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Simulation of Spatial and Temporal Variability of Chronic Copper Toxicity to *Daphnia magna* and *Pseudokirchneriella subcapitata* in Swedish and British Surface Waters

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ABSTRACT

Water Quality Criteria (WQC) for metals are usually based on single species laboratory toxicity data. The influence of water characteristics of the surface waters on bioavailability to freshwater organisms is hence neglected, along with regional and temporal variability of these water characteristics. A methodology is presented to account for regional and temporal variability in the WQC setting for copper in the United Kingdom and Sweden. Bioavailability models were applied in a Monte-Carlo approach to account for temporal variability and a Geographic Information System was used to account for geographical variability on the chronic copper toxicity to *Daphnia magna* and *Pseudokirchneriella subcapitata*. Fifth percentiles of distributions of the No Observed Effect Concentration (NOEC) for both model species were derived in both study regions. For *P. subcapitata*, it was demonstrated that this fifth percentile can vary by a factor 10 in the UK study region. The ratio of these NOEC fifth percentiles (*D. magna* percentile divided by *P. subcapitata* percentile) was used to compare the ecotoxicity of copper to two model species. This ratio showed the highest variability (a factor 5) within the Swedish study region. The findings of this research stress the need for the use of region-specific WQC for copper.

Key Words: bioavailability models, temporal and geographical variability, $NOEC_5$, inter-regional and intra-regional variability, Monte-Carlo simulation.

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INTRODUCTION

As a result of copper's ubiquity and its intrinsic properties, regulatory agencies have established Water Quality Criteria (WQC) for copper. In the context of this article, with WQC is meant: concentration limits that are intended to protect aquatic life. The derivation of such WQC is usually predominately based on standardized data analysis procedures that are applied to single species laboratory toxicity data obtained with a wide variety of species and organism types. These ecotoxicity tests are typically performed using standard test media, which tend to maximize the bioavailability of metals. It is well known, however, that different bioavailability-modifying characteristics of test media and surface waters (*e.g.*, dissolved organic carbon [DOC] or pH) can affect the toxicity of copper (Winner 1985; Meador 1991; Di Toro *et al.* 2001; De Schamphelaere and Janssen 2004). Because these factors alter the bioavailability of copper, a generic WQC derived from standard ecotoxicity tests may be, depending on the characteristics of the receiving natural waters, over as well as under protective. Standard approaches for deriving WQC for metals for regulatory purposes, usually implemented at regional or national level, may not be appropriate to accurately assess their true impact on the ecological quality of aquatic ecosystems. It is suggested that extrapolating ecotoxicity results from laboratory to field conditions should be performed using toxicity-related bioavailability models like the Biotic Ligand Model (BLM) (*e.g.*, De Schamphelaere *et al.* 2003) to account for the spatial heterogeneity of surface waters. Additionally, the current metal WQC are usually assumed to be fixed in time. As demonstrated through extensive monitoring campaigns (*e.g.*, Probst 1992) bioavailability modifying water characteristics like pH and DOC exhibit seasonal and annual variations. This is currently not accounted for in the implementation of metal WQC.

The aim of this study is to develop a methodology to account for both temporal and spatial variability, representative of European surface waters, of the ecotoxicity of copper to aquatic species. In addition, the influence of water type on the relative sensitivity to copper of common aquatic species belonging to different trophic levels is assessed.

METHODOLOGY

Bioavailability-Corrected Toxicity Assessment

Chronic toxicity test results and associated bioavailability models were used for the evaluation of spatial and temporal variability of the copper toxicity to two model species. The use of chronic test data for environmental risk assessment procedures is recommended in the Technical Guidance Document (TGD) (EC 2003). Additionally, chronic bioavailability models have been developed for two standard species: the freshwater flea *Daphnia magna* and the green alga *Pseudokirchneriella subcapitata* (De Schamphelaere *et al.* 2003; De Schamphelaere and Janssen 2004). Both models were used to calculate NOECs for *D. magna* and *P. subcapitata* based on available information on water characteristics in two European regions: southern Sweden and southern UK. Input for these models contain pH, DOC, temperature, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Fe^{2+}, Cl^{-}, SO_4^{2-}, and alkalinity. The output of the models are predicted No Observed Effect Concentrations (NOECs) of dissolved copper (μg/L).

Physico-Chemical Water Characteristics

Both Swedish and UK water characteristics were obtained from the **S**urface **WA**ter **D**atabase (Van Sprang *et al.*, in preparation). The information in this database originated from various environmental agencies and institutes and contains monitoring data for Belgium, France, Germany, Northern Ireland, The Netherlands, Sweden, and the United Kingdom.

