

The effect of temperature on environmental risk assessment using species sensitivity distributions

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Abstract

The toxicity of a chemical depends on environmental factors such as temperature through a change in bioavailability, toxicokinetics and toxicodynamics of the chemical and indirect by a change in the biology of an organism. Although many studies confirm the interaction between temperature and toxicity, the effect is usually not included in toxicological risk assessment. The purpose of the current study is to assess the effect of temperature on species sensitivities towards compounds using the species sensitivity distribution (SSD) approach.

Two model compounds have been selected for this study. Toxicity data for a broad range of aquatic species were collected for the heavy metal cadmium (Cd) and the organic biocide pentachlorophenol (PCP). The results were divided per compound into a high- and low temperature group (respectively $\leq 20^{\circ}\text{C}$ and $> 20^{\circ}\text{C}$) based on the median test temperature. Per temperature group, geometric mean 96h-LC50 values were calculated for each species. The values were used to construct temperature group-dependent SSDs. A comparison of toxicant sensitivity between the temperature groups was made by means of the 5% and 50% hazardous concentration (HC₅ and HC₅₀). This was done twice for each compound, using all available species and only species tested at both high and low temperature.

For Cd, the HC₅ and HC₅₀ were significantly decreased by a factor 2 in the high temperature group compared to the low temperature group. When the same species were used in the temperature-specific SSDs, the HC₅ for high temperature was a factor 5 lower although the HC₅₀ was only decreased by a factor 1.4. For PCP, the differences between the HC₅ and HC₅₀ of different temperature groups were a factor 0.65 and 1.80 respectively and were not statistically significant.

Results suggest that in cases where temperatures of water bodies are (temporarily) elevated above 20°C, the effect of temperature on sensitivity should be taken into account. When upholding a precautionary principle it would be advisable to apply a safety factor of minimal 2 to environmental quality standards for chemical pollution in waters with increased temperature when striving to protect 95% of the species. By taking relevant environmental conditions such as temperature into account, environmental risk assessment can become more realistic.

Introduction

Aquatic organisms are exposed to large variations in chemical, biological and physical conditions. The fluctuations in factors such as light availability, temperature, oxygen concentrations and food availability can cause different degrees of stress (Segner 2007). Temperature variability represents one of the most significant environmental stresses for organisms, especially in the aquatic environment. With some exceptions like aquatic mammals and aquatic birds, essentially all aquatic organisms are ectotherms. Their body temperature greatly depends on the surrounding water temperature. A change of temperature will thus directly influence internal processes such as increasing metabolic rates and may result in alteration of physiology, depending on the optimum living temperature for a certain organism (Sokolova, Lannig 2008).

Usually, organisms are adapted to natural stressors in their habitats. However, when additional stresses are combined, an additional or synergistic effect may occur (Cairns Jr., Heath & Parker 1975). Still relatively little is known about interactions between different stressors. However, it can have important implications for exposed individuals and populations.

Exposure to toxic compounds is such an additional stressor that may interact with temperature stress. Temperature can directly influence the toxic effects to organisms by changing bioavailability, influence uptake over membranes, alter fate in the body of the organism (toxicokinetics) and alter the mechanism by which toxicants produce unique cellular effects within organisms (toxicodynamics and organism biology) (Cairns Jr., Heath & Parker 1975, Dyer, Belanger & Carr 1997, Heugens et al. 2001). Bioavailability is especially related to temperature for organic compounds because it influences compound solubility and therefore environmental partitioning (for example, sorption to particulate matter). Toxicokinetics is the study of time-dependant processes (absorption, distribution, storage, biotransformation and elimination) related to toxicants as they interact with living organisms. Temperature affects for instance uptake and elimination rates and therefore influences the internal concentrations in organisms. Temperature may alter toxicodynamics for instance by the interaction between a toxicant molecule and a receptor, where the binding strength between the two is temperature dependent.

Several studies have reviewed research papers to the combined effect of temperature and toxicant stress to aquatic species (Cairns Jr., Heath & Parker 1975, Heugens et al. 2001, McLusky, Bryant & Campbell 1986). For instance, Cairns reported a mainly higher lethality at elevated temperatures for ammonia, cyanide and zinc. Furthermore, also an overall increased sensitivity was found by Cairns for tests performed with the organochlorine and organophosphorus compounds and phenols. However, for these compounds some studies reporting no or negative effects of temperature on toxicity were also found. No clear trend was found by reviewing studies reporting on several metals (cadmium, copper, nickel, chromium and mercury) (Cairns Jr., Heath & Parker 1975). Heugens and Sokolova reviewed all known studies about temperature and toxicity at that time and did find a trend. Almost all studies focusing on cadmium and zinc reported a combined effect of toxicants and temperature on mortality (Sokolova, Lannig 2008, Heugens et al. 2001). This was also found for organochlorine compounds. Overall, a positive correlation between toxicity and temperature was found for 70% of the 151 studies reviewed by Heugens et al. and Sokolova and Lannig, for metals this was even 80% (Sokolova, Lannig 2008, Heugens et al. 2001).

As temperature affects sensitivity of single species, one would expect that sensitivity is also affected at a multi-species level. However, literature combining results of altered toxicant sensitivity at different temperatures for a group of species is scarce. No general approach exists to analyze the extent of the interaction for a group of species. However, the interaction may have implications for legislation of chemicals in the environment at different ambient temperatures. Currently, effects of temperature on toxicity are not taken into account which may lead to an increased environmental risk.

In the European Union, current environmental quality standards for toxicants in the environment are directed of protecting the ecosystem status, taking the protection of 95% of the species as a minimum target (European Chemicals Bureau 2003). Usually, the median or lower estimate of the hazardous concentration for 5% (HC₅) of the species is taken, which implicates that a maximum of 5% of the species are potentially affected at this environmental concentration. The HC₅ is derived from a Species Sensitivity Distribution (SSD) which describes the range of sensitivities (usually expressed as the No Observed Effect Concentration (NOEC)) of a representative number of species from different phyla towards a compound. This way, the interspecies variation in toxicant sensitivity is taken into account (Posthuma, Suter & Traas 2001).

SSDs used for derivation of Environmental Quality Standards (EQS) are static and do not consider changing environmental conditions. Specific experimental temperatures are not taken into account in current approach, which may lead in

an over- or underestimation of the ecotoxicological risk depending on the environmental condition.

SSDs have also been used to quantify ecological risk by determination or comparison of the fraction of affected species. For instance, SSDs were also used to assess non-toxic stressors such as the impact of thermal pollution (De Vries et al. 2008), to assess the differences between field- and laboratory data (Selck et al. 2002, Schroer et al. 2004), and to compare the sensitivity between salt- and freshwater species (Wheeler et al. 2002).

This study aims at comparing SSDs derived from toxicity data using different experimental temperatures. By determination of the HC₅ and HC₅₀ for different temperature groups, the extent of the temperature-toxicant interaction the compounds could be revealed.

Two model compounds, the heavy metal cadmium (Cd) and the organic pollutant pentachlorophenol (PCP), were selected to study the interaction between temperature and toxicants. For both compounds, increased sensitivity was indicated; the selection is explained in detail in the method section.

Although temperature effects may be compound specific, these two different compounds provide a good starting point towards the comparison of the effect of temperature on toxicant sensitivity. Many metals have common toxic cellular mechanisms and elicit similar protective responses in a wide variety of organisms. For instance, interference with aerobic metabolism, including energy demand, oxygen supply and mitochondrial function, forms a physiological base for the temperature-toxicity interaction in all organisms (reviewed by (Sokolova, Lannig 2008, Cairns Jr., Heath & Parker 1975)). This is not only the case for metals, but also for other pollutants and environmental stressors that require an elevated energy demand for stress and/or negatively affect energy supply.

The toxicodynamics of Cd and PCP have been elucidated over the last few decades. Both compounds have been shown to be uncouplers of mitochondrial oxidative phosphorylation (Jacobs et al. 1956, Weinbach, Garbus 1964). Furthermore, Cd inhibits mitochondrial respiration and creates oxidative damage by reducing cellular oxidant capacity (Jacobs et al. 1956). Also, Cd is genotoxic and mainly exerts toxicity by nonspecific binding to sulfhydryl groