Available online at www.sciencedirect.com
ScienceDirect
Environment International 34 (2008) 390–396
ENVIRONMENT INTERNATIONAL
www.elsevier.com/locate/envint

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

Frederik De Laender [a,b,*], Karel A.C. De Schamphelaere [a], Peter A. Vanrolleghem [b,c], Colin R. Janssen [a]

[a] Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University (UGent) Jozef Plateaustraat 22, B-9000 Gent, Belgium
[b] Department of Applied Mathematics, Biometrics and Process Control (BIOMATH), Ghent University (UGent) Coupure Links 654, B-9000 Gent, Belgium
[c] modelEAU, Département de genie civil, Université Laval, Laval, QC, Canada

Received 14 June 2007; accepted 18 September 2007
Available online 31 October 2007

Abstract

Species sensitivity distributions (SSDs) are statistical distributions which extrapolate single-species toxicity test results to ecosystem effects. This SSD approach assumes that ecological interactions between populations, such as grazing and competition, do not influence the sensitivity of ecosystems. The validity of this assumption in a simple freshwater pelagic ecosystem was tested using ecosystem modelling. 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 were 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 an 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 tend to be equal for toxicants directly affecting zooplankton and fish, e.g. insecticides. For the 254 hypothetical toxicants for which the tested assumption was false, a predicted no effect concentration (PNEC) calculated as the lowest single-species EC_{10} divided by an application factor of 10 was on average a factor 10 lower than the corresponding ecosystem-NOEC calculated by the ecosystem model.

Keywords: Risk; SSD; Modelling; Ecology

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 (Forbes and Calow, 2002). 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

* Corresponding author. Laboratorium voor Milieutoxicologie en Aquatische Ecologie, Plateaustraat 22, B-9000 Gent, Belgium. Tel.: +32 9 264 3707; fax: +32 9 264 3766.
E-mail address: f.delaender@nioo.knaw.nl (F. De Laender).

0160-4120/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2007.09.006

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, 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 paper, 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 (NOECs) for the populations present in the ecosystem, as such taking into account ecological interactions. These population-NOECs were calculated by an ecosystem modelling approach. 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.

2. Materials and methods

2.1. Considered ecosystem

The ecosystem for which hypothesis T1 was tested is a simple lentic pelagic system consisting of populations 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).

2.2. Ecosystem model

A mechanistic dynamic ecosystem model was constructed using 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 based on USEPA (2002). By connecting different objects and defining the trophic links between them, a customized food web can be designed. 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 the supporting documents. The ecosystem modelled in the present study included two phytoplankton objects (spring phytoplankton: small-celled and fast growing; and summer phytoplankton: large-celled and slow growing), three zooplankton objects (rotifers: fast growing; large cladocerans: slow growing; large copepods: slow growing), and one planktivorous fish object. Ecological interactions were set according to Sommer et al. (1986). 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. 1.

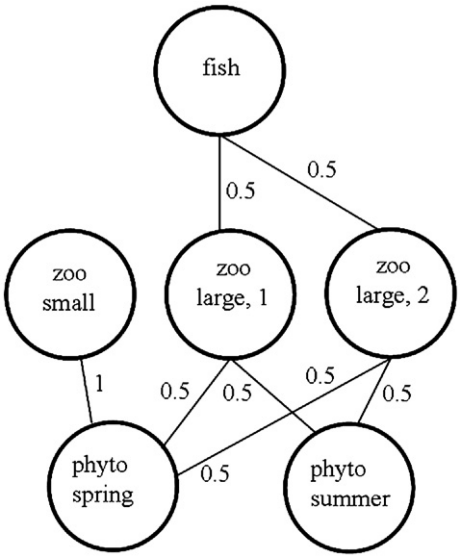

Fig. 1. Food web diagram of the considered ecosystem. Nodes represent the populations present and lines represent feeding links between them. The preference of a population for a connected population is given by the preference factor alongside the connection. Zooplankton and phytoplankton are coded by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

The ecosystem model was calibrated to obtain a realistic succession of seasonal events for this type of system, as 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. Parameter values resulting in population dynamics reflecting those events are given in the supporting document.

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 of the toxic effect sub-models and the values assigned to their parameters is given in Table 1.