The physico-chemical parameters reported in most data sets are pH, alkalinity, major ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Fe^{2+}, Cl^{-}, SO_4^{2-}), dissolved and/or total organic carbon (TOC), and metal concentrations. For southern Sweden, only TOC was available, whereas the dissolved carbon fraction was determined in the UK-monitoring program. The most frequently determined metals were As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn; other metals were measured occasionally. The SWAD consists of time series of (monthly) measured water characteristics at 140 Swedish locations and 278 UK locations. Using the bioavailability models, NOECs were calculated for every month at every location. Time series of monthly measurements of five successive years (1997–2001) were selected for southern Sweden, whereas time series of monthly measurements of four successive years (1999–2002) were used for southern UK. These measurements were not always complete, and locations for which DOC, pH, alkalinity, or Na^{+} (most important model inputs) were missing were omitted. The applied bioavailability models have been developed and calibrated for a specified range of water characteristics (see Table 1). Water characteristics in some locations were not always within the calibration limits of the models. To determine which locations had physico-chemical values outside this range, median values were calculated for all water characteristics and for every location. In 49 of the 140 southern Swedish locations and in 71 of the 278 southern UK locations, these median water characteristics were within the models' calibration domain. The main reason for removing the locations in Sweden was the Na concentration and pH, which exceeded calibration limits (60% of removed locations). The remaining 40% of the omitted Swedish locations had ($Ca^{2+} + Mg^{2+}$) values exceeding the calibration limits. For the United Kingdom, Na concentrations and pH were too high in 80% of the removed locations; the remaining 20% was discarded due to exceedence of the ($Ca^{2+} + Mg^{2+}$) limit. In both study regions, the removal of the locations was due to the extreme physico-chemical water characteristics. In the UK study region, waters are mainly characterized by high pH (7.6), whereas in the Swedish study region waters have a high TOC content (8.8 mg/L).

Table 1. BLM calibration domain for pH, Ca^{2+} and Mg^{2+}, and Na^{+} (De Schamphelaere and Janssen 2004).

Water characteristic	Domain
pH	<8.5 and >5.5
Ca^{2+} and Mg^{2+}	$([Ca^{2+}] + [Mg^{2+}])^{*}1000 > 0.1$ M
Ca^{2+} and Mg^{2+}	$([Ca^{2+}] + [Mg^{2+}])^{*}1000 < 5$ M
Na	<20 mM and >0.1 mM

Geographical References

Each measurement is characterized geographically by a set of coordinates. For Sweden the coordinates were in the format of the Swedish national grid, RT90. All UK sites are defined using the UK National Grid Reference. More information on these national grids can be found on the internet (http://www.landmateriet.se for Sweden and http://www.ordnancesurvey.co.uk for the UK). Based on these geographical references, spatial variability of water characteristics could be assessed using a Geographic Information System (GIS). In this study, ArcView 3.2a was used (ESRI Inc., 1996, California, USA) to visualize spatial trends in the water characteristics.

Temporal Variability

To assess the influence of temporal variability of the water characteristics on the toxicity of copper to the two model species a characterization of the temporal variability of the bioavailability-determining water characteristics is needed. For this, the temporal variability of bioavailability-determining water characteristics at a certain location was propagated through the bioavailability models. The time series data are assumed to be a random sample of site-specific water characteristics. Cumulative probability functions were fitted to the water characteristics (pH, DOC, Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, alkalinity, SO_4^{2+}, and temperature) for each location in Sweden and the United Kingdom using the statistical software Statistica (Statsoft Inc. 2000).

It is important to understand the interpretation of these cumulative probability functions. The cumulative probability function of for example pH gives the probability F of having a pH of pH_F or less. The same interpretation holds for all other water characteristics, as shown in Figure 1. Goodness of fit of these probability distribution functions for the various water characteristics was assessed using statistical tests (Anderson-Darling, Kolmogorov-Smirnov, and Chi-squared test). For Ca^{2+}, Mg^{2+},

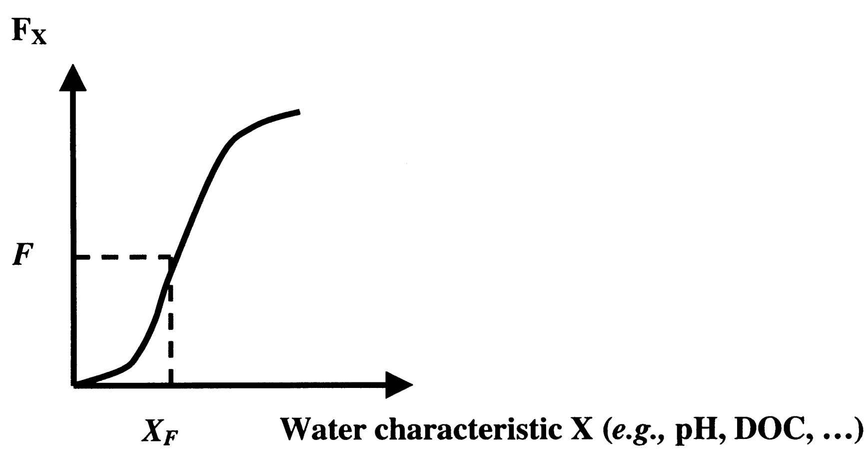


Figure 1. Cumulative probability function of a water characteristic X (*e.g.*, pH, DOC, ...). It represents the probability F of a water characteristic having a value $\leq X_F$.

Na^+, K^+, Cl^-, alkalinity, and SO_4^{2+}, lognormal distributions gave the best fit. For temperature, pH, and DOC a normal distribution was most appropriate.

