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Highlighted Article

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

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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 paper, 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 that are four times lower than the observed NOECs.

Keywords: Ecological effect assessment; Ecosystem modeling; Toxic effect sub-model

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 Lokke, 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; 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

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very resource demanding they cannot be used as a routine practice in lower tiers. Especially in view of REACH (Registration, Evaluation and Authorization 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; and (2) 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–response 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–response functions, established in single-species tests using organisms that are taxonomically and ecologically representative for the considered model populations. These external concentration–response 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 invertebrates 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. (2004a) 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, 1992, 1999; DeAngelis et al., 1989; Hanratty and Liber, 1996). 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 invertebrates and vertebrates was used by Traas et al. (2004a). These authors make the mortality rates of invertebrates 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–response 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–response data exhibit a sigmoidal pattern (Newman and Unger, 2003), a linear function does not represent the actual concentration–response data. Naito et al. (2003) argued that linear concentration–response 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–response function may be indicative of the mode of action of a toxicant (Vanwijk 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 paper, we constructed four ecosystem models that 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–response function used. 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 observed 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 observed in the microcosm experiment. Because the process of water quality criteria setting seeks to determine the maximum chemical concentration that 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.

2. Material and methods

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 Schaeffers, 2001). Briefly, indoor aquatic microcosms with a volume of about 1 m^3 containing

water (750 L) and a sediment layer (20 cm), were permanently exposed to six levels of copper sulphate (5, 10, 20, 40, 80 and 160 μg Cu L^{-1}); two replicates per concentration were used. Biomass dynamics of various community elements (phytoplankton, cladocerans, rotifers, copepods, and the macrophyte *Elodea densa*), were monitored during the 110 days exposure period. Four untreated replicates were used as controls. Photoperiod (between 8 and 17 h day^{-1}) and temperature (between 17 and 22 °C) were controlled to simulate a season starting with spring and ending with autumn.

2.2. Ecosystem model

A mechanistic 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. By connecting different objects and defining the trophic links between them, a customized food web was designed. The number of populations that can be modelled is unlimited and available objects are: phytoplankton, macrophytes, zooplankton, planktivorous fish and piscivorous fish. Additionally, the growth kinetics of these objects can be further differentiated by parameter tuning (e.g., slow growing vs. fast growing). The phytoplankton object contains the processes photosynthesis, respiration, excretion, mortality, sinking, and grazing by zooplankton. The zooplankton object describes grazing on phytoplankton and detritus, defecation, respiration, excretion, mortality, and grazing by planktivorous fish. The fish object describes grazing on zooplankton or planktivorous fish, defecation, respiration, excretion, mortality, and predation by piscivorous fish. All differential equations are based on Park (1974) and USEPA (2000) and are described in detail in the supporting documents. The planktonic system used in the present study was composed of two phytoplankton objects (small, fast-growing phytoplankton, versus large, slow-growing phytoplankton), 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). These authors found that large-bodied zooplankton (most copepods and cladocerans) can graze on both small and large phytoplankton, while small-bodied zooplankton can only ingest small phytoplankton. These findings were imported in the ecosystem model by means of preference factors (see supporting document for detailed equations), which vary between 0 and 1, indicating the fraction in the diet consisting of the respective food source. The sum of preference factors per zooplankton population equals 1. In our models, both copepods and cladocerans have equal preference factors for small and $phytoplankton_{large}$ (i.e., 0.5). The preference factor of rotifers (small zooplankton) for $phytoplankton_{small}$ was set to 1. The resulting customized food web was used for all four ecosystem models evaluated in this study (Fig. 1).

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

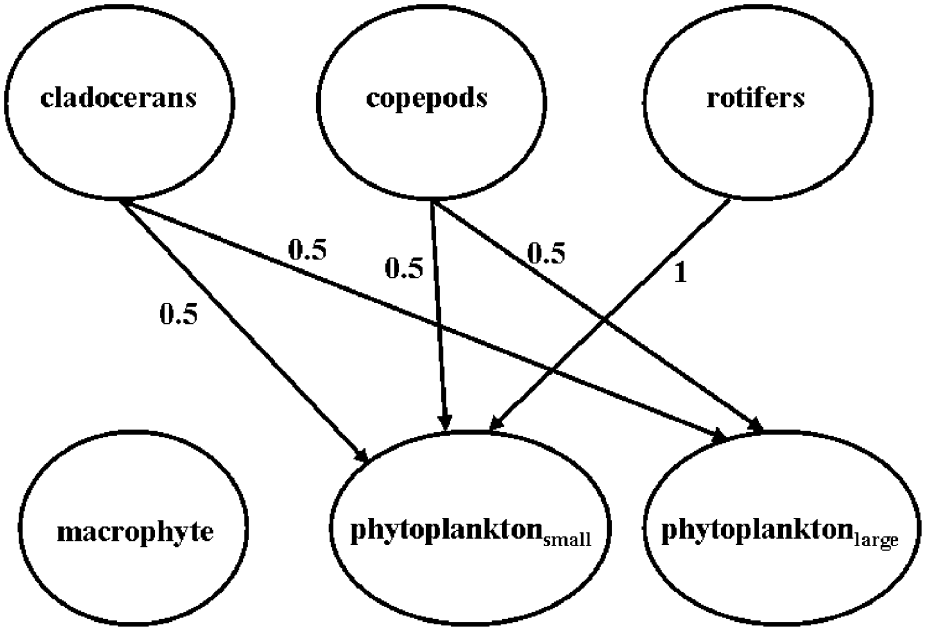

Fig. 1. Scheme of the customized food-web representing the trophic links in the microcosm experiment performed by Schaeffers (2001). Numbers alongside the arrows indicate zooplankton preferences for the respective food source. Technical implementation of these preference factors can be found in the supporting documents.

phytoplankton and macrophytes, and mortality effects on macrophytes. In the LOGC and LINC ecosystem models, both mortality and sublethal toxicant effects on invertebrates are included. In the other two ecosystem models, LOG and LIN, only mortality effects are included. 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 characteristics of the toxic effect sub-models of the four ecosystem models are summarized in Table 1.

2.4. Parameters of the toxic effect sub-models

Data on the effects of copper on aquatic biota were collected from literature (Table 2). 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; De Schamphelaere 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 Schamphelaere 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 Schamphelaere and Janssen (2004), and De Schamphelaere 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 Schamphelaere 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 previous study examining copper effects on the same macrophyte (De Laender et al., submitted). A slope value for concentration–response curves of metals was taken from Smit et al. (2001) and assumed to be representative of the slope of concentration–response functions for both mortality and sublethal effects. An overview of the used bioavailability normalized toxicity data is presented in Table 2. Characteristics of the microcosm water are provided in the supporting documents.

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 by Sommer et al. (1986). These events are, chronologically: (1) spring bloom of small phytoplankton, (2) bloom of small zooplankton, resulting in a 'clear water phase', (3) a summer bloom of large phytoplankton, followed by (4) a bloom of larger zooplankton. The advantage of such an approach is that no measured biomass dynamics are required for application of the method. To obtain this succession of events, growth related parameters of the different populations, i.e. mortality rate and ingestion rate for invertebrates and photosynthesis rate for phytoplankton and the macrophyte, were calibrated. A complete list of parameter values can be found in the supporting documents. 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 populations' biomass status in the reference situation with that in the different copper treatments, its biomass under

Table 1
Equations used in the toxic effect sub-models of the four ecosystem models, LIN, LINC, LOG, and LOGC, with P_{max} = maximum photosynthesis rate at a toxicant concentration 'tox' (d^{-1}); $P_{max,0}$ = intrinsic maximum photosynthetic rate (d^{-1}); tox = toxicant concentration; $EC_{50,\ pmax}$ = effect concentration for a 50% reduction in photosynthesis rate; slope = slope of the respective concentration-effect function; Kmort = mortality rate at a toxicant concentration 'tox' (d^{-1}); $Kmort_0$ = intrinsic mortality rate (d^{-1}); ln = natural logarithm; time = duration of toxicity assay (d); LC_{50} = lethal concentration for 50% of the organisms, as determined in the acute toxicity assay; C_{max} = maximum ingestion rate at a toxicant concentration 'tox' (d^{-1}); $C_{max,0}$ = intrinsic maximum ingestion rate (d^{-1})

Model	Photosynthesis effect	Mortality effect	Ingestion effect
LIN	$P_{max} = P_{max,0}\left\{1 - \frac{tox}{2EC_{50,Pmax}}\right\}$	$Kmort = Kmort_0 + \frac{(ln(2)/time) - Kmort_0}{LC_{50}} tox$	–
LINC			$C_{max} = C_{max,0}\left\{1 - \frac{tox}{2EC_{50,Cmax}}\right\}$
LOG	$P_{max} = \frac{P_{max,0}}{1+(tox/EC_{50,Pmax})^{slope}}$	$Kmort = \frac{1}{time} ln\left\{1 + \left(\frac{tox}{LC_{50}}\right)^{slope}\right\}$	–
LOGC			$C_{max} = \frac{C_{max,0}}{1+(tox/EC_{50,Cmax})^{slope}}$

Table 2
Collected toxicity data after normalization to the water characteristics of the microcosm study

Model population	Parameters of sub-models				
	Log(EC_{50}, photosynthesis) ($\mu g\,L^{-1}$)	Log(LC_{50}) ($\mu g\,L^{-1}$)	Log(EC_{50}, grazing rate) ($\mu g\,L^{-1}$)	Sm (−)	Acute test duration (days)
$Phytoplankton_{small}$	1.76 (0.20)	–	–	1	–
$Phytoplankton_{large}$	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

In the case of EC_{50}'s and LC_{50}'s, numbers represent the means of the normal distributions expressed as $\mu g\,L^{-1}$, 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 the text.

*In absence of experimental data, effects on macrophyte mortality were taken from a previous study examining copper effects on the same macrophyte (De Laender et al., submitted), resulting in macrophyte mortality rates of 1e−3; 1e−3; 1e−3; 2e−3; 1e−2; 1e−2–2e−2; 3e−2–4e−2 at 0; 5; 10; 20; 40; 80; 160 $\mu g\,L^{-1}$.

both scenario's was averaged over the exposure period. Relative differences of a populations' biomass between the control and the treatments were calculated as follows:

$$RD_{tox,i} = \frac{X_{tox,i} - X_{ref,i}}{X_{ref,i}}, \quad (1)$$

with $RD_{tox,i}$ the relative difference of the average biomass concentration of population '*i*' when exposed to a toxicant concentration 'tox', with its biomass concentration in the control. $X_{tox,i}$ the 110 day average biomass concentration of population *i*, when exposed to a toxicant concentration 'tox'. $X_{ref,i}$ the 110 day average biomass concentration of population '*i*' in the control, i.e. the reference value.

2.6. Comparison of experimental and predicted effects

To account for variability of each of the used single-species toxicity test result, the four ecosystem models were run in a Monte-Carlo setting. Characteristics of the statistical distributions describing this variability are given in Table 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. As an example, plots of standard deviations of the simulations vs. number of runs are provided in Fig. 1 of the supporting document. Each of these 100 simulations was compared with the reference 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 (Eq. (1)).

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. In the context of ecological effect assessments, both increases and decreases of phytoplankton biomass are considered undesirable: the former because of increased eutrophication risk, the latter because of a loss of primary production. For the macrophyte and invertebrates, biomass decreases are considered as undesirable.

The $NOEC_{\alpha}$ for decrease of a populations' biomass was defined as the largest concentration at which less than 100 $(1-\alpha)$% 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 $(1-\alpha)$% of the RD values for this population were larger than 0.2. The effect 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 vary 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 can never account for such indirect effects.

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 any of the populations: 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 using the methodology discussed in the previous paragraphs.

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 that aims at determining a maximum toxicant concentration that does not adversely affect the ecosystem.

3. Results

3.1. Control vs. treatments population biomass: phytoplankton and macrophyte

The microcosm data show that the $\text{phytoplankton}_{\text{small}}$ biomass increases with increasing copper concentrations (Fig. 2). Up to $80\,\mu g\,L^{-1}$, all four-ecosystem models predict an increase of $\text{phytoplankton}_{\text{small}}$, although the observed increase (300–1000%) is larger than the predicted increase (100–200% for all four ecosystem models). At $160\,\mu g\,L^{-1}$, a complete collapse of $\text{phytoplankton}_{\text{small}}$ is predicted, although observations indicate an increase of 2000%.

Experimental RD values for the $\text{phytoplankton}_{\text{large}}$ decrease with increasing copper concentration, indicating a loss of biomass (Fig. 2). Results from all four-ecosystem models exhibit this decrease. Only at $20\,\mu g\,L^{-1}$ 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. 2).

3.2. Control vs. treatments population biomass: zooplankton

The microcosm data indicate that cladoceran biomass decreases drastically at concentrations $\geqslant 40\,\mu g\,L^{-1}$ (Fig. 2). At concentrations $<40\,\mu g\,L^{-1}$, 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. 2). Experimental RDs at 5 and $10\,\mu g\,L^{-1}$ 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. 2). This can probably be explained by the very low rotifer densities ($<0.5\,\mu g\,L^{-1}$) 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.

