlying these extrapolation techniques is related to the toxicant type. For toxicants directly targeting zooplankton and fish, these assumptions are likely to be valid, while the opposite holds for toxicants directly targeting phytoplankton. These findings, together with results from practical ecosystem studies aid in understanding the significance of applying current extrapolations techniques. Hence, they can assist effect assessors in applying current techniques so that more accurate predicted no effect concentrations for chemicals are obtained.

Chapter XI

Appendix

XI. 1. Introduction

This appendix lists the data used to perform the experiments described in the previous chapters. Section XI.2 gives the references to the studies with artificial ecosystems discussed in chapter II. Also the toxicity data considered in chapter II are listed in that section. In section XI.3, a description of the state and driver variables is given. In section XI.4, a description of all parameters is provided. The values assigned to them throughout this dissertation are listed in sections XI.5 and XI.6. In section XI.5, this is done for the parameters with a constant value throughout chapters III to VIII. Section XI.6 carefully lists those parameters which have received different values in the different chapters. It also lists the parameter values resulting from the quantitative calibration in chapter IX. In XI.7, a justification of the experimental NOEC-data used in chapter VI is provided.

XI. 2. supporting data for chapter II

XI. 2. 1. Considered ecosystem studies between 1990 and 2006

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XI. 2. 2. Considered toxicity data

Table XI.2.2.1: Results of ecosystem studies addressing the first issue from Fig II.3: the relationship between $EC_{x,ECO1}$ and $EC_{x,single\text{-}species}$, i.e. between effect concentrations for the abundance of populations within an ecosystem study-setting and effect concentrations derived in a single-species test, performed alongside the ecosystem study. Endpoints of both EC_xs are abundance-based. Data originate from Fairchild *et al.*, 1992; Van Wijngaarden *et al.*, 1996; Schroer *et al.*, 2004; Vanderhoeven *et al.*, 1997; and Hose *et al.*, 2003.

reference	species	single-species endpoint	lower 95% CI	upper 95% CI	ECO1 endpoint	lower 95% CI	upper 95% CI
Fairchild et al	bluegill	96hLC50	0.19	0.42	LC50-4 months	0.25	0.67
van Wijngaarden et al.	*Caenis horaria*	96h-EC10	0.3	0.6	32d-EC10	0.13	0.54
		96h-EC50	0.4	0.5	32d-EC50	0.25	0.5
	Chaeborus obscuripes	96h-EC10	0.2	0.6	32d-EC10	0.4	0.4
		96h-EC50	0.6	0.8	32d-EC50	0.4	0.4
	Cloeon dipterum	96h-EC10	0.1	0.2	32d-EC10	0.07	0.74
		96h-EC50	0.2	0.2	32d-EC50	0.17	0.5
	Simocephalus vetulus	96h-EC10	0.2	0.4	32d-EC10	0	23.9
		96h-EC50	0.3	0.5	32d-EC50	0.02	16.7
Schroer et al.	*Ceanis horaria*	48h-EC10	0.0036	0.0114	weeks-EC10	0.0016	0.0148
		96h-EC10	0.0012	0.0111	weeks-EC50	2.6	288
		48h-EC50	0.0128	0.0251			
		96h-EC50	0.0077	0.024			
	Chaoborus obscuripes	48h-EC10	0.0003	0.0013	weeks-EC10	0.0008	0.0073
		96h-EC10	0.0006	0.0022	weeks-EC50	3.5	22.7
		48h-EC50	0.0018	0.0044			
		96h-EC50	0.002	0.0039			
	Cloeon dipterum	48h-EC10	0.0037	0.014	weeks-EC10	0.0021	0.034
		96h-EC10	0.0746	0.0746	weeks-EC50	7.2	67.4
		48h-EC50	0.0172	0.0358			
		96h-EC50	0.0883	0.0883			
	Gammarus pulex	48h-EC10	0.0074	0.027	weeks-EC10	0.0004	0.0144
		96h-EC10	0.007	0.0247			
		48h-EC50	0.016	0.0349			
		96h-EC50	0.0159	0.0367			
Vanderhoeven et al.	*Daphnia pulex*	48h-EC10	0.11	0.19	48h-EC10	0.03	0.18
	Daphnia pulex	7d-EC10	0.11	0.15	7d-EC10	0.006	0.05
	Daphnia pulex	48h-EC50	0.3	0.47	48h- EC50	0.2	0.46
	Daphnia pulex	7d-EC50	0.21	0.29	7d-EC50	0.13	0.85
Hose et al.	*Atalophlebia spp.*	12h-LC50	20.3	33.9	12h-LC50	15.5	40.6
	Atalophlebia spp.	48h-LC50	12.1	15.2	48h-LC50	13.5	17.1

XI. 3. State and driver variables

state variables	**description**
$Bio_{population}$	biomass concentration of given population(mg L^{-1})
N	nitrogen concentration (mg N L^{-1})
P	phosphorus concentration (mg P L^{-1})
POM	particulate organic matter (mg L^{-1})
DOM	dissolved organic matter (mg L^{-1})
NH_3-N	ammonia-nitrogen (mg L^{-1})
NO_3-N	nitrate-nitrogen (mg L^{-1})
PO_4-P	phosphate phosphorus (mg L^{-1})
POM	particulate organic matter (mg L^{-1})
SOM	settled organic matter (mg L^{-1})

driver variables	**description**
Photoperiod	the fraction of day with sunlight (-)
Solar	average daily incident solar radiation (cal m^{-2} day^{-1})
Temperature	water temperature (°C)
Oxygen	oxygen concentration in the water columns (mg L^{-1})

XI. 4. Parameters - description

parameter	description
Area	surface area of reservoir (m^2)
*C_{max0}	maximum ingestion rate for given consumer at control (d^{-1})
$DecayMax_{DOM}$	maximum rate of DOM conversion to nutrients (d^{-1})
$DecayMax_{POM}$	maximum rate of POM conversion to nutrients (d^{-1})
$DecayMax_{SOM}$	maximum rate of SOM conversion to nutrients (d^{-1})
Depth	depth of the reservoir (m)
$Ecoeff_{DOM}$	extinction coefficient of DOM (m^{-1} mg^{-1} L)
$Ecoeff_{macrophytes}$	extinction coefficient of macrophytes (m^{-1} mg^{-1} L)
$Ecoeff_{phytoplankton}$	extinction coefficient of phytoplankton (m^{-1} mg^{-1} L)
$Ecoeff_{POM}$	extinction coefficient of POM (m^{-1} mg^{-1} L)
$Extinction_{water}$	extinction by pure water (m^{-1})
*$EgestionCoeff_{resource\ i}$	fraction of consumed resource i lost through egestion (-)
Emort	exponential coefficient for stress-related increased mortality (-)
ESed	exponential factor for accelerated sinking (-)
*Exc	excretion / photosynthesis ratio (-)
Excr	constant relationship between excretion and respiration (-)
*$FHalfSat_{resource\ i}$	half saturation constant for consumption of resource i (mg L^{-1})
$K_{denitri}$	maximum rate of denitrification (m d^{-1})
*K_N	Michaelis-Menten constant for nitrogen limitation (mg L^{-1})
K_{nitri}	maximum rate of nitrification (m d^{-1})
K_O	Michaelis-Menten constant for oxygen limitation (mg L^{-1})
*K_P	Michaelis-Menten constant for phosphorus limitation (mg L^{-1})
KT	a coefficient for decreasing acclimation as water temperature approaches T_{ref} (-)
*L_m	optimal light intensity for phytoplankton photosynthesis (cal m^{-2} $day^{?1}$)
$MinBio_{resource\ i}$	minimum resource concentration at which given consumer begins consuming (mg L^{-1})
*$Mort_0$	intrinsic mortality (d^{-1})
Org2Ammonia	a default conversion factor between organic matter and NH_3-N (-)
Org2Phos	a default conversion factor between organic matter and PO_4-P (-)
pH_{max}	maximum pH for given process
pH_{min}	minimum pH for given process
$Pref_{resource\ i}$	preference of given consumer for resource i (-)
*Psmax,0	maximum photosynthesis at control (d^{-1})
Q10	rate of change per 10°C temperature change (-)