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

Table 1
Equations used in the toxic effect sub-models of the applied ecosystem model, with P_{max}=maximum photosynthesis rate (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 (d^{-1}); ln=natural logarithm; time=duration of toxicity assay (d), set to two days for all zooplankton and fish; 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"

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

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).

of the "true" SSD_1, a conventional species sensitivity distribution ($cSSD_1$) was fitted to this set of 6 EC_{10}s:

$$cSSD_1 \sim \left(\widehat{\mu}_1, \widehat{\sigma}_1\right)$$

with:

$$E\left[\widehat{\mu}_1\right] = \mu_1$$

$$E\left[\widehat{\sigma}_1\right] = \sigma_1.$$

Next, the same 6 EC_{10}s were used in the toxic effect sub-models of the 6 populations in the ecosystem model (Table 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 (reference) situation with its status at the different exposure concentrations, relative differences (RDs) were calculated:

$$RD_{tx,p} = \frac{X_{tx,p} - X_{ref,p}}{X_{ref,p}} \quad (1)$$

with:

$X_{tx,p}$ the time-averaged biomass concentration of population 'p', when exposed to a toxicant concentration 'tx'

$X_{ref,p}$ the time-averaged biomass concentration of population 'p' in the unexposed case, i.e. the reference value

Because 20% is the minimum detectable difference for most population characteristics in the field (Suter, 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 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. Note that these population-NOECs were determined using an ecosystem model, as such taking into account ecological interactions in this NOEC calculation. A cumulative plot of those 6 population-NOECs was defined as the eco-Species Sensitivity Distribution for tx_1 (eco-SSD_1):

$$\text{eco-}SSD_1 \sim \left(\mu_{1,eco}\sigma_{1,eco}\right).$$

Using these definitions, the hypothesis T1 was rephrased as:

$$\widehat{\mu}_1 = \mu_{1,eco} \Lambda \widehat{\sigma}_1 = \sigma_{1,eco}.$$

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

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.

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 5% 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 the 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.

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:

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

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

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 $EC_{10,zooplankton}$) primarily 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.

Stepwise discriminant function analyses (Jennrich, 1977) were used to determine which variable (r_{PZ} or r_{ZF}) discriminates best between two or more

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 ($\widehat{\mu}_i = \mu_{eco,i}$; group 0) and those for which the means of cSSD and eco-SSD differ ($\widehat{\mu}_i \neq \mu_{eco,i}$; 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 ($\widehat{\sigma}_i = \sigma_{i,eco}$; group 2) and those for which the standard deviations of cSSD and eco-SSD differ ($\widehat{\sigma}_i \neq \sigma_{i,eco}$; group 3). Because in both analyses there are only two groups, the stepwise discriminant function analyses are analogous to multiple regression. In a two-group case a linear function is fitted to the data. For example, in the first analysis this function is:

$$\text{Group} = a + b_1 \cdot r_{PZ} + b_2 \cdot r_{ZF}$$

where a, b_1 and b_2 are coefficients that are changed so that r_{PZ} and r_{ZF} predict if a toxicant belongs to group 0 or 1. Note that 'Group' is a binary variable which can only have 0 or 1 as value. After this fitting procedure, the variable with the largest (standardized) regression coefficient is that one that contributes most to the prediction of group membership. This variable will thus have the highest discriminative power. 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 built in order to classify toxicants into groups 0 and 1 (Breiman et al., 1984). The same was done to classify toxicants into groups 2 and 3. A classification tree is a data mining technique to classify cases, in this case toxicants, into groups based on a set of variables, in this case r_{PZ} and r_{ZF}. A classification tree consists of a set of split conditions which are connected by branches (Fig. 3). To classify a toxicant into a group, one starts at the top split condition and runs through the tree until an end node is reached. This end node shows the group into which the toxicant is classified. Split conditions are based on the variables considered in the analysis, in this case r_{PZ} and r_{ZF} and determine how the tree should be ran through. The r_{PZ} and r_{ZF} values of two-third of the 1000 toxicants together with their group membership (e.g. group 0 or 1) were used to construct the tree. This two-third is called the training-set. Split conditions were calibrated to maximize the amount of correctly classified training-set toxicants. Afterwards, the remaining one-third of the 1000 toxicants (test-set), 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.