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. 3. 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.

3.4. Population-NOECs: phytoplankton and macrophyte

Both LIN and LINC estimate a $\text{NOEC}_{\text{small phytoplankton}}$ of $10\,\mu g\,L^{-1}$, while LOG and LOGC predicts the $\text{NOEC}_{\text{small phytoplankton}}$ of $20\,\mu g\,L^{-1}$ accurately (experimental $\text{NOEC}_{\text{small phytoplankton}}$ is $20\,\mu g\,L^{-1}$). Predicted values of $\text{NOEC}_{\text{large phytoplankton}}$ (Fig. 4), differ most between LIN/LINC and LOG/LOGC models. The latter predict a $\text{NOEC}_{\text{large phytoplankton}}$ of $20\,\mu g\,L^{-1}$, while the former

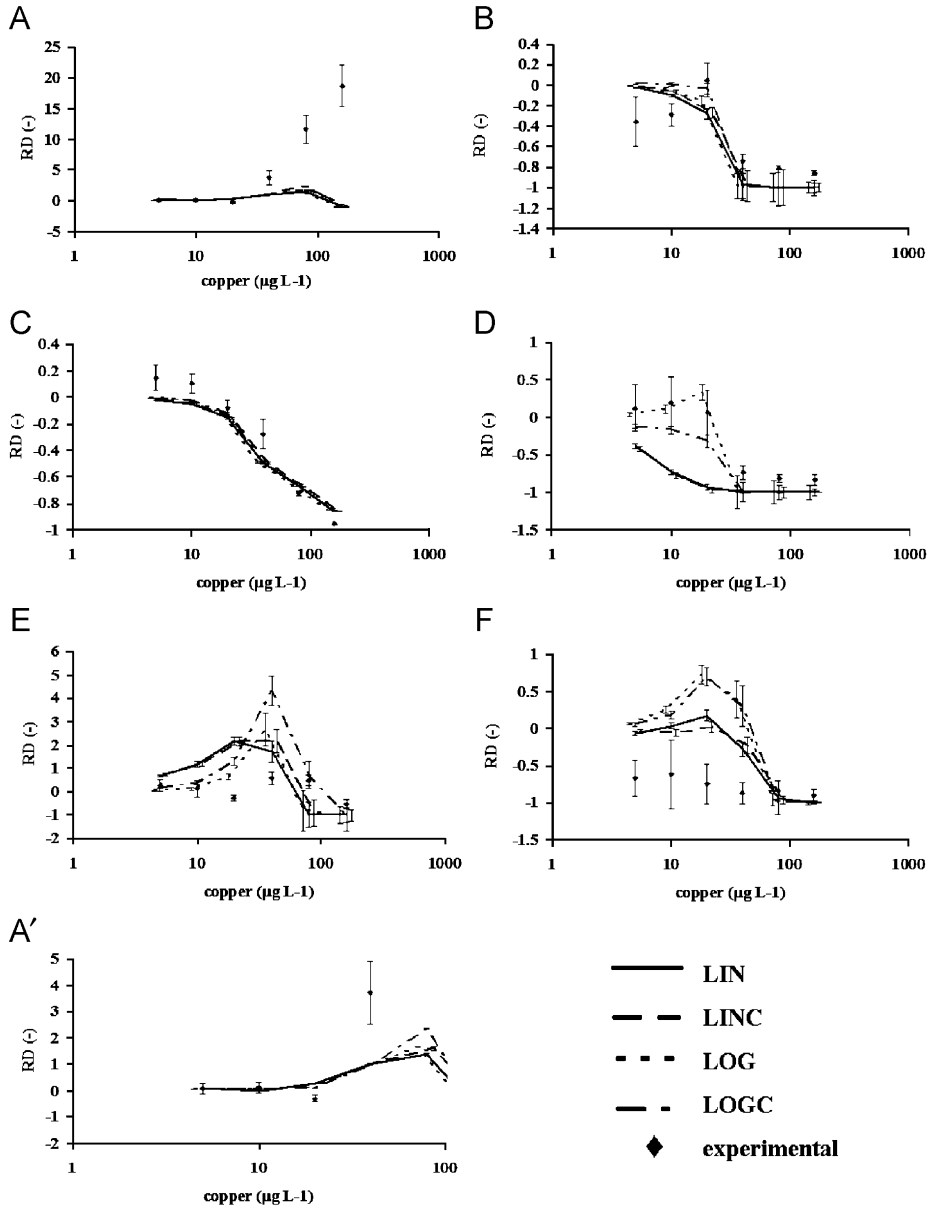

Fig. 2. Biomass changes, relative to a reference condition, as a function of copper for the six populations in the ecosystem: small phytoplankton (A); large phytoplankton (B); the macrophyte (C); cladocerans (D); copepods (E); rotifers (F). Observed relative differences (RD) and associated standard errors are indicated by ◆ and error bars, respectively. A more detailed graph is added for the small phytoplankton biomass change as a function of copper concentrations between 5 and 40 µg L^{-1} (A′). Line codes are given in the legend.

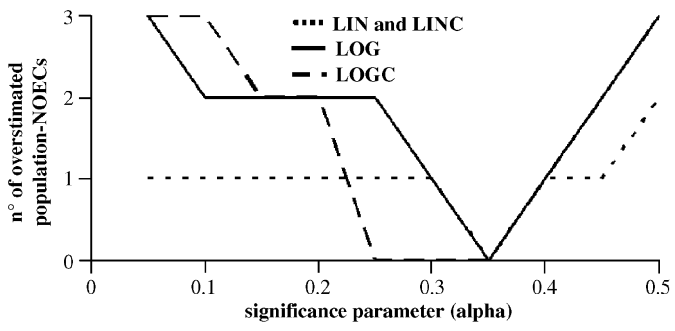

Fig. 3. The number of populations for which population-NOECs were predicted higher by the models than those observed in the microcosm study, for different alpha levels.

models result in $NOEC_{large\ phytoplankton}$ of 10 µg L^{-1}. The experimental $NOEC_{large\ phytoplankton}$ is 20 µg L^{-1} (Fig. 4).

At 40 µg L^{-1}, all ecosystem models predict a significant decline in macrophyte biomass, (Fig. 4). All ecosystem models result in a $NOEC_{macrophyte}$ of 20 µg L^{-1}.

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. 4). The $NOEC_{cladocerans}$ derived with the LOG model was thus equal to the observed value: 20 µg L^{-1}. LOGC already predicted an effect at 20 µg L^{-1}, while according to both

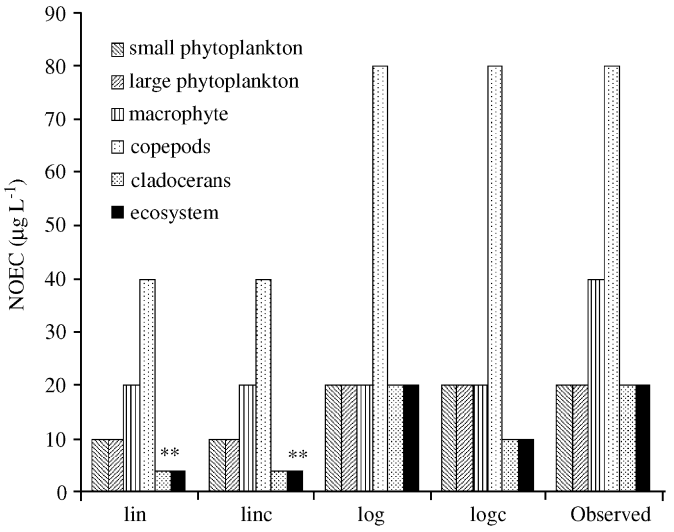

Fig. 4. Population-NOECs for small phytoplankton, large phytoplankton, the macrophyte, copepods, and cladocerans as predicted by the four models (lin, linc, log, and logc), observed in the microcosm experiment (DATA). Ecosystem-NOECs are represented by the black bars. Values of $<5\,\mu g\,L^{-1}$ are plotted as $4\,\mu g\,L^{-1}$ and indicated by *.

LIN and LINC effects are expected at the lowest treatment concentration ($5\,\mu g\,L^{-1}$).

The experimental $NOEC_{copepods}$ is $80\,\mu g\,L^{-1}$ (Fig. 4). At $80\,\mu g\,L^{-1}$, a decline of copepod biomass is estimated by LIN(C), resulting in a $NOEC_{copepods}$ prediction of $40\,\mu g\,L^{-1}$. Application of the other two ecosystem models yields a $NOEC_{copepods}$ equal to the experimental value.

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 $20\,\mu g\,L^{-1}$ 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. 4. 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 ($20\,\mu g\,L^{-1}$), while LOGC is a factor 2 more conservative ($10\,\mu g\,L^{-1}$).