Propagation of Temporal Variability using Monte-Carlo Simulation

The effect of temporal variability on the chronic copper toxicity to *D. magna* and *P. subcapitata* was evaluated. The temporal variability of the water characteristics (temporal variability on input) was propagated through the bioavailability models (see section "Bioavailability corrected toxicity assessment") to examine the temporal variability of the NOEC values of both model species (temporal variability on output). This propagation was executed by means of Monte-Carlo simulation (Cullen and Frey 1999). The number of required shots to reach a desired accuracy was determined by plotting the Monte-Carlo output *versus* the number of shots. After 100 shots the Monte-Carlo output converged. This was the case for both study regions. Probability distributions were fitted to the water characteristics of the remaining 49 Swedish and 71 UK locations. Characteristics of some input distributions on two representative locations (one with high, respectively low TOC content) are given in Table 2. Due to the fitting, however, it was observed that the range of the Monte-Carlo shots slightly exceeded the calibration range of both used models (2 to 6% of the shots were outside the BLM calibration range). Therefore, the cumulative probability distributions of the water characteristics were truncated according to the models' calibration domain (Table 1). The results of the Monte-Carlo analysis were site-specific cumulative probability functions of the predicted NOEC for both species. A general scheme can be found in Figure 2.

Although only TOC measurements were available in the case of Sweden, Jonsson *et al.* (2001) and Köhler *et al.* (2002) have shown that TOC mainly consists (90 to 99%) of DOC. The remaining 1 to 10% OC is assumed to have the same binding capacity as DOC. As such, TOC values are used as DOC values for calculations in the Sweden case. For BLM predictions it was assumed that DOC contains 50% of active fulvic acid and 50% of organic matter not reacting with Cu. This assumption

Table 2. Characteristics of some input distributions and of the resulting NOEC distributions on two representative locations (one with high, respectively low TOC content, both located in Sweden): mean (standard deviation between brackets).

Water characteristic	Lat: 55.85; Long: 12.79	Lat: 58.97; Long: 14.17
pH	7.82 (0.17)	6.52 (0.16)
TOC (mg/L)	4.38 (1.09)	18.07 (4.30)
Ca^{2+} (mM)	2.34 (0.58)	0.27 (0.67)
Mg^{2+} (mM)	0.35 (0.60)	0.09 (0.60)
Na^+ (mM)	1.06 (1.46)	0.87 (1.52)
Modelled species' NOEC distribution		
D. magna (μg/L)	55 (1)	197 (1)
P. subcapitata (μg/L)	14 (1)	155 (1)

Distribution types are given in the section on temporal variability. Lat and Long stand for latitude and longitude, representing the geographic coordinates of both locations.

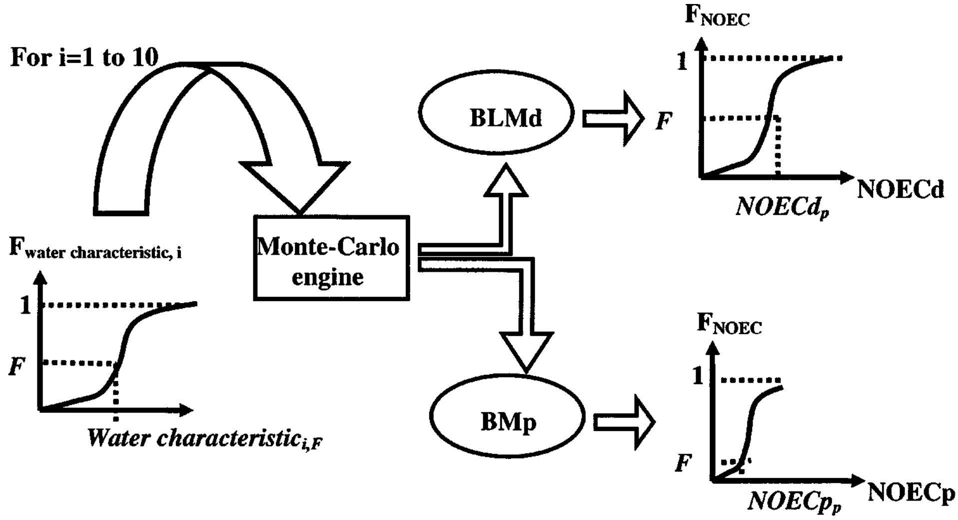


Figure 2. Scheme of the assessment of the temporal variability of the NOEC, when cumulative probability distributions of all water characteristics (from 1 to 10) are given. NOECd and NOECp represent the NOEC values of *Daphnia magna* and *Pseudokirchneriella subcapitata*, respectively. BLMd and BMp represent the *D. magna* BLM and the *P. subcapitata* bioavailability model, respectively.

has previously been shown adequate for prediction of Cu toxicity to *D. magna* and *P. subcapitata* in natural waters (De Schamphelaere *et al.* 2004; De Schamphelaere *et al.* 2003).

Similar to the cumulative probability distributions of the water characteristics, these NOEC distributions represent the temporal variability of the respective species NOEC values. From this type of distribution, one can deduce the probability of a species' NOEC being smaller than or equal to a certain concentration. *Vice versa*, one can deduce which value of a species' NOEC occurs in a certain fraction of time or less. The last reasoning can be especially useful in risk assessment procedures because regulators can put a maximum value on the percentage of time during which NOECs should not exceed a certain value.