3. Results and discussion

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. $\widehat{\mu} > \mu_{eco,i}$ and $\widehat{\sigma} > \sigma_{i,eco}$. Therefore, in the results of the discriminant analysis and decision tree approach, groups 0 and 1 were redefined as toxicants for which $\widehat{\mu} > \mu_{eco,i}$ and those for which $\widehat{\mu} > \mu_{eco,i}$, respectively. Similarly, groups 2 and 3 were redefined as toxicants for which $\widehat{\sigma}_i > \sigma_{i,eco}$ and those for which $\widehat{\sigma} > \sigma_{i,eco}$, respectively. The difference between $\widehat{\mu}_i$ and $\mu_{eco,i}$ was on average 0.6 log-units and the difference between $\widehat{\sigma}_i$ 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 $\widehat{\sigma}_i$ 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 was 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 source (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 related zooplankton populations. These shifts in sensitivity explain the lower variance of eco-SSDs compared to the cSSDs.

3.2. Comparing 'safe concentrations' derived from cSSD with ecosystem-NOECs derived from the ecosystem model

PNECs derived as the lowest single-species EC_{10} divided by an application factor of 10 (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 $\widehat{\mu}_i > \mu_{eco,i}$, the HC_5 was larger than the ecosystem-NOEC. For all toxicants for which only $\widehat{\sigma}_i > \sigma_{i,eco}$, the HC_5 was found to be smaller than the ecosystem-NOEC. For 28 of the 30 toxicants for which both $\widehat{\mu}_i > \mu_{eco,i}$ and $\widehat{\sigma}_i > \sigma_{i,eco}$, 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. Thus, 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 paper 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.

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. 2), it appears that the power to discriminate between group 0 toxicants (i.e. for which $\widehat{\mu} > \mu_{eco,i}$) and group 1 toxicants (i.e. for which $\widehat{\mu} > \mu_{eco,i}$) 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

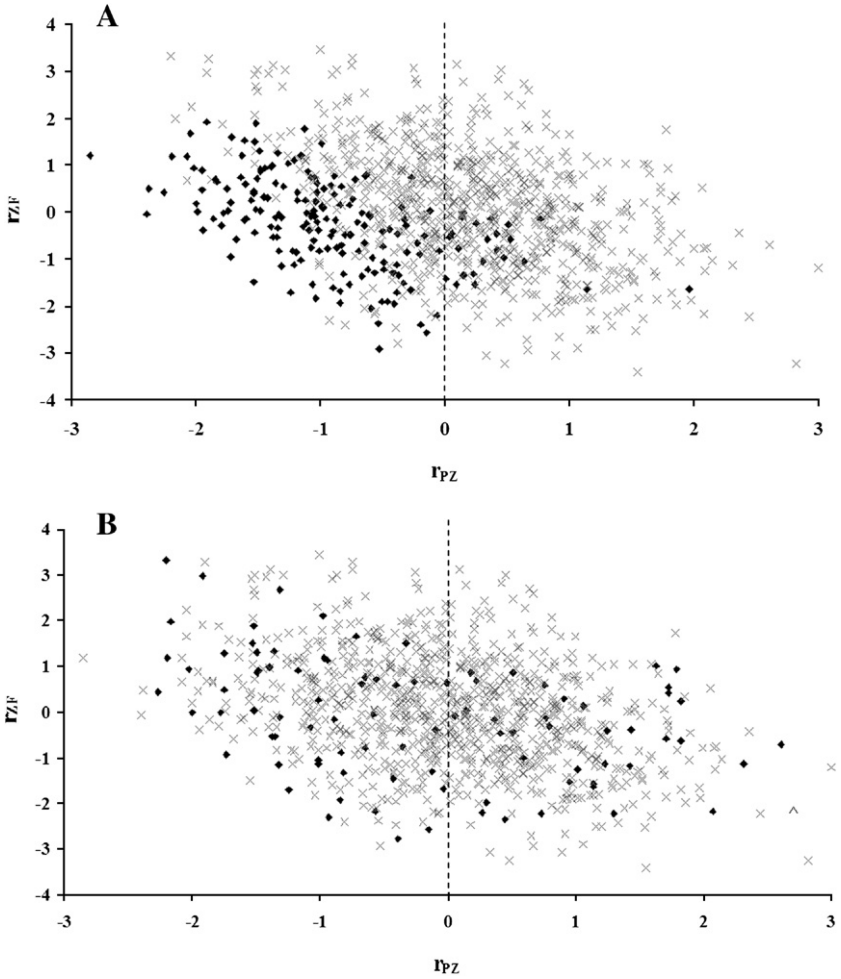

Fig. 2. A: Scatterplot of the 1000 considered toxicants based on their r_{PZ} and r_{ZF} value. A black symbol indicates that $\widehat{\mu}_i > \mu_{eco,i}$ for that toxicant. A grey symbol indicates that $\widehat{\mu}_i = \mu_{eco,i}$ for that toxicant. A dashed line indicates $r_{PZ}=0$. B: Scatterplot of the 1000 considered toxicants as a function of their r_{PZ} and r_{ZF} value. A black symbol indicates that $\widehat{\sigma}_i > \sigma_{eco,i}$ for that toxicant. A grey symbol indicates that $\widehat{\sigma}_i = \sigma_{eco,i}$ for that toxicant. A dashed line indicates $r_{PZ}=0$.

more to the right of $r_{PZ}=0$ (Fig. 2A). 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 single-species toxicity test results are sufficient to classify a toxicant in group 0 or group 1.