***parameters receiving different values in the different chapters**

XI. 4. Parameters – description – continued

parameter	description
Resp	fraction of energy lost to dynamic action (-)
Resp0	intrinsic respiration (d^{-1})
Sed	intrinsic sinking or sedimentation rate (m d^{-1})
TempResp	exponential coefficient for increased respiration because of increased water temperature ($°C^{-1}$)
T_{max}	maximum temperature for given population or process (°C)
T_{obs}	the temperature at which a given process rate was determined (°C)
T_{opt}	optimum temperature for given population (°C)
T_{ref}	reference temperature, below which there is no acclimation (°C)
Volume	volume of water in reservoir (m^3)
XM	the maximum acclimation (°C)

***parameters receiving different values in the different chapters**

XI. 5. Parameters - general values

XI. 5. 1. zooplankton and fish

parameter	$zooplankton_{small}$	$zooplankton_{large}$	planct.fish	pisc.fish	reference
Excr	0.17	0.17	0.05	0.05	Scavia and Park (1976)
KT	5	5	5	5	Kitchell et al. (1972)
$MinBio_{resource\ i}$	0.05	0.05	0.1	0.25	USEPA (2002); Walz (1995)
$Pref_{resource\ i}$	~	~	~	~	
Q10	2	2	2	2	DeNicola (1996)
Resp	0.25	0.18	0.172	0.172	Hewett & Johnson (1992); USEPA (2002)
Resp0	0.03	0.014	0.04	0.04	Collins and Wlosinski (1983); Hewett & Johnson (1992); USEPA (2002)
TempResp	0.065	0.065	0.065	0.065	USEPA (2002)
T_{max}	34	34	36	36	Collins and Wlosinski (1983)
T_{opt}	26	26	27	27	Collins and Wlosinski (1983)
T_{ref}	5	5	2.5	2.5	Collins and Wlosinski (1983)
XM	5	5	5	5	Kitchell et al. (1972)

~the value of these parameters is mentioned in the text

XI. 5. 2. Organic matter, nutrient cycling, and physicochemical quantities

parameter	**value**	**reference**
Area	#	
$DecayMax_{DOM}$	0.29	USEPA (2002)
$DecayMax_{POM}$	0.29	USEPA (2002)
$DecayMax_{SOM}$	0.04	USEPA (2002)
Depth	1	micro- or mesocosm
$Ecoeff_{DOM}$	0.03	USEPA (2002)
$Ecoeff_{macrophytes}$	0.05	LeCren & Lowe-McConnell (1980)
$Ecoeff_{phytoplankton}$	0.014	Collins and Wlosinski (1983)
$Ecoeff_{POM}$	0.12	Verduin (1982)
$Extinction_{water}$	0.016	Wetzel (2001)
$K_{denitri}$	0.1	Di Toro (2001)
K_{nitri}	0.135	Effler (1996)
K_O	0.1	Bowie et al. (1985)
KT	5	Kitchell et al. (1972)
Org2Ammonia	0.079	Redfield (1958)
Org2Phos	0.018	Redfield (1958)
pH_{max}	8.5	Lyman et al., (1982)
pH_{min}	5	Lyman et al., (1982)
Q10	2	DeNicola (1996)
Sed	0.15	Collins and Wlosinski (1983)
T_{max}	60	Collins and Wlosinski (1983)
T_{obs}	25	Collins and Wlosinski (1983)
Volume	#	

[*]parameters receiving different values in the different chapters
[#]a fixed Area/Volume ratio of 1 was used in all chapters
[~]the value of these parameters is mentioned in the text

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	macrophyte	reference
Emort	0.04	0.04	0.01	USEPA (2002)
ESed	1.1	1.1		Wetzel (2001)
KT	5	5	5	Kitchell et al. (1972)
Q10	2	2	2	DeNicola (1996)
Resp0	0.02	0.01	0.01	Collins and Wlosinski (1983); Hewett & Johnson (1992); USEPA (2002)
Sed	0.15	0.15		Collins and Wlosinski (1983)
TempResp	0.065	0.065	0.065	USEPA (2002)
T_{max}	30	40	40	Collins and Wlosinski (1983)
T_{opt}	8	20	20	Collins and Wlosinski (1983)
T_{ref}	2	10	10	Collins and Wlosinski (1983)
XM	5	5	5	Kitchell et al. (1972)

XI. 6. Parameters – specific values per chapter

XI. 6. 1. Chapters IV and V

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	macrophyte	zooplankton$_{small1}$	zooplankton$_{large1}$	zooplankton$_{large2}$
$C_{max,0}$				4	1.8	1.8
EgestionCoeff$_{resource\ i}$				0.15	0.3	0.3
Exc	0.03	0.02	0.3			
FHalfSat$_{resource\ i}$				0.5	1	1
K_N	0.05	0.002	0.002			
K_P	0.01	0.002	0.002			
L_m	48	100	100			
$Mort_0$	0.06	0.02	0.001	0.06	0.03	0.03
$PS_{max,0}$	3.8	1.8	0.2			

XI. 6. 2. Chapter VI – study 1

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	zooplankton$_{small1}$	zooplankton$_{large1}$	zooplankton$_{large2}$	plank .fish	pisc.fish
$C_{max,0}$			4	1.8	1.8	1.3	1.2
EgestionCoeff$_{resource\ i}$			0.2	0.2	0.2	0.1	0.1
Exc	0.025	0.02					
FHalfSat$_{resource\ i}$			0.5	1	1	5	5
K_N	0.05	0.002					
K_P	0.01	0.002					
L_m	48	100					
$Mort_0$	0.06	0.02	0.06	0.03	0.03	0.0001	0.001
$PS_{max,0}$	3.8	1.8					

XI. 6. 3. Chapter VI – studies 2 and 9

parameter	phytoplankton$_{spring1}$	phytoplankton$_{spring2}$	phytoplankton$_{summer1}$	zooplankton$_{small1}$	zooplankton$_{large1}$
$C_{max,0}$				4	1.8
EgestionCoeff$_{resource\ i}$				0.2	0.2
Exc	0.025	0.025	0.02		
FHalfSat$_{resource\ i}$				0.5	1
K_N	0.05	0.05	0.002		
K_P	0.01	0.01	0.002		
L_m	50	50	100		
$Mort_0$	0.06	0.06	0.02	0.06	0.03
$PS_{max,0}$	4	4	1.8		

XI. 6. 4. Chapter VI – studies 3 and 4

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	zooplankton$_{small1}$	zooplankton$_{large1}$	planct.fish	k
$C_{max,0}$			4	1.8	1.2	
EgestionCoeff$_{resource\ i}$			0.2	0.2	0.1	
Exc	0.025	0.02				
FHalfSat$_{resource\ i}$			0.5	1	5	
K_N	0.05	0.002				
K_P	0.01	0.002				
L_m	50	100				
$Mort_0$	0.06	0.02	0.04	0.03	0.0001	
$PS_{max,0}$	4	1.8				

XI. 6. 5. Chapter VI – study 5

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	phytoplankton$_{summer2}$	macrophyte	zooplankton$_{small1}$	zooplankton$_{small2}$	zooplankton$_{large1}$
$C_{max,0}$					4	4	1.8
EgestionCoeff$_{resource\ i}$					0.2	0.2	0.2
Exc	0.025	0.02	0.02	0.4			
FHalfSat$_{resource\ i}$					0.5	0.5	1
K_N	0.02	0.002	0.002	0.002			
K_P	0.005	0.002	0.002	0.002			
L_m	48	100	100	100			
$Mort_0$	0.06	0.02	0.02	0.001	0.06	0.03	0.03
$PS_{max,0}$	3.8	1.8	1.8	0.2			

XI. 6. 6. Chapter VI – studies 6 and 7

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	zooplankton$_{small1}$	zooplankton$_{large1}$	zooplankton$_{large2}$
$C_{max,0}$			4	1.8	1.8
EgestionCoeff$_{resource\ i}$			0.2	0.2	0.2
Exc	0.025	0.02			
FHalfSat$_{resource\ i}$			0.5	1	1
K_N	0.05	0.002			
K_P	0.01	0.002			
L_m	48	100			
$Mort_0$	0.06	0.02	0.04	0.03	0.03
$PS_{max,0}$	3.8	1.8			

XI. 6. 7. Chapter VI – study 8

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	zooplankton$_{small1}$	zooplankton$_{large1}$	zooplankton$_{large2}$	planct.fish	k
$C_{max,0}$			4	1.8	1.8	1.2	
EgestionCoeff$_{resource\ i}$			0.2	0.2	0.2	0.1	
Exc	0.025	0.02					
FHalfSat$_{resource\ i}$			0.5	1	1	5	
K_N	0.05	0.002					
K_P	0.01	0.002					
L_m	48	100					
$Mort_0$	0.06	0.02	0.04	0.03	0.03	0.0001	
$PS_{max,0}$	3.8	1.8					

XI. 6. 8. Chapter VI – study 10

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	zooplankton$_{small1}$	zooplankton$_{large1}$	planct.fish	l	k	c.fish
$C_{max,0}$			4	1.8	1.1		0.1	
EgestionCoeff$_{resource\ i}$			0.2	0.2	0.1		0.1	
Exc	0.025	0.02						
FHalfSat$_{resource\ i}$			0.5	1	0.25		0.25	
K_N	0.05	0.002						
K_P	0.01	0.002						
L_m	48	100						
$Mort_0$	0.06	0.02	0.04	0.03	0.0001		0.008	
$PS_{max,0}$	4.5	1.5						

XI. 6. 9. Chapter VI – study 11

parameter	phytoplankton$_{spring1}$	phytoplankton$_{summer1}$	phytoplankton$_{summer2}$	macrophyte	zooplankton$_{small1}$	zooplankton$_{large1}$	zooplankton$_{large2}$
$C_{max,0}$					4	1.8	1.8
EgestionCoeff$_{resource\ i}$					0.2	0.2	0.2
Exc	0.025	0.02	0.02	0.3			
FHalfSat$_{resource\ i}$					0.5	1	1
K_N	0.05	0.002	0.002	0.002			
K_P	0.01	0.002	0.002	0.002			
L_m	48	100	100	100			
$Mort_0$	0.06	0.02	0.02	0.001	0.04	0.03	0.03
$PS_{max,0}$	4.5	1.5	1.5	0.2			

XI. 6. 10. Chapters VII and VIII

parameter	$phytoplankton_{spring1}$	$phytoplankton_{summer1}$	$zooplankton_{small1}$	$zooplankton_{large1}$	$zooplankton_{large2}$	planct.fish
$C_{max,0}$			4	1.8	1.8	1.3
$EgestionCoeff_{resource\ i}$			0.2	0.2	0.2	0.158
Exc	0.025	0.02				
$FHalfSat_{resource\ i}$			0.5	1	1	5
K_N	0.05	0.002				
K_P	0.01	0.002				
L_m	45	105				
$Mort_0$	0.06	0.02	0.04	0.03	0.03	0.0001
$PS_{max,0}$	3.8	1.8				

XI. 6. 11. Chapter IX – parameters resulting from quantitative calibration

parameter	**O.M, nutrients, physicochemical**	**diatoms**	**greens**	**macrophyte**	**copepods**	**cladocerans**
Area	1					
$C_{max,0}$					1.2	2
$DecayMax_{DOM}$	0.95					
$DecayMax_{POM}$	0.04					
$DecayMax_{SOM}$	0.04					
$DecayMax_{SiOM}$	0.006					
Depth	1					
$Ecoeff_{DOM}$	0.03					
$Ecoeff_{macrophytes}$	0.05					
$Ecoeff_{phytoplankton}$	0.014					
$Ecoeff_{POM}$	0.12					
$Extinction_{water}$	0.016					
$EgestionCoeff_{resource\ i}$					0.6;0.4;0.4[a]	0.5;0.3;0.3[a]
Emort		0.04	0.06	0.01		
ESed		1.1	1.1			
Exc		0.026	0.03	0.53		
Excr						
$FHalfSat_{resource\ i}$					0.5	1.2
$K_{denitri}$	0.1					
K_N		0.003	0.004	0.005		
K_{nitri}	0.135					
K_O	0.1					
K_P		0.0003	0.002	0.002		
KT	5					
L_m		64	95	200		
$MinBio_{resource\ i}$					0.05	0
$Mort_0$		0.03	0.03	0.001	0.06	0.02
Org2Ammonia	0.079					
Org2Phos	0.018					
pH_{max}	8.5					
pH_{min}	5					
$Pref_{resource\ i}$	~					
$PS_{max,0}$		3	2.3	0.5		
Q10		1.8	2	3.1	2.6	2.6

[a]Egestion coefficients differ between food sources.
The egestion coefficients are given in the order: particulate organic matter; green algae; diatoms

parameter	**O.M, nutrients, physicochemical**	**diatoms**	**greens**	**macrophyte**	**copepods**	**cladocerans**
Resp					0.2	0.18
Resp0		0.022	0.006	0.02	0.014	0.014
Sed	0.18 for POM	0.04	0.08			
TempResp	0.065					
T_{max}	60 for process	30	42	44	34	36
T_{obs}	25					
T_{opt}		10	25	15	26	30
T_{ref}						
Volume	1					
XM	5					

XI. 7. Justification of the used NOEC data and grouping of species into model populations in chapter VI

In chapter VI, a comparison is made between predicted no observed effect concentrations (NOECs) and experimentally derived NOECs reported in the literature. Per population, one NOEC is derived, and compared with an experimentally derived NOEC for that population. However, in most papers, multiple NOECs may be given per population, or NOECs have to be derived from the reported observations. To strengthen the results of this validation study, this has been done in one consistent way, which is described in what follows.

XI. 7. 1. General grouping rules:

XI. 7. 1. 1. zooplankton

Large bodied cladocerans and copepods both belong to 'large zooplankton'; rotifers and small cladocerans both belong to 'small zooplankton'. In cases where the single-species sensitivity of cladocerans and copepods is different, they are modelled as two separate model populations. The cut-off value for body size of zooplankton was 0.7 mm. A useful website on zooplankton taxonomy is http://planktonweb.ifas.ufl.edu/taxonomy.htm.

XI. 7. 1. 2. phytoplankton

Phytoplankton species were modelled as spring or summer populations, depending on (1) the species given in Sommer *et al*. (1986) or (2) on the reported dynamics in the paper of the considered study.

XI. 7. 2. Detailed overview of assigned NOECs (µg L^{-1})

Study 1: Boyle *et al*. (1996)

Spring phyto 1: NOEC $\geq$ 10 µg L^{-1}
Summer phyto 1: NOEC < 10 µg L^{-1}
Small zoo 1: rotifers: NOEC < 10 µg L^{-1}
Large zoo 1: copepods: NOEC < 10 µg L^{-1}

Large zoo 2: cladocerans: NOEC < 10 µg L^{-1}
Planct. fish: bluegill: NOEC < 10 µg L^{-1}
Pisc. fish: bass: NOEC ≥ 10 µg L^{-1}

In this paper, two different treatment regimes, both with the same exposure concentration (10 µg L^{-1}), resulted in the same observed biological effects. Rotifers, copepods, and cladocerans were affected at 10 µg L^{-1}. The treatment had no significant effect on the chlorophyll concentration during spring. However, during summer, there was a significant effect of 10 µg L^{-1} on chlorophyll concentration. Biomass of bass was not significantly different between treatments and controls. In contrast, the biomass of its prey, bluegill, was significantly different between treatments and controls.

Study 2: Hamilton *et al.* (1988)

Spring phyto 1: diatoms: NOEC < 100 µg L^{-1}
Spring phyto 2: chryptophyta and chrysophyta excluding diatoms: NOEC≥ 100 µg L^{-1}
Summer phyto 1: dinoflagellates and chlorophyta: NOEC < 100 µg L^{-1}
Small zoo 1: rotifers: NOEC ≥ 100 µg L^{-1}
Large zoo 1: copepods and cladocerans: NOEC ≥ 100 µg L^{-1}

Diatoms experienced a significant abundance decrease for 50 days. Abundance of chryptophyta and chrysophyta excluding diatoms did never exhibit a statistical difference, except on two sampling dates. Chlorophyta abundance was reduced at 60% of the sampling dates. Dinoflagellate abundance showed a significant decrease in 66% of the sampling dates after a second application. Out of the six rotifer species present, only one changed in abundance on three out of 7 sampling dates. Although the abundance of cladocerans and copepods was consistently 50% lower in the exposure concentrations, no significant effects could be detected.