Spatial Variability

For all 49 Swedish locations and 71 UK locations, the fifth percentiles of both NOEC distributions (for *D. magna* and *P. subcapitata*) were determined using Statistica (Statsoft Inc. 2000). Hereafter, a fifth percentile of a NOEC distribution will be termed "$NOEC_5$." These $NOEC_5$ values can be visualized using a Geographical Information System (GIS) (ArcView 3.2a, ESRI Inc., 1996, California, USA). Summary statistics of the $NOEC_5$ values in both study regions were calculated for both model species: that is, minimum, maximum, and median value. These summary statistics were then used to examine inter- and intra-regional variability. Inter-regional variability was defined as the variability of the $NOEC_5$ between the two study regions. Intra-regional variability was defined as the variability within one study region. Intra-regional variability was examined by comparing the minimum with the maximum $NOEC_5$ for a study region. Inter-regional variability was examined by comparing median $NOEC_5$s of both study regions. To examine possible causes of this variability of $NOEC_5$ values, the standard deviation of the derived site-specific pH and DOC distributions were calculated. The standard deviation of the means of all site-specific distributions in each study region was also calculated. Finally, the standard deviation of the standard deviations of all site-specific distributions in each study region were calculated.

To examine the chronic copper toxicity to *P. subcapitata* in relation to that observed for *D. magna*, the $NOEC_5$s of *P. subcapitata* were divided by the $NOEC_5$s of *D. magna* for each location. This quotient was examined visually using the GIS, allowing assessment of spatial variability of the relative copper toxicity to the latter species. Again, summary statistics were derived and subsequently used to examine inter- and intra-regional variability of the relative ecotoxicity of copper.

RESULTS AND DISCUSSION

Variability of Water Characteristics

To demonstrate the variability of water characteristics within a region, the example of southern Sweden was elaborated. Figures 3 and 4 show the toxicity modifying water characteristics in the study region of southern Sweden. Both figures consist of two maps, representing the average values of the water characteristic in summer and winter, respectively. Summer concentrations were defined as concentrations measured during June through August. Winter concentrations were defined as concentrations measured during December through February. Comparing summer with winter TOC concentrations, a clear shift from one concentration range to another is observed at some locations. For example, at the encircled location in Figure 3, the average TOC concentration in winter was twice that of the TOC concentration in summer. A similar comparison for pH is presented in Figure 4: the encircled locations all shifted to a higher pH in summer.

Figures 3 and 4 show that water characteristics in southern Sweden vary spatially. In Figure 3, the western part of southern Sweden is characterized by lower TOC concentrations compared to the eastern part, as indicated by the arrow. From Figure 4, it can be concluded that pH values are also higher in the eastern region of southern Sweden.

Figures 3 and 4 demonstrate that there is spatial as well as temporal variability in toxicity modifying water characteristics in the southern part of Sweden. This may result in changes in the toxicity of copper to the two model species.

Variability of NOEC Values: NOEC Distributions

All calculated NOEC distributions were characterized by a log-normal distribution with a standard deviation between 0.1 and 0.25 (log μg/L) for both model species. Specific NOEC distributions resulting from the water characteristics on the representative locations are given in Table 2. Summary statistics of the $NOEC_5$s are given in Figure 5A and illustrate that, for *D. magna*, the variability within a region (intra-regional) is more pronounced in southern UK (a factor 10) than in southern Sweden (a factor 6). Standard deviation of the $NOEC_5$ of *D. magna* be-

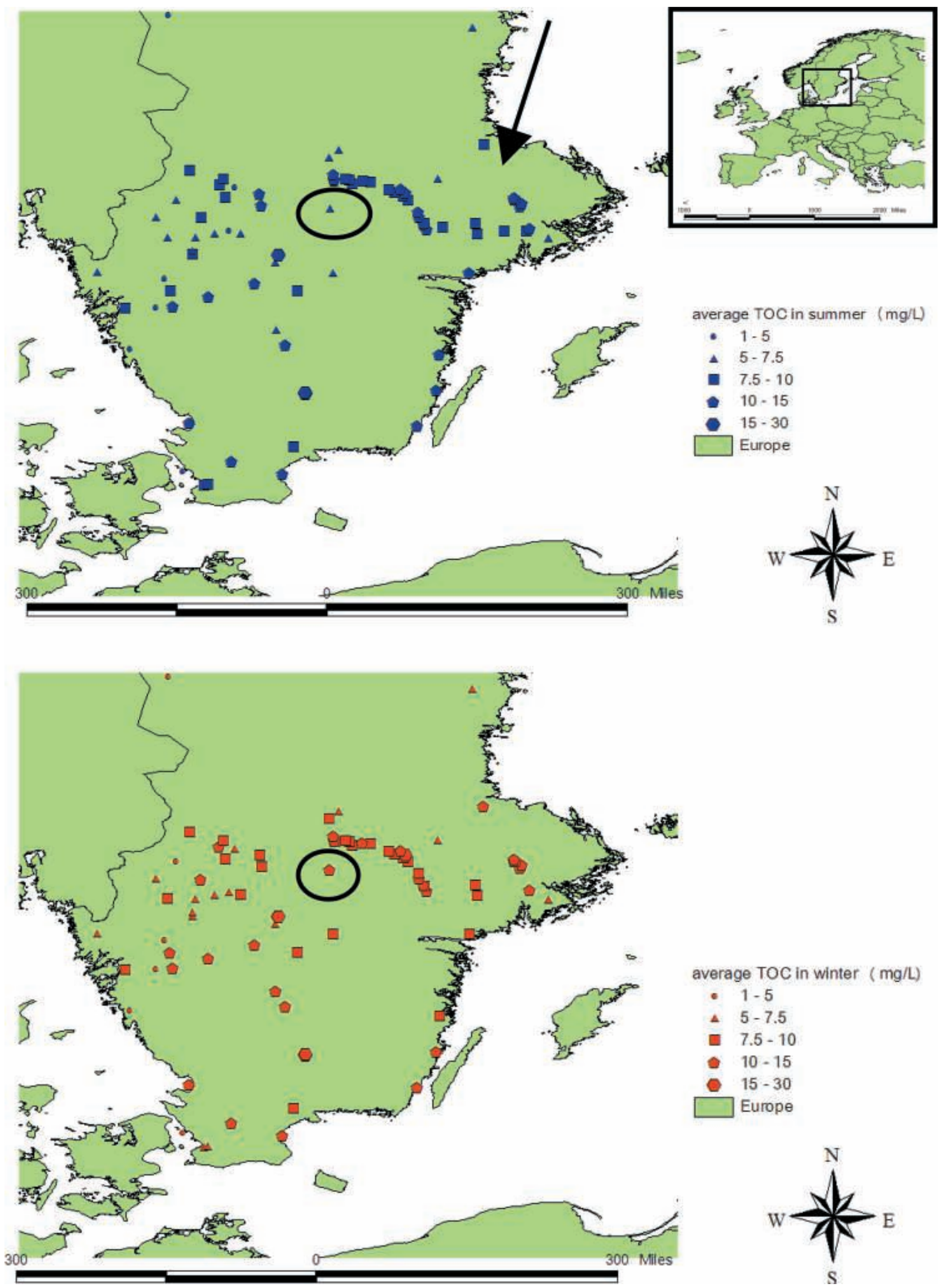


Figure 3. Seasonal variations in TOC concentrations (mg/L) in southern Sweden. Upper: TOC concentrations in summer. Lower: TOC concentrations in winter. The arrow indicates a region with higher TOC concentrations. The encircled location shows a shift to a lower TOC concentration in summer.

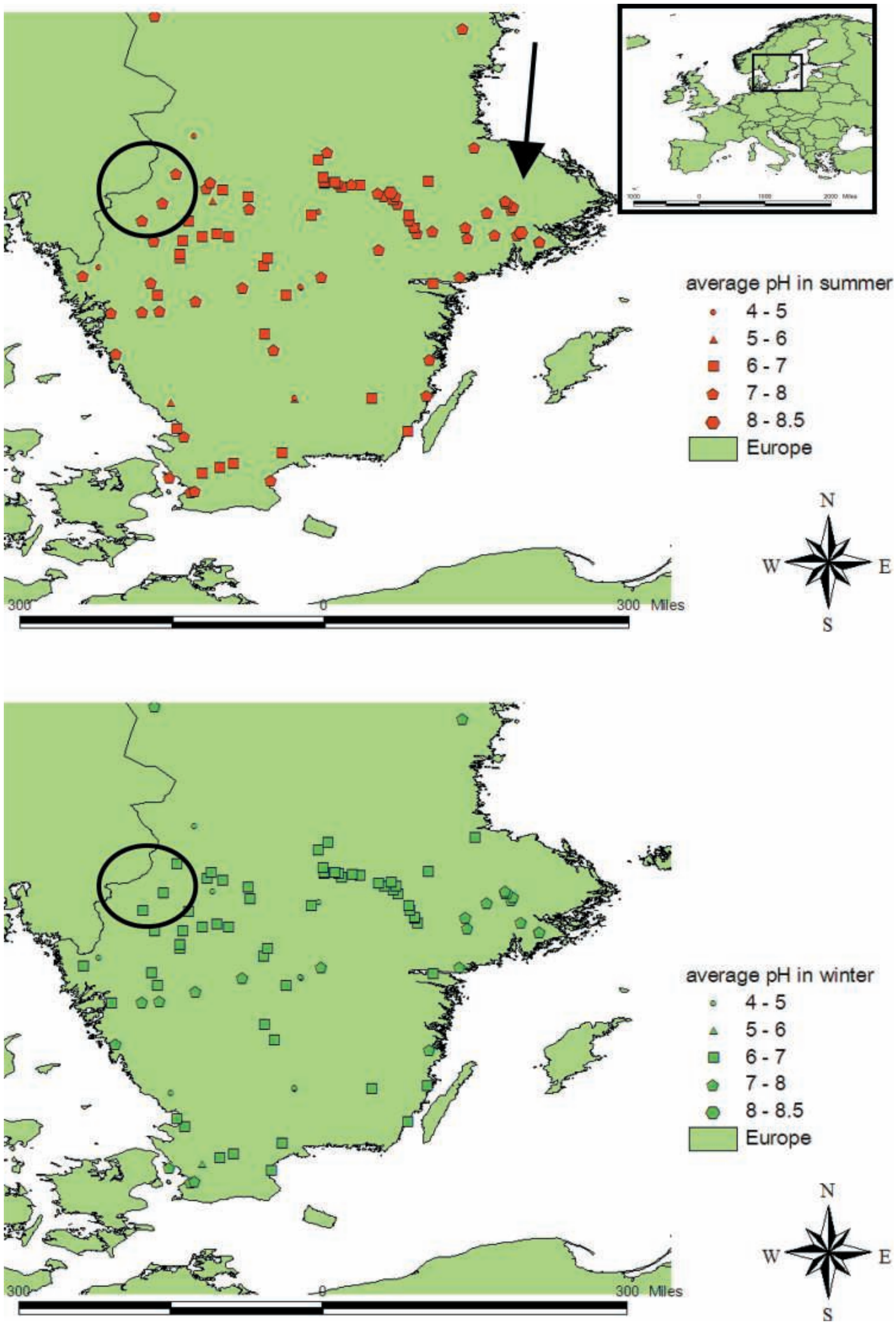


Figure 4. Seasonal variations in pH in southern Sweden. Upper: pH in summer. Lower: pH in winter. The arrow indicates a region with higher pH values. The encircled locations show a shift to a higher pH range in summer.

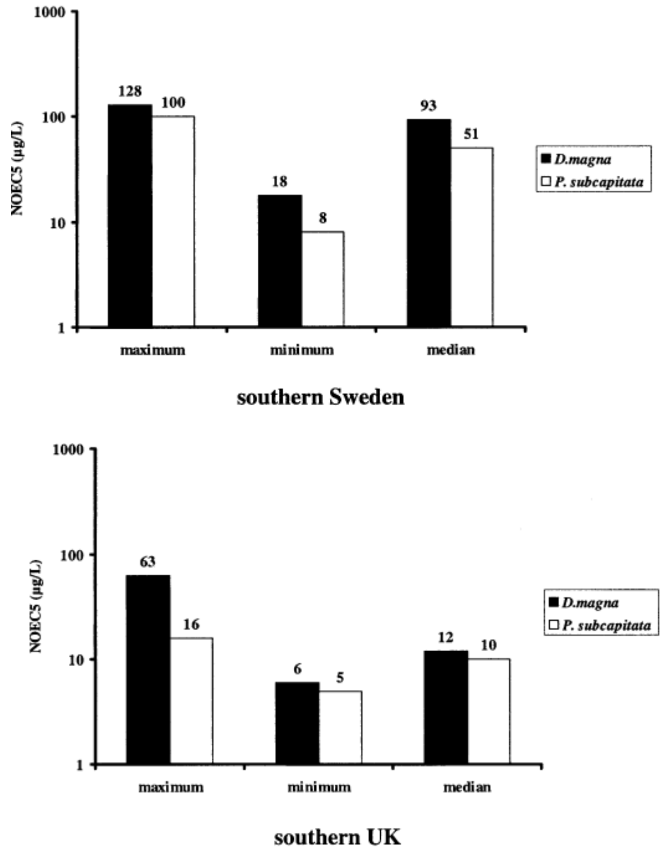
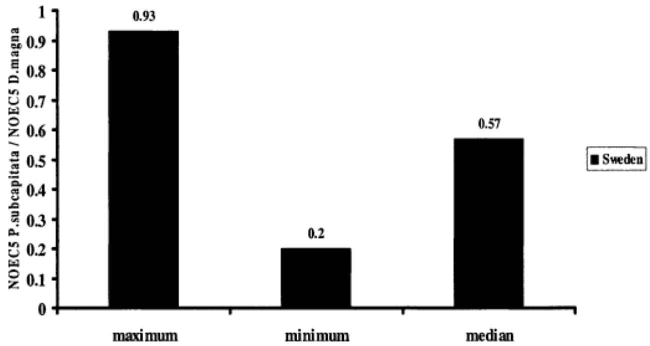
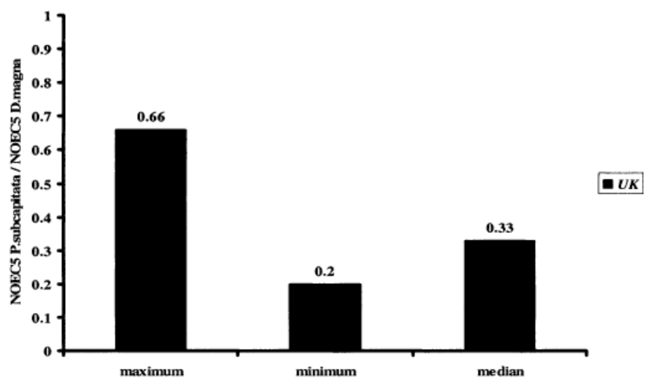


Figure 5A. Summary statistics of the fifth percentiles of the NOEC distributions for *Daphnia magna* and *Pseudokirchneriella subcapitata* in southern Sweden (upper) and southern UK (lower): maximum, minimum and median $NOEC_5$ within a region were calculated to assess inter-regional and intra-regional variation.