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

3.4. For which toxicants is T1 valid? Classification tree approach

In Fig. 3 the resulting classification tree predicting if $\widehat{\mu} > \mu_{eco,i}$ (coded class "1") or if $\widehat{\mu} > \mu_{eco,i}$ (coded class "0") based on r_{PZ} and r_{ZF} is shown. The tree consists of split conditions (ellipses) and end nodes (boxes). One has to start at the top of the tree and let the split conditions decide which way to proceed. If a split condition is fulfilled, this results in a continuation via the left branch. The tree is followed until an end node is reached. This end node gives the resulting classification (underlined). For example, the dashed line indicates the pathway for a toxicant for which $r_{PZ}=2$. Since the first split condition ($r_{PZ} \leq -0.9385$) is not fulfilled ($r_{PZ}=2$), one has to continue to the left branch of the tree. As such, a second split condition is reached ($r_{PZ} \leq -0.3861$), which is also not fulfilled in this example. As a result, a toxicant with $r_{PZ}=2$ is classified as class "0", i.e. $\widehat{\mu} = \mu_{eco,i}$ for that toxicant. In a similar way, a toxicant for which $r_{PZ}=-2$ and $r_{ZF}=0$ will be classified as class "1", i.e. $\widehat{\mu} \neq \mu_{eco,i}$ for that toxicant, as indicated by the dotted line in Fig. 3.

The number of training-set toxicants which were correctly and erroneously classified using these split conditions is also given in these end nodes. For example, in the box situated in the left side of the tree, 35 toxicants were classified correctly (i.e. in class 1), while 2 were classified erroneously. Toxicants classified in end nodes marked with an asterisk, 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.

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

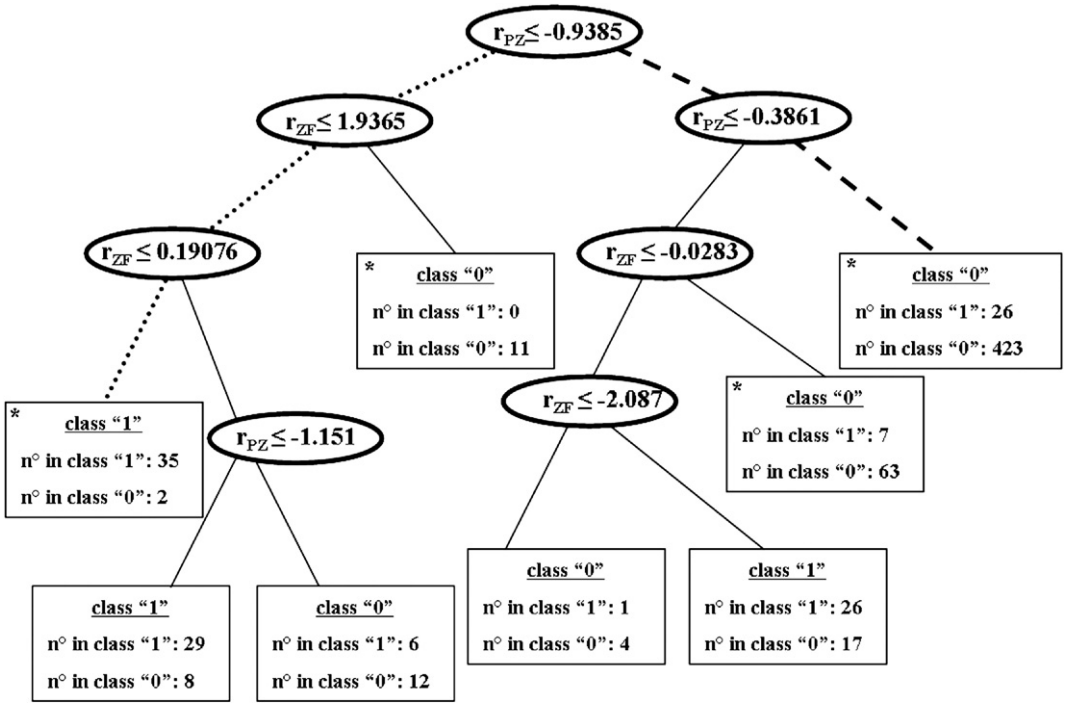

Fig. 3. Classification tree predicting if $\widehat{\mu}_i > \mu_{eco,i}$ (coded class "1") or if $\widehat{\mu}_i = \mu_{eco,i}$ (coded class "0") based on r_{PZ} and r_{ZF}. If a split condition (ellips) is fulfilled, this results in a continuation to the left branch. The tree is followed until an 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 is 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 $r_{ZF}=0$.

$r_{PZ}=-2$ 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 $r_{ZF}\approx 0$. 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 $\widehat{\mu}_i > \mu_{eco,i}$ as indicated by the dotted line in Fig. 3. Thus, for these toxicants, T1 is not valid: when accounting for ecological interactions, the mean ecosystem sensitivity for herbicides ($\mu_{eco,i}$) is predicted to be different from the mean ecosystem sensitivity when ecological interactions are not accounted for ($\widehat{\mu}_i$). Hence, the applications of the cSSD approach for herbicides may lead to inaccuracies caused by differences in parameters between cSSD and eco-SSD. Conversely, toxicants primarily targeting zooplankton and fish (e.g. $r_{PZ}=2$ and $r_{ZF}=0$), have $\widehat{\mu}_i = \mu_{eco,i}$, as indicated by the dashed line in Fig. 3. This suggests that the mean of eco-SSD and cSSD is comparable for these toxicants. 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

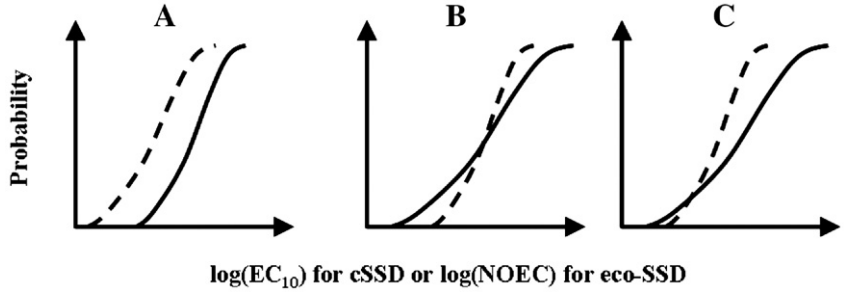

Fig. 4. Visualisation of possible differences between parameters of cSSD (bold line) and eco-SSD (dashed line): A: $\widehat{\mu}_i > \mu_{eco,i}$; B: $\widehat{\sigma}_i > \sigma_{eco,i}$; C: $\widehat{\mu}_i > \mu_{eco,i} \wedge \widehat{\sigma}_i > \sigma_{eco,i}$.

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. 4B). In contrast, the opposite may hold when the mean of the eco-SSD is lower than the mean of the cSSD (Fig. 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 cSSD than for eco-SSD (Fig. 4C), it is difficult to *a priori* predict which of both approaches (cSSD or eco-SSD) will result in the lowest 'safe concentration'. Yet, the different locations of cSSD and eco-SSD, as indicated by the difference between $\widehat{\mu}_i$ and $\mu_{i,eco}$ should primarily be regarded as an indication of the violation of T1 for a substantial amount (25%) of toxicants. The possible implications of this violation for water quality standards give valuable insights. However, underlying assumptions of the cSSD approach are many (Forbes and Calow, 2002). Thus the way in which these assumptions influence water quality standard derivation will depend on the validity of all of these assumptions, and not only on the validity of the assumption examined here.

Acknowledgements

Frederik De Laender is the recipient of a PhD-grant of the Flemish Institute for the Promotion of Scientific and Technological Research in Industry (IWT, Belgium).

Website: www.iwt.be. Karel De Schamphelaere is currently supported by a post-doctoral fellowship from the Fund for Scientific Research-Flanders (FWO-Vlaanderen, Belgium). Peter Vanrolleghem is Canada Research Chair in Water Quality Modelling.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envint.2007.09.006.

References

Balczon JM, Pratt JR. A comparison of the responses of 2 microcosm designs to a toxic input of copper. Hydrobiologia 1994;281:101–14.