Study 3: Webber *et al.* (1992)

Spring phyto 1: NOEC = 0.18 µg L^{-1}
Summer phyto 1: NOEC = 0.18 µg L^{-1}
Small zoo 1: copepod nauplii and rotifers: NOEC ≥ 0.69 µg L^{-1}
Large zoo 1: cladocerans and copepods: NOEC = 0.18 µg L^{-1}
Planct. fish: bluegill: NOEC ≥ 0.69 µg L^{-1}

Phytoplankton increased at the highest concentration (0.69 µg L^{-1}). Nauplii were characterized by very low abundance compared to rotifer abundance. We thus considered the NOEC for rotifers to be representative for this model population. Cladocerans and copepods were absent at concentrations above 0.18 µg L^{-1}. Population size (number and weight) of bluegill was similar for all treatments.

Study 4: Fairchild *et al.* (1992)

Spring phyto 1: NOEC = 0.25 µg L^{-1}
Summer phyto 1: NOEC = 0.25 µg L^{-1}
Small zoo 1: rotifers: NOEC $\geq$ 1.71 µg L^{-1}
Large zoo 1: copepods and cladocerans: NOEC $\geq$ 1.71 µg L^{-1}
Planct. fish: bluegill: NOEC = 0.67 µg L^{-1}

A decrease of phytoplankton was appreciated at 0.67 µg L^{-1}. Rotifers did not decrease at any concentration. During the exposure period, the zooplankton mainly consisted of copepods. No significant effects on abundance of copepods and cladocerans were observed. On 4 out of 6 treatment days, there was an increased mortality of bluegill at 1.71 µg L^{-1}. Because details on bluegill biomass were not provided, a NOEC of 0.67 µg L^{-1} was assumed.

Study 5: Brock *et al.* (2004)

(Spring phyto 1: *Fragilaria ulna*: NOEC < 1.8 µg L^{-1})
Summer phyto 1: *Gomphonema sp*: NOEC = 56 µg L^{-1}
Summer phyto 2: *Anabaena cylindrica*: NOEC = 18 µg L^{-1}
Macrophyte: *Myriophillum spicatum*: NOEC $\geq$ 180 µg L^{-1}
Small zoo 1: *Chydorus sphaericus, Lecane sp., Mytilana ventralis, Polyarthra remata*: NOEC = 18 µg L^{-1}
Small zoo 2: *Polyarthra remata* and *Trichocerca*: NOEC $\geq$ 180 µg L^{-1}
Large zoo 1: other species: NOEC $\geq$ 180 µg L^{-1}

In this study, NOECs were derived for every sampling date. The date-specific NOEC with the highest frequency of occurrence was taken. If the frequency of occurrence was equal for two or more NOEC-values, the lowest NOEC was chosen. The same strategy was followed if species belonging to the same model population had different NOECs.

The NOEC of < 1.8 µg L^{-1} for *Fragilaria ulna* was not accounted for due to the low abundance of this species. Moreover, this NOEC was reported as unreliable by Brock *et al.* (2004). Amongst the phytoplankton, only the more or less prolonged differences observed for *Anabaena cf. cylindrica* and *Gomphonema pumilum* were clearly treatment-related (Brock *et al*., 2004). No consistent treatment-related effects were observed for the macrophyte. Significant deviations from control for large zooplankton were only established on 1 sampling date. Brock *et al*. consider this effect to be only short-lived.

Study 6: Sierzen and Lozano (1998)

Spring phyto 1: NOEC ≥ 20 µg L^{-1}
Summer phyto 1: NOEC ≥ 20 µg L^{-1}
Small zoo 1: rotifers: NOEC ≥ 20 µg L^{-1}
Large zoo 1: copepods: NOEC ≥ 20 µg L^{-1}
Large zoo 2: cladocerans: NOEC = 4 µg L^{-1}

Based on the taxonomical determination of the cladoceran species, cladocerans consisted mainly out of large zooplankton. The total abundance of cladocerans was affected at nearly all sampling dates at 20 µg L^{-1}, resulting in a NOEC of 4 µg L^{-1}. There were no strong responses of total copepod or rotifer abundance to any pesticide application. No effects on phytoplankton were reported.

Study 7: Hanazato and Kasai (1995)

Spring phyto 1: cryptophyceae and centrales: NOEC ≥ 200 µg L^{-1}
Summer phyto 1: : chlorococcales and volvocales: NOEC < 20 µg L^{-1}
Small zoo 1: rotifers: NOEC ≥ 200 µg L^{-1}
Large zoo 1: copepods: NOEC = 20 µg L^{-1}
Large zoo 2: cladocerans: NOEC < 20 µg L^{-1}

Rotifers did not exhibit a decrease at the tested concentrations. Copepod abundance was reduced significantly by the high-dose (200 µg L^{-1}) treatment. Cladocerans were mostly large-bodied and their abundance was depressed markedly at the lowest treatment concentration (20 µg L^{-1}). Spring phytoplankton predominantly consisted of centrales, which were not affected at any concentration. Summer phytoplankton increased in all treatments concentrations.

Study 8: Tanner and Knuth (1995)

Spring phyto 1: NOEC $\geq$ 4 µg L^{-1}
Summer phyto 1: NOEC $\geq$ 4 µg L^{-1}
Small zoo 1: rotifers: NOEC $\geq$ 4 µg L^{-1}
Large zoo 1: cladocerans: NOEC $\geq$ 4 µg L^{-1}
Large zoo 2: copepods: NOEC $\geq$ 4 µg L^{-1}
Planct. fish: bluegill: NOEC $\geq$ 4 µg L^{-1}

Cladocerans, copepods, and rotifers exhibited a succession of peaks and only copepodites were significantly reduced one week after the treatment, after which they returned to control levels. Azinphos-methyl did not affect biomass of bluegills at any concentration. No effects were reported on phytoplankton.

Study 9: Juttner *et al*. (1995)

Spring phyto 1: diatoms: NOEC = 68 µg L^{-1}
Spring phyto 2: cryptophyceae: NOEC = 68 µg L^{-1}
Summer phyto 1: other species: NOEC = 68 µg L^{-1}
Small zoo 1: copepod nauplii and rotifers: NOEC = 182 µg L^{-1}
Large zoo 1: cladocerans: NOEC = 68 µg L^{-1}

Cryptophyceae decreased at concentrations > 68 µg L^{-1}. Diatoms increased at concentrations > 68 µg L^{-1}. Other phytoplankton species decreased at concentrations above 68 µg L^{-1}. Copepod nauplii and rotifers had different NOECs, and following the general rule the NOEC was 182 µg L^{-1}. Cladocerans were found to decrease in all enclosures, but no information is given on the significance of this trend. Since indirect effects (reduced food abundance) are given as an explanation for this phenomenon, the same NOEC as its food source was assumed.

Study 10: Denoyelles *et al.* (1982)

Spring phyto 1: *Cryptomonas sp.* and *Mallomonas sp.*: NOEC = 20 µg L^{-1}
Summer phyto 1: *Peridinium sp.*: NOEC = 20 µg L^{-1}
Small zoo 1: rotifers: NOEC $\geq$ 500 µg L^{-1}

Large zoo 1: 75% copepods and 25% cladocerans: NOEC = 20 µg L^{-1}
Planct. fish: bluegill: NOEC ≥ 500 µg L^{-1}
Pisc. fish: bass: NOEC ≥ 500 µg L^{-1}

An increase in abundance of *Cryptomonas sp.* and *Mallomonas sp*, was observed. However, it is unclear at what concentration this trend became significant. At 20 µg L^{-1}, the increase occurs in the first 10 days of the treatment period. After this 10-day period, the abundance of *Cryptomonas sp.* and *Mallomonas sp* returns to control levels. At 500 µg L^{-1}, there is a decrease during the first 10 days. However, after this 10-day period, there is an increase in abundance of these species during 40 days. *Peridinium sp.* is absent at the 500 µg L^{-1} treatment. Rotifers do not decrease at any concentration. Copepods (75%) and cladocerans declined in the 500 µg L^{-1} treatment. Responses higher up the food chain than zooplankton were not reported.

Study 11: Cuppen *et al.* (1997) and Vandenbrinck *et al.* (1997).

Spring phyto 1: *Cocconeis*: NOEC =50 µg L^{-1}
Summer phyto 1: *Chlamydomonas*: NOEC = 15 µg L^{-1}
Summer phyto 2: *Phormidium foveolarum*: NOEC = 50 µg L^{-1}
Macrophyte: *Elodea nuttallii*: NOEC = 15 µg L^{-1}
Small zoo 1: rotifers: NOEC = 50 µg L^{-1}
Large zoo 1: cladocerans: NOEC ≥ 150 µg L^{-1}
Large zoo 2: copepods: NOEC ≥ 150

The dominant species in the zooplankton samples belonged to the groups of cladocerans, copepods, and rotifers, while ostracods occurred in low numbers. For that reason, ostracods were not considered in this paper. The NOEC for decrease of rotifers in week 1-4 post treatment was 15. However, in week 5 to 11 post treatment, a NOEC of 50 was observed, suggesting recovery. Since predicted NOECs are based on changes in mean biomass, the NOEC was considered equal to 50. Cladocerans and copepods never declined in abundance because of chemical treatment, resulting in a NOEC ≥ 150. Compared to the control microcosms, a nonsignificant increase in biomass of *E. nuttallii* was observed in the microcosms treated with the lowest two doses. A significant decrease in those with the two highest doses led to a NOEC of 15 µg L^{-1}. Phytoplankton NOECs are listed in Vandenbrinck *et al.* (1997).

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Summary

Concurrently with the increasing human population and its associated activity, the number of chemical substances found in water bodies has augmented substantially during the last century. Because these different chemicals have a wide range of biological modes of action, it is not unlikely that their presence in water bodies results in adverse effects on aqueous ecosystems. Within the field of ecotoxicology, ecological effect assessment of chemicals aims at assessing and predicting these effects. It has become a routine practice to perform such assessments solely relying on single-species toxicity test results. Results from this type of tests reflect the **direct effect** of a chemical on one isolated species in a laboratory setting. These test results are mostly expressed as concentrations resulting in x% effect (EC_x) on a given test population. Current approaches to extrapolate single-species toxicity test results to ecosystem-level effects are based on a set of largely untested assumptions which **ignore ecological interactions** between different populations. One such an approach is to use a statistical distribution of single-species toxicity test results of different species for a given chemical, termed species sensitivity distribution (SSD).

It has been shown that ecological effects are determined by (1) **ecological interactions** and (2) **direct effects**. Hence, predictions of ecological effects by current extrapolation methods (e.g. the use of SSDs) will most likely be inaccurate. Therefore, ecological effect assessments relying on such inaccurate predictions result in accurate assessments of chemical risk to aquatic ecosystems.

The use of **dynamic ecosystem models** has been proposed as an alternative to current approaches. These models may consist of (1) a food web model; (2) toxic effect sub-models; and (3) a model for nutrient and detritus cycling. The advantage of these models in comparison to current approaches is that they **can account for ecological interactions** between populations by incorporating feeding and competition relationships. Unfortunately, no information exists on which type of toxic effect sub-model results in the most accurate predictions of an ecosystem model. Also, quantitative validations of effects predicted by such ecosystem models are scarce. As a result, it is unknown if and how ecosystem models can contribute to ecological effects assessments.

Because current approaches for ecological effect assessments rely on the relationship between single-species toxicity test results and effects on ecosystems, this dissertation starts with a review of studies which were designed to examine this relationship (**chapter II**). These studies can be divided in (1) experimental ecosystem studies and (2) studies using ecosystem models. For both study types, changes in abundances or biomasses of populations were most frequently studied.

Experimental ecosystem studies with insecticides report EC_xs for invertebrate species (with biomass or abundance as endpoint) which differ less than a factor two of single-species EC_xs for the same species based on the same endpoint. Results from the few modelling studies found indicate that EC_xs within an ecosystem can be lower than corresponding single-species EC_xs. The overestimated effects produced by the reviewed ecosystem models and the focus on toxicants for which prey are more sensitive than predators can explain the difference between results from experimental ecosystem studies and results from modelling studies.

In **chapter III and IV** the development and application of a new ecosystem model is described. A dynamic ecosystem model is constructed in such a way that it can be customized to represent different lentic (i.e. non-running) aquatic ecosystems. Also the toxic effect sub-models can be customized. The ecosystem model aims at accurately predicting ecological effects, rather than pursuing the exact replication of observed population dynamics. Ecological effects of copper, which were observed in a previously conducted ecosystem study, were predicted accurately by the developed ecosystem model (**chapter IV**). In contrast, extrapolation based on single-species toxicity test results alone did not accurately predict ecosystem effect levels.

While a default toxic effect sub-model was chosen in chapter IV, an effort was made in **chapter V** to determine which toxic effect sub-model is most suited for the developed ecosystem modelling approach. To this end, four ecosystem models were constructed, each with a different toxic effect sub-model. The capacity of each of these models to predict biomass changes and no observed effect concentrations (NOECs) established in an experimental microcosm was evaluated. The ecosystem model with a toxic effect sub-model incorporating effects on zooplankton mortality using a logistic concentration-effect function was superior to the other three models since it made accurate NOEC predictions for most populations. Additional incorporation of sub-lethal effects on zooplankton did not result in better predictions. Ecosystem models using linear concentration-effect functions predict biomass decreases already occurring at concentrations which are 4 times lower than the observed NOECs.

In **chapter VI** the ecosystem model which gave the best predictions in chapter V was further validated using literature data. Predicted NOECs were compared with population and ecosystem - NOECs observed in 11 experimental ecosystems. For each of those studies, the model was customized to account for the specific ecological interactions within the studied system. Population-NOEC predictions were accurate, or at least protective (i.e. smaller than the observed

one), for 60 and 86% of all considered populations, respectively. For all 11 studies, a protective ecosystem-NOEC could be derived, i.e. accurate and conservative predictions in 8 and 3 cases, respectively. It was concluded that the inclusion of the relevant populations and taking the median of model outputs can increase the accuracy of model predictions.

In **chapter VII** the validity of a theoretical assumption underlying species sensitivity distributions (SSDs), used for deriving "safe concentrations" based on single-species toxicity test results, was examined in a simple freshwater lentic pelagic ecosystem. The tested assumption was that ecological interactions do not alter a sensitivity distribution. For each of 1000 hypothetical toxicants, a lognormal SSD was fitted to chronic single-species toxicity test results, i.e. without taking into account ecological interactions and therefore termed 'conventional SSD' (cSSD). Next, corresponding sensitivity distributions, which do take ecological interactions into account, were constructed (eco-SSDs) using the ecosystem modelling-approach described and validated in the previous chapters. For 254 of the 1000 hypothetical toxicants, mean and/or variance of the cSSD were significantly higher than mean and/or variance of the eco-SSD, as such rejecting the general validity of the tested assumption. A classification tree approach further indicated that especially for toxicants exerting direct effects on phytoplankton (e.g. herbicides), the cSSD may have a higher mean than the eco-SSD. Conversely, means of eco-SSD and cSSD are likely to be equal for toxicants targeting zooplankton and fish.

A second theoretical assumption underlying SSDs was tested in **chapter VIII**. Here, the tested assumption was that ecosystem structure (i.e. species composition) is as or more sensitive than ecosystem function. This test was performed using the same ecosystem as that used in chapter VII. NOECs were calculated for ecosystem structure and function for each of the 1000 hypothetical toxicants. For 979 of these toxicants, the ecosystem structure-NOEC was lower than or equal to the ecosystem function-NOEC, indicating that the tested assumption is valid. For 239 of these 979 toxicants, both NOECs were equal. For half of the 1000 toxicants, structure of lower trophic levels (i.e. phytoplankton) appears to be more sensitive than structure of higher trophic levels (i.e. fish). As such, ecosystem structure-NOECs are primarily determined by the sensitivity of the structure of lower trophic levels. In contrast, ecosystem functions associated with higher trophic levels (e.g., total ingestion by fish) are more sensitive than functions associated with lower trophic levels (e.g., total photosynthesis by phytoplankton) for 749 toxicants. Top-down regulation of ecosystem structure and cascading effects from lower trophic level functions to

higher trophic level ecosystem functions are discussed as possible explanations for these two contrasting findings.

When applying SSDs in ecological effect assessments, it is generally accepted that a concentration corresponding to a percentile y of this SSD is a hazardous concentration for y% of the species within an ecosystem (HC_y). To elucidate the ecological significance of this concept, the ecosystem model developed and validated in the previous chapters was used in a practical ecosystem study (**chapter IX).** The ecological effects of different HC_ys of copper on ecosystem structure (biomass) and function (photosynthesis of phytoplankton, $PS_{all\ phytoplankton}$; and ingestion by zooplankton, $I_{all\ zooplankton}$) were estimated with the developed ecosystem model. Zooplankton biomass and the associated ecosystem function rate ($I_{all\ zooplankton}$) remained unaffected when the system was exposed to concentrations $\leq HC_{30}$ of an SSD based on EC_{20}s derived from single-species tests. Phytoplankton biomass and $PS_{all\ phytoplankton}$ increased at concentrations $> HC_5$ or HC_{30} of an SSD based on EC_{20}s or EC_{10}s, respectively. Thus, exposing the ecosystem studied to other percentiles than the commonly chosen HC_5 does not necessarily result in ecological effects on 5% of the species.

A summary of the conclusions drawn in the different chapters is provided in **chapter X**. In general, it is concluded that the type of ecosystem models constructed in this dissertation can serve as an ecology-based method to accurately **predict ecological effects**, provided that the relevant populations are included in the model and a logistic toxic effect sub-model is used to integrate direct effects on mortality of animals. The use of ecosystem models in ecological effect assessments benefits from the limited data needs of such models. The amount of standard single-species toxicity test results needed to use such an approach is comparable to the amount of data needed to apply conventional extrapolation approaches. Moreover, the models developed here can also **support** current extrapolation approaches. The validity of the assumptions underlying these extrapolation approaches is related to the toxicant type. For toxicants directly targeting zooplankton and fish, these assumptions are likely to be valid, while the opposite holds for toxicants directly targeting phytoplankton. These findings, together with results from experimental ecosystem studies aid in understanding the significance of current extrapolations approaches. As such, they can assist risk assessors in applying approaches that more accurately predict no effect concentrations for chemicals.

Samenvatting

De stijgende bevolkingsdruk en de daarmee gepaard gaande menselijke activiteiten maakt dat het aantal chemische stoffen in oppervlaktewaters is toegenomen. Deze stoffen hebben een waaier aan chemische en toxicologische eigenschappen waardoor het niet onwaarschijnlijk is dat ongewenste effecten optreden in ecosystemen. In het vakgebied van de ecotoxicologie beoogt ecologische effectenevaluatie inzicht te krijgen in hoe deze effecten ontstaan teneinde ze beter te kunnen inschatten of voorspellen. Effectenevaluaties zijn meestal enkel gebaseerd op resultaten van "single-species" tests. Zulke single-species tests laten toe om **directe effecten** van chemicaliën op één geïsoleerd species te onderzoeken in gecontroleerde omstandigheden. Resultaten van deze tests worden meestal uitgedrukt als concentraties die x% effect hebben op een test populatie (EC_x). Huidige methodes om meerdere single-species EC_x-waarden te extrapoleren naar ecosysteemeffecten, bijvoorbeeld de soorten-gevoeligheidsdistributie (SGD), zijn gebaseerd op een aantal ongeteste veronderstellingen die **ecologische interacties** tussen populaties van species **niet in rekening** brengen. Nochtans is bekend dat ecologische effecten bepaald worden door (1) **ecologische interacties** en (2) **directe effecten** op populaties in het ecosysteem. Daarom is het onwaarschijnlijk dat voorspellingen van ecologische effecten door middel van huidige extrapolatietechnieken (bvb SGDs) accuraat zijn. Bijgevolg kunnen ook afgeleide ecologische risico's in twijfel worden getrokken.

Een alternatief voor deze momenteel toegepaste technieken kan het gebruik van **dynamische ecosysteemmodellen** zijn. Dergelijke ecosysteemmodellen bestaan uit (1) een voedselweb model; (2) een toxisch effect sub-model: en (3) een model voor de detritus- en nutriëntencyclus. Het voordeel van een dergelijke aanpak ten opzichte van huidige technieken is dat **ecologische interacties** tussen populaties in rekening kunnen worden gebracht door incorporatie van processen zoals consumptie en competitie. Helaas bestaat er geen informatie over welk type toxisch effect sub-model moet gebruikt worden opdat een ecosysteemmodel accurate voorspellingen oplevert. Ook kwantitatieve validatie-oefeningen zijn schaars in de beschikbare literatuur. Bijgevolg is het onvoldoende gekend of en hoe ecosysteemmodellen kunnen gebruikt worden in ecologische effectenevaluaties.

Aangezien huidige extrapolatietechnieken gebaseerd zijn op de relatie tussen resultaten van single-species tests en ecosysteemeffecten, wordt in deze thesis een overzicht gegeven van de studies die deze relatie onderzoeken (**hoofdstuk II**). Deze studies kunnen onderverdeeld worden in (1) experimentele ecosysteemstudies en (2) studies met ecosysteemmodellen. Beide types studies behandelen meestal veranderingen in abundantie (of biomassa) van een soort als gevolg

van blootstelling aan chemicaliën. Resultaten van experimentele ecosysteemstudies duiden aan dat voor insecticiden, EC_x-waarden van invertebraten binnen een ecosysteem (mét ecologische interacties) maximaal een factor twee verschillen van deze afgeleid in single-species tests met deze groep organismen (zonder ecologische interacties). Anderzijds leren de schaarse studies die gebruik maken van ecosysteemmodellen ons dat de EC_x voor biomassa of abundantie binnen een ecosysteem ook lager kan zijn dan de EC_x afgeleid uit een single-species test. De chemicaliën die in deze studies bestudeerd werden hadden grote indirecte effecten op predators, waardoor het verschil tussen (directe) effecten in een single-species test en (directe en indirecte) effecten in een ecosysteem vergroot wordt.

In **hoofdstukken III en IV** wordt de ontwikkeling van een nieuw ecosysteemmodel beschreven. Een dynamisch ecosysteemmodel is zo opgebouwd dat het op eenvoudig wijze kan aangepast worden om het dynamisch gedrag van verschillende ecosystemen te simuleren. Ook sub-modellen voor effecten van toxicanten kunnen aangepast worden al naargelang de toepassing. Het ecosysteemmodel is bedoeld om op een accurate wijze ecologische effecten te voorspellen, eerder dan de geobserveerde populatiedynamiek exact te reproduceren. In een demonstratie van het ecosysteemmodel (**hoofdstuk IV**) werden ecologische effecten van koper in een experimentele ecosysteemstudie accuraat voorspeld. Resultaten van single-species tests alleen bleken daartoe niet in staat.

Een standaard sub-model voor toxische effecten werd gekozen in hoofdstuk IV. In **hoofdstuk V** echter werd onderzocht welke sub-modellen het best geïncorporeerd worden in het ecosysteemmodel opdat accurate voorspellingen zouden bekomen worden. Daartoe werden vier ecosysteemmodellen geconstrueerd met elk een ander sub-model voor toxische effecten. De voorspellende kracht van elk van deze modellen werd geëvalueerd door voorspelde 'no observed effect concentrations' (NOECs) te vergelijken met NOECs waargenomen in een experimentele ecosysteemstudie. Het ecosysteemmodel met een sub-model voor toxische effecten dat effecten op mortaliteit van zooplankton beschrijft met een logistische functie bleek te resulteren in de beste predicties. Het additioneel incorporeren van subletale effecten op zooplankton in een sub-model voor toxische effecten verhoogde de predictieve capaciteit niet. Het gebruik van lineaire functies in sub-modellen voor toxische effecten resulteerde in NOEC voorspellingen die een factor 4 lager lagen dan de geobserveerde NOECs.

In **hoofdstuk VI** werd het ecosysteemmodel dat de beste predicties gaf in hoofdstuk V onderworpen aan een grootschalige validatie met data uit de literatuur. Voorspelde NOECs werden vergeleken met populatie- en ecosysteem-NOECs gerapporteerd in 11 experimentele ecosysteemstudies. Het ecosysteemmodel werd telkens aangepast opdat het rekening zou houden met de aanwezige populaties in de experimentele ecosystemen. Populatie-NOECs werden accuraat voorspeld in 60% van de gevallen en waren beschermend in 86% van de gevallen, i.e. lager dan de experimentele populatie-NOECs. Voor alle 11 studies kon een beschermende ecosysteem-NOEC voorspeld worden, bovendien was deze accuraat voorspeld in 8 van de 11 gevallen.

In **hoofdstuk VII** werd een theoretische veronderstelling die aan de basis ligt van de SGD techniek, gebuikt voor de extrapolatie van single-species EC_x-waarden naar een veilige concentratie in het milieu, getoetst in een eenvoudig ecosysteem. De geteste veronderstelling was dat ecologische interacties de gevoeligheidsdistributie niet beïnvloeden. Voor 1000 hypothetische toxicanten werd een lognormale SGD gefit aan single-species EC_{10}s. Merk op dat deze SGD geen ecologische interacties bevat en daarom de naam 'conventionele SGD' krijgt (cSGD). Vervolgens werden zogenaamde eco-SGDs geconstrueerd voor elk van de 1000 toxicanten. Deze eco-SGDs brengen ecologische interacties wél in rekening, gezien ze berekend werden met het ecosysteemmodel dat in vorige hoofdstukken ontwikkeld en gevalideerd werd. Voor 254 van de 1000 toxicanten bleken het gemiddelde en/of de variantie van de cSGD hoger dan deze van de eco-SGD. Bijgevolg werd besloten dat de geteste veronderstelling niet algemeen geldig is, i.e. ecologische interacties beïnvloeden wel degelijk de SGD. Een additionele analyse met classificatieboom-technieken toonde aan dat dit vooral het geval is voor toxicanten die een direct effect uitoefenen op fytoplankton (bvb herbiciden). Voor toxicanten die een direct effect uitoefenen op zooplankton en vissen (bvb insecticiden) werd de geteste veronderstelling wel geldig bevonden.

Een tweede theoretische veronderstelling die aan de basis ligt van de SGD techniek werd getoetst in **hoofdstuk VIII**. In hetzelfde ecosysteem als dat beschouwd in het vorige hoofdstuk werd de veronderstelling getest dat de structuur van een ecosysteem (i.e. zijn samenstelling) gevoeliger is dan de functies die het vervult. Deze studie werd opnieuw uitgevoerd door gebruik te maken van het voorheen ontwikkelde en gevalideerde ecosysteemmodel. NOECs werden voorspeld voor zowel de structuur als de functies van het beschouwde ecosysteem en dit voor 1000 hypothetische toxicanten. Voor 979 gevallen bleek de geteste veronderstelling correct, aangezien de NOEC voor

structuur lager lag dan de NOEC voor functie. Voor de helft van de 1000 toxicanten bleek de structuur van lagere trofische niveaus (bijvoorbeeld fytoplankton biomassa) gevoeliger dan de structuur van hogere trofische niveaus (bijvoorbeeld biomassa van vissen). Bijgevolg kan men stellen dat de gevoeligheid van de structuur van het gehele ecosysteem vaak bepaald wordt door de gevoeligheid van de structuur van de lagere trofische niveaus. Voor ecosysteemfuncties werd net het omgekeerde gevonden: ecosysteemfuncties geassocieerd met hogere trofische niveaus (bijvoorbeeld consumptie door vissen) waren gevoeliger dan functies geassocieerd met lagere trofische niveaus (bijvoorbeeld primaire productie) in 749 van de 1000 gevallen. Top-down regulatie en een cascadeproces van hogere naar lagere trofische niveaus worden gesuggereerd als mogelijke verklaringen voor de geobserveerde verschillen.

Wanneer SGDs worden gebruikt voor ecologische effectenevaluatie neemt men meestal aan dat een percentiel y van deze SGD een gevaar inhoudt voor y% van de species in een ecosysteem. Daarom wordt deze concentratie de 'hazardous concentration' voor y% van de species genoemd (HC_y). Om de ecologische betekenis van deze HC_y te onderzoeken werd het voorheen ontwikkelde en gevalideerde ecosysteemmodel gebruikt in een praktische ecosysteemstudie (**hoofdstuk IX**). De effecten op ecosysteemstructuur (biomassa) en functie (fotosynthese door fytoplankton, $PS_{\text{alle fytoplankton}}$ en ingestie door zooplankton, $I_{\text{alle zooplankton}}$) die optreden wanneer een eenvoudig ecosysteem blootgesteld wordt aan verschillende HC_ys voor koper werden voorspeld. Biomassa van zooplankton en $I_{\text{alle zooplankton}}$ vertoonden geen nadelige effecten wanneer het ecosysteem werd blootgesteld aan concentraties $\leq HC_{30}$ van een SGD gebaseerd op EC_{20}s. Biomassa van fytoplankton en $PS_{\text{alle fytoplankton}}$ waren hoger bij concentraties > HC_5 of HC_{30} van een SGD respectievelijk gebaseerd op EC_{20}s en EC_{10}s. Bijgevolg kan gesteld worden dat koperconcentraties verschillend van de HC_5 niet noodzakelijkerwijs resulteren in ecologische effecten op 5% van de aanwezige species.

In **hoofdstuk X** wordt een overzicht gegeven van de conclusies bekomen in de verschillende hoofdstukken en wordt een algemeen besluit geformuleerd. Er werd geconcludeerd dat de ecosysteemmodellen die in deze thesis werden geconstrueerd kunnen gebruikt worden voor het **voorspellen** van **ecologische effecten van chemicaliën**. Voorwaarden voor het slagen van dergelijke aanpak zijn dat de relevante populaties in het model opgenomen worden en een logistisch toxisch effect sub-model gebruikt wordt. De datavereisten van de ontwikkelde benadering zijn beperkt en vergelijkbaar met de hoeveelheid data die huidige technieken vereisen. Naast een voorspellende rol kunnen de ontwikkelde modellen ook **een ondersteunende rol**

spelen voor huidige statistische extrapolatietechnieken. Zo bleek de geldigheid van enkele theoretische veronderstellingen die aan de basis liggen van een huidige extrapolatietechniek afhankelijk te zijn van het type toxicant. Voor toxicanten die een direct effect uitoefenen op zooplankton en vis bleken de geteste veronderstellingen correct. Voor toxicanten die een direct effect uitoefenen op fytoplankton was dit niet het geval. Deze conclusies, samen met de resultaten van de praktische ecosysteemstudie, laten toe de beperkingen en toepassingen van de huidige extrapolatietechnieken te evalueren. Tenslotte kan gesteld worden dat de ontwikkelde modellen toelaten om ecologische effecten van chemicaliën op een meer accurate manier in te schatten.

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Personalia

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Professional employment

2003-now: Scientific researcher at the Laboratory for Environmental Toxicology
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Publications

Peer-reviewed articles (A1):

1. **De Laender F**., De Schamphelaere K.A.C., Verdonck F.A.M., Heijerick D.G., Van Sprang P.A., Vanrolleghem P.A., Janssen C.R. (2005). Simulation of spatial and temporal variability of chronic copper toxicity to Daphnia magna and Pseudokirchneriella subcapitata in Swedish and British surface waters. *Human and Ecological Risk Assessment*, 11: 1177–1191.

2. Deleebeeck N.M.E, Muyssen B.T.A., **De Laender F**., Janssen C.R., De Schamphelaere K.A.C. (2007). Comparison of nickel toxicity to cladocerans living in soft versus hard surface waters. Ecotoxicology and Environmental Safety, *Aquatic Toxicology, accepted*.

3. **De Laender F**, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Comparison of different toxic effect sub-models in ecosystem modelling used for ecological effect assessments and water quality standard setting, *Ecotoxicology and Environmental Safety, in press.*

4. **De Laender F**, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Ecological significance of different SSD-percentiles for copper in a simple ecosystem. *Ecotoxicology and Environmental Safety, submitted.*

5. **De Laender F**, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Validation of an ecosystem modelling approach as a tool for ecological effect assessments. *Chemosphere, accepted.*

6. **De Laender F**, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Do ecological interactions change the species sensitivity distribution of a simple freshwater ecosystem? A validation of a theoretical assumption underlying species sensitivity distribution models. *Environment International, accepted.*

7. **De Laender F**, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Is ecosystem structure the target of concern in ecological effect assessments? A validation of a theoretical assumption underlying species sensitivity distribution models. *Environmental Science and Technology, submitted.*

8. **De Laender F**., De Schamphelaere, K.A.C., Vanrolleghem, P.A., Janssen, C.R. Relating single-species toxicity test results to ecosystem effects: a review of current methodologies. *Ecotoxicology and Environmental Safety, submitted.*

9. **De Laender F**, De Schamphelaere K.A.C., Janssen C.R., Vanrolleghem P.A. An ecosystem modelling approach for deriving water quality criteria. Water Science and Technology (56) 6, *in press.*

Peer-reviewed conference proceedings

10. **De Laender F**., Goethals P.L.M., Vanrolleghem P.A., Janssen C.R. (2005). Ecological effect assessment through the combination of mechanistic and data driven models. Proceedings of Cost Action 636 meeting, 18-22 April 2005, Silkeborg, Denmark

Platform presentations (presenting author)

1. De Laender F, De Schamphelaere K.A.C., Schaefers C., Vanrolleghem P.A., Janssen C.R. Understanding copper effects on the functioning of aquatic ecosystems: a modelling approach. *15th Annual Meeting of SETAC-Europe, 23-26 April 2005, Lille, France.*

2. De Laender F, Goethals P.L.M.,Vanrolleghem P.A., Janssen C.R. Ecological effect assessment through the combination of mechanistic and data driven models. *COST 626 meeting, 18-22 April 2005, Silkeborg, Denmark.*

3. De Laender F, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Derivation of safe concentration for freshwater communities using modelling. *16th Annual Meeting of SETAC-Europe, 7-11 May 2006, The Hague, Netherlands.*

4. De Laender F, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Can ecosystem models improve ecological risk assessment and water quality criteria setting? *27th Annual Meeting of SETAC-North America,* 5-9 November 2006, *Montreal, QC, Canada.*

5. De Laender F, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Do we have to incoporate ecological interactions in the sensitivity assessment of ecosystems? An examination of the SSD approach using ecosystem modelling; *17th Annual Meeting of SETAC-Europe, 20-24 May 2007, Porto, Portugal.*

6. De Laender F, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Validation of ecosystem modelling as a tool for ecological effect assessments; *17th Annual Meeting of SETAC-Europe, 20-24 May 2007, Porto, Portugal.*

7. De Laender F, De Schamphelaere K.A.C., Janssen C.R., Vanrolleghem P.A. Validation of ecosystem modelling as a tool for ecological effect assessments. *7th International IWA Symposium on Systems Analysis and Integrated Assessment in Water Management, 7- 9 May, 2007, Washington DC - USA.*

Poster presentations (presenting author)

1. De Laender F., Verdonck F.A.M., De Schamphelaere K.A.C., Janssen C.R. and Vanrolleghem P.A. Geography- referenced bioavailability modelling in risk assessment: a case study of copper in Swedish surface waters.
7th International Conference on the biogeochemistry of trace elements., Uppsala, Sweden. 15-17 June 2003. Won second price.
13th Annual Meeting of SETAC-Europe, 27 April- 1 May 2003, Hamburg, Germany.

2. De Laender F., Verdonck F.A.M., De Schamphelaere K.A.C., Heijerick D.G., Janssen C.R., Vanrolleghem P.A. The influence of copper toxicity on a two species system in Swedish surface waters. *14th Annual Meeting of SETAC-Europe, 18-22 April 2004, Prague, Czech republic.*

3. De Laender F., De Schamphelaere K.A.C., Verdonck F.A.M., Heijerick D.G., Vanrolleghem P.A., Janssen C.R. Geo-referencing of chronic zinc toxicity: towards catchment based standards. *14th Annual Meeting of SETAC-Europe, 18-22 April 2004, Prague, Czech republic.*

4. Heijerick D.G., Van Sprang P.A., De Schamphelaere K.A.C., De Laender F., Janssen C.R., Vanrolleghem P.A. Development and use of the surface water database (SWAD) and the soil/sediment database (S-SED) in risk assessment procedures. *14th Annual Meeting of SETAC-Europe, 18-22 April 2004, Prague, Czech republic.*

5. De Laender F., De Schamphelaere K.A.C., Verdonck F.A.M., Heijerick D.G., Vanrolleghem P.A., Janssen C.R. Geographical and temporal variability of zinc bioavailability: towards region-based water quality standards. *Beltox meeting, 1 April 2004, Liege, Belgium.*

6. De Laender F, De Schamphelaere K.A.C., Schaefers C., Vanrolleghem P.A., Janssen C.R. Do Species Sensitivity Distributions protect ecosystem function? A case study for copper. *15th Annual Meeting of SETAC-Europe, 23-26 April 2005, Lille, France.*

7. De Laender F, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Influence of the slope of concentration response relationships on community effects. *16th Annual Meeting of SETAC-Europe, 7 -11 May 2006, The Hague, Netherlands.*

8. De Laender F, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Does laboratory based probabilistic effect assessment correctly predict field community effects? A theoretical exercise. *16th Annual Meeting of SETAC-Europe, 7 -11 May 2006, The Hague, Netherlands.*

9. De Laender F, De Schamphelaere K.A.C., Vanrolleghem P.A., Janssen C.R. Foodweb-mediated effects of linuron in a freshwater ecosystem. *27th Annual Meeting of SETAC-North America,* 5-9 November 2006, *Montreal, QC, Canada.*

Foreign study visits

15 September 2005: Sokoine State University of Agriculture in Morogoro, Tanzania.

Goal: oral presentation of PhD research

30 October - 5 December: University of Wisconsin, Madison, USA,

Goal: Collaboration with Professor Anthony R. Ives at the Lab for theoretical ecology. Professor Ives is an internationally acclaimed scientist in the field of theoretical ecology. Multivariate Autoregressive (MAR) were used to extract feeding relationships out of ecosystem data. This study visit was funded by a travel grant from the Fund for Scientific Research - Flanders (FWO-Vlaanderen, Belgium).

Attended conferences and workshops

1. 14th Annual Meeting of SETAC-Europe, 18-22 april 2004, Prague, Czech republic.

2. BELTOX, Role of Bioavailability in Environmental Risk Assessment, 1 April 2004, Liege, Belgium.

3. 15th Annual Meeting of SETAC-Europe, 23 -26 April 2005, Lille, France.

4. COST 626 meeting, 18 22 April 2005, Silkeborg, Denmark.

5. 16th Annual Meeting of SETAC-Europe, 7-11 May 2006, The Hague, The Netherlands.

6. 27th Annual Meeting of SETAC-North America, 5-9 November 2006, Montreal, QC, Canada.

7. 17th Annual Meeting of SETAC-Europe, 20-24 May 2007, Porto, Portugal.

Scientific Awards

Second price in poster contest at the 7th International Conference on the biogeochemistry of trace elements., Uppsala, Sweden. 15-17 June 2003 for
"De Laender F., Verdonck F.A.M., De Schamphelaere K.A.C., Janssen C.R. and Vanrolleghem P.A. Geography- referenced bioavailability modelling in risk assessment: a case study of copper in Swedish surface waters. "

SETAC Travel Award for the 27th annual meeting of SETAC North America.

Membership of professional organisations

2003-present:	Member of the Society of Environmental Toxicology and Chemistry (SETAC).