tween the Swedish locations is 1.4, whereas in the UK case this standard deviation is 2.4, leading to the same conclusion. Conversely, intra-regional variability for *P. subcapitata* is more pronounced in southern Sweden (a factor 10) than in southern UK a factor 3). Standard deviation of the $NOEC_5$ of *P. subcapitata* between the Swedish locations is 2.5, whereas between the UK locations it is 2. To examine the reason for this difference, the standard deviation of the means of the site-specific DOC and pH distributions in both study regions are given in Table 3. The standard deviation of the standard deviations of the site-specific DOC and pH distributions in both study regions are also summarized in Table 3. Comparison of these statistical properties indicates that standard deviations of the site-specific standard deviations of DOC distributions differ more between both study regions than the standard deviations of the site-specific means of DOC distributions. Southern UK, the region where intra-regional variability is more pronounced for *D. magna*, is characterized by a higher standard deviation of the site-specific standard deviations of the DOC



southern Sweden



southern UK

Figure 5B. Summary statistics of quotients of $NOEC_5$s of *Daphnia magna* and *Pseudokirchneriella subcapitata* for southern Sweden (upper) and southern UK (lower). The two values >1 are not included in the upper graph.

distributions. Southern Sweden, the region where intra-regional variability is more pronounced for *P. subcapitata*, is characterized by a higher standard deviation of the site-specific standard deviations of pH.

Inter-regional variability, based on the median $NOEC_5$s, differs with a factor 5 and 8 for *P. subcapitata* and *D. magna*, respectively.

Table 3. Comparison of standard deviations of both site-specific means and of standard deviations in both study regions.

Property	Water characteristic	UK-value	Sweden-value
Standard deviation of mean	pH (−)	0.29	0.73
Standard deviation of mean	DOC or TOC (mg/L)	2.38	3.29
Standard deviation of standard deviation	pH (−)	0.06	0.18
Standard deviation of standard deviation	DOC or TOC (mg/L)	3.28	1.50

Using the 5th percentile for this analysis (instead of another percentile) is in fact a worst case scenario on the effect side: it is desired to be protective for the 1 exceptional (worst case) day on 20 days. Of course, taking the 1st percentile would even be more protective. Which percentile is optimal for risk derivation is not examined here and can therefore be a subject of future research.

Variability of the Relative Ecotoxicity of Copper

From the comparison between $NOEC_5$s of *P. subcapitata* and the $NOEC_5$s of *D. magna* in southern Sweden, it is concluded that in most cases (96%) the $NOEC_5$ of *P. subcapitata* was lower than that of *D. magna*. In southern UK, a higher $NOEC_5$ for *D. magna* than for *P. subcapitata* is observed at all locations. The reason for the two exceptions in Sweden is the combination of a low DOC content (<5 mg/L), which favors toxicity to *D. magna* (De Schamphelaere and Janssen 2004), and the low pH (≈6.0), which reduces toxicity to *P. subcapitata* (De Schamphelaere *et al.* 2003). As discussed in the methodology section, the ratio of the two $NOEC_5$s ($NOEC_5$ of *P. subcapitata* divided by $NOEC_5$ of *D. magna*) was calculated for each location (Figure 6). The two locations where the $NOEC_5$ is higher for *P. subcapitata* than for *D. magna* (*i.e.*, the quotient ≥1), are indicated with a hexagonal symbol. The northern part of southern UK is mainly characterized by triangular and point symbols, indicating that quotients have values of 0.3 or less.

For southern Sweden, the median of the 49 quotients was 0.57, whereas for southern UK, the median of the 71 quotients was 0.33 (Figure 5B). The maximum quotient that was calculated for the region of southern Sweden was 0.93 (excluding the two locations with a quotient ≥1). The maximum quotient in southern UK was 0.66. Minimum quotients were, both in southern Sweden as in southern UK, around 0.2. This comparison of the relative sensitivity ratios may serve as an indication of the large spatial variability of copper toxicity in southern Sweden (a factor 5) compared to that in southern UK (a factor 3).

Possibilities for Implementation in Risk Assessment

In general, when a geo-referenced database of water characteristics is available, the presented methodology can be applied to derive site specific NOEC distributions for both or, more generally, for other species if appropriate models are available. These distributions can be combined with a (site specific) exposure concentration to derive a risk for different species to be affected by copper. If an Exposure Concentration Distribution is available then the latter risk can be refined by also incorporating temporal and spatial variability of exposure concentrations. The authors see the developed methodology as a starting point for a tool that can be applied to yield risks for species of interest in risk assessment procedures. Whether the highest risk is chosen as representative for ecosystem effects, or a combination of these risks is preferred, is an issue beyond the scope of this article.

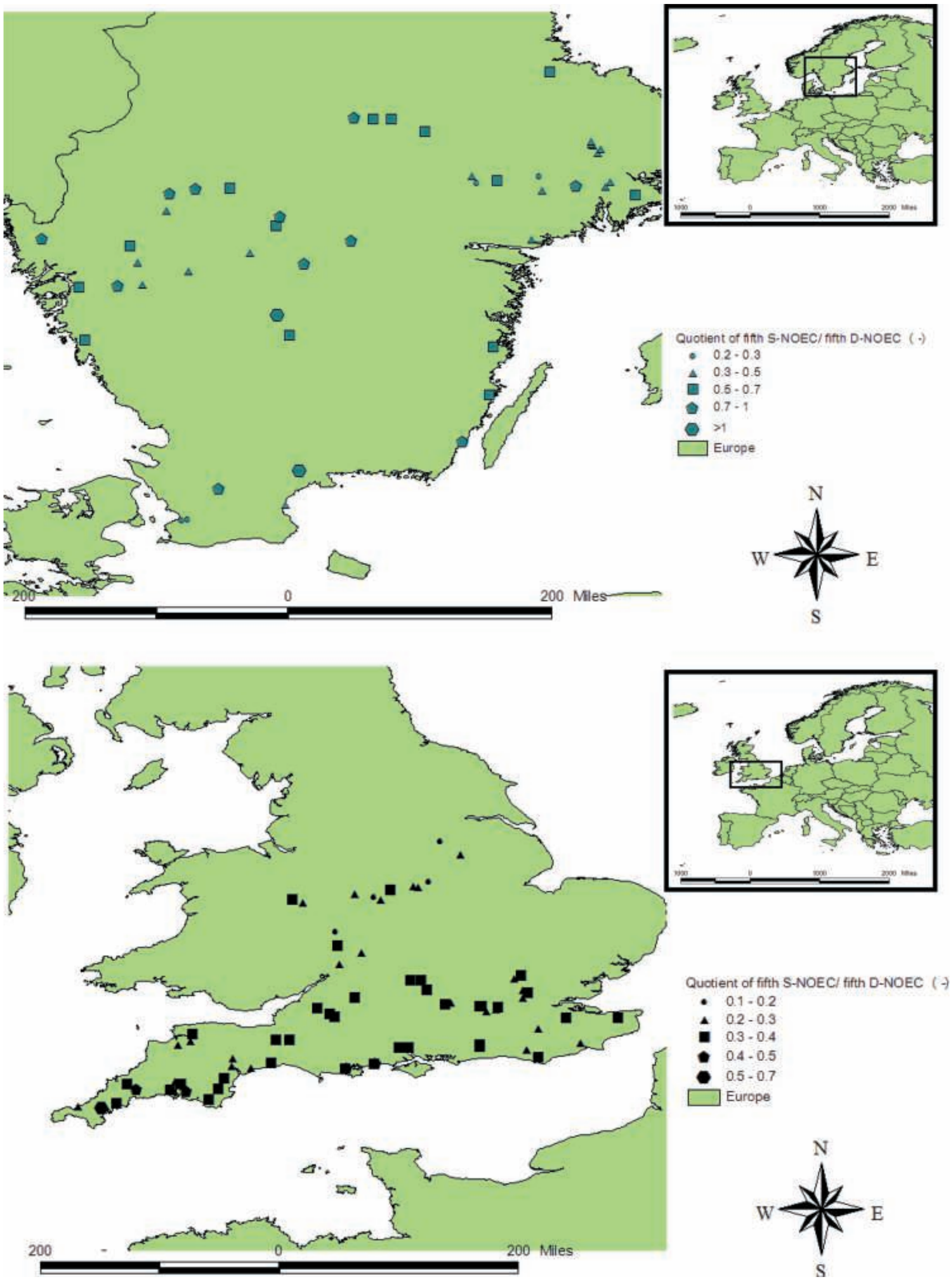


Figure 6. A quotient of fifth percentiles of the NOEC distributions (fifth percentile of *Pseudokirchneriella subcapitata* NOEC distributions divided by fifth percentile of *Daphnia magna* NOEC distributions) in all locations in southern Sweden (upper figure) and in southern UK (lower figure).

CONCLUSIONS

The results presented here stress the need for using region-specific water quality standards. Different (local) conditions of physico-chemical water characteristics lead to differences in the toxicity of copper to these two model species.

We have demonstrated that the median $NOEC_5$ for *P. subcapitata* and *D. magna* in southern Sweden was respectively 5 and 8 times higher than that in southern UK. For *D. magna*, intra-regional variability of the $NOEC_5$ is more pronounced in southern UK than in southern Sweden. For *P. subcapitata*, the opposite holds true: intra-regional variability of the $NOEC_5$ is higher in southern Sweden than in southern UK.

The quotient of the NOECs of both model species also varies geographically, indicating that this quotient is not a fixed value. This finding has implications on the current generic practices in (probabilistic) risk assessment. In these practices, the assumption is made that under all circumstances copper is more toxic to one species than to another. This assumption is based on a comparison of the NOEC values obtained under standard test conditions. However, because of the variability in physico-chemical characteristics of the surface waters, we observed that the intra-regional variability (the quotient of the $NOEC_5$s of both species) in southern Sweden was as high as a factor 5. For the inter-regional variability a factor of 2 difference in median values was noted.

Furthermore, the presented methodology can be used to derive species specific risks, which can be considered for the application in ecological risk assessment, and as such in WQC derivation.

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