Breiman L, Friedman JH, Olshen RA, Stone CJ. Classification and regression trees. New York: Chapman and Hall; 1984.

Chang KH, Nagata T, Hanazato T. Direct and indirect impacts of predation by fish on the zooplankton community: an experimental analysis using tanks. Limnology 2004;5:121–4.

Duboudin C, Ciffroy P, Magaud H. Effects of data manipulation and statistical methods on species sensitivity distributions. Environ Toxicol Chem 2004a;23:489–99.

Duboudin C, Ciffroy P, Magaud H. Acute-to-chronic species sensitivity distribution extrapolation. Environ Toxicol Chem 2004b;23:1774–85.

EU. Technical Guidance Document (TGD). Technical guidance document in support of commission directive 93/67/EEC on risk assessment for new notified substances and commission regulation (EC) N° 1488/94 on risk assessment for existing substances and directive 98/8/EC of the European parliament and of the council concerning the placing of biocidal products on the market. Part II. Environmental risk assessment. Luxembourg: Office for Official Publication of the European Communities; 2003.

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

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

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

Gonzalez-Doncel M, Ortiz J, Izquierdo JJ, Martin B, Sanchez P, Tarazona JV. Statistical evaluation of chronic toxicity data on aquatic organisms for the hazard identification: the chemicals toxicity distribution approach. Chemosphere 2006;63:835–44.

Hamilton PB, Jackson GS, Kaushik NK, Solomon KR, Stephenson GL. The impact of 2 applications of atrazine on the plankton communities of *in situ* enclosures. Aquat Toxicol 1988;13:123–39.

Hose GC, Hyne RV, Lim RP. Toxicity of endosulfan to *Atalophlebia* spp. (ephemeroptera) in the laboratory, mesocosm, and field. Environ Toxicol Chem 2003;22:3062–8.

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

Jennrich RI. Stepwise discriminant analysis. In: Enslein K, Ralston A, Wilf HS, editors. Statistical Methods for Digital Computers. New York: John Wiley & Sons; 1977. p. 77–95.

Kefford BJ, Palmer CG, Warne MS, Nugegoda DT. What is meant by "95% of species"? An argument for the inclusion of rapid tolerance testing. Hum Ecol Risk Assess 2005;11:1025–46.

Lange R, Hutchinson TH, Scholz N, Solbe J. Analysis of the ECETOC aquatic toxicity (EAT) database – II – comparison of acute to chronic ratios for various aquatic organisms and chemical substances. Chemosphere 1998;36:115–27.

Maltby L, Blake N, Brock TCM, van den Brink PJ. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. Environ Toxicol Chem 2005;24:379–88.

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

Posthuma L, Suter GW, Traas TP. Species sensitivity distributions in ecotoxicology. Boca Raton: Lewis; 2002.

Schmitt-Jansen M, Altenburger R. Predicting and observing responses of algal communities to photosystem II-herbicide exposure using pollution-induced community tolerance and species-sensitivity distributions. Environ Toxicol Chem 2005;24:304–12.

Selck H, Riemann B, Christoffersen K, Forbes VE, Gustavson K, Hansen BW, et al. Comparing sensitivity of ecotoxicological effect endpoints between laboratory and field. Ecotox Environ Saf 2002;52:97–112.

Smit MGD, Hendriks AJ, Schobben JHM, Karman CC, Schobben HPM. The variation in slope of concentration–effect relationships. Ecotox Environ Saf 2001;48:43–50.

Sommer U, Gliwicz ZM, Lampert W, Duncan A. The Peg-model of seasonal succession of planktonic events in fresh waters. Arch Hydrobiol 1986;106:433–71.

Suter GW. Ecological risk assessment. Boca Raton: Lewis Publishers; 1993.

USEPA. O. o. w. Aquatox: modelling environmental fate and ecological effects in aquatic ecosystems: technical documentation. report no. EPA-823-R-01-007; 2002.

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

Van Donk E, Prins H, Voogd HM, Crum SJH, Brock TCM. Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated fresh-water microcosms.1. Responses of plankton and zooplanktivorous insects. Arch Hydrobiol 1995;133:417–39.

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

Wagner C, Lokke H. Estimation of ecotoxicological protection levels from NOEC toxicity data. Water Res 1991;25:1237–42.

Werner EE, Hall DJ. Optimal foraging and size selection of prey by bluegill sunfish (*Lepomis Macrochirus*). Ecology 1974;55:1042–52.