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

4. Discussion

4.1. Control vs. treatments population biomass: phytoplankton and macrophyte

The increase of $phytoplankton_{small}$ in experimental enclosures exposed to metals has also been observed by Jak et al. (1996). However, according to the concentration–response functions (Table 1), copper does not increase maximal photosynthesis rate (P_{max}) of $phytoplankton_{small}$ at these concentrations. For example, at $40\,\mu g$ copper L^{-1}, the concentration–response functions indicate a 30–40% decrease of P_{max}, while at this concentration, a $phytoplankton_{small}$ biomass increase of 150–250% 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 $phytoplankton_{small}$, $phytoplankton_{large}$ biomass is found to decrease with increasing copper concentration. Yet, both $phytoplankton_{large}$ and $phytoplankton_{small}$ are grazed upon by the same zooplankton (as rotifer biomass is negligible, see previous paragraphs). Hence, they should experience the same reduction of grazing pressure. Moreover, P_{max}-EC_{50}s of both populations are the same (Table 2), indicating equal direct copper effects on P_{max} of $phytoplankton_{large}$ and $phytoplankton_{small}$. A possible explanation for the decrease of $phytoplankton_{large}$ biomass is therefore the superiority of $phytoplankton_{small}$ in competing for nutrients, as observed in other experimental studies (e.g., Havens, 1994a, b).

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–response 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.

4.3. NOEC derivations

The large influence of alpha 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 5–7 larger than CV's of microcosm observations. The large variability of the ecosystem model predictions hampers the early detection of population effects at $\alpha = 0.05$–0.25, leading to severe underestimations of effects. Yet, applying alpha-levels of 0.4–0.5 on the microcosm data—which has a

smaller variability would result in experimental population-NOECs below $5\,\mu g\,L^{-1}$.

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$_{small}$ biomass that is too low. Note that also the NOEC for increase of phytoplankton$_{small}$ biomass is predicted too low. The possible mechanism behind this phenomenon is explained in the previous paragraph.

The difference between model predictions (predicted $NOEC_{macrophyte} = 20\,\mu g\,L^{-1}$) and microcosm observations (experimental $NOEC_{macrophyte} = 40\,\mu g\,L^{-1}$) may be due to the use of a phytoplankton EC_{50} in the toxic effect submodels of this macrophyte.

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 $<5\,\mu g\,L^{-1}$). Naito et al. (2003), who used the comprehensive aquatic systems model (CASM) equipped with a linear toxic effect submodel (i.e. comparable with the LIN model in this paper) 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.

4.5. NOECs derived using other cut-off values

Because we acknowledge that the 20% cut-off value, although often cited, is not definitive, NOECs-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 (Fig. 2, supporting document), only the experimental NOECs of the macrophyte and of phytoplankton$_{large}$ are lower than those derived using 20% as a cut-off. The experimental NOEC for decrease of the macrophyte is $20\,\mu g\,L^{-1}$ using the 10% cut-off. The experimental NOEC for decrease of phytoplankton$_{large}$ is $5\,\mu g\,L^{-1}$ using the 10% cut-off. This last NOEC is overpredicted by all four models. However, from Fig. 2B, it can be seen that at $20\,\mu g\,L^{-1}$, experimentally observed biomass of phytoplankton$_{large}$ returns to its control range. Hence, it can be questioned if the experimentally observed decrease at $10\,\mu g\,L^{-1}$ 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.

4.6. Concluding remarks

Based on the comparison observed vs. predicted data given in previous paragraphs, the largest differences in the predictive capacity of the ecosystem models are attributable to the different types of concentration–response function. LOG and LOGC models gave more accurate predictions of population-NOECs and ecosystem-NOECs than LIN and LINC models, when using an alpha-level of 0.35. Apparently, implementation of the correct shape of concentration–response 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 is dependent 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 paper, 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 paper 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 $EC_{50,\ photosynthesis}$ 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 that is not always available in open literature.

As such, the model described in this paper relies on (1) default ecosystem dynamics; and (2) single-species toxicity test results to predict ecological effects at different chemical concentrations. RD values can be predicted with this model for every population at a series of different concentrations. From these simulated RD-values, NOECs can be derived, both on a population and ecosystem level. Nevertheless, if

such NOEC-predictions are to serve as an alternative to results from current statistical extrapolation techniques, their validity has to be assessed in other, more complex ecosystems and for other toxicant types. Indeed, as the number of populations in a system increases, the quality of the used single-species toxicity data and assumptions on ecological interactions are expected to become more important.

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Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2007.08.020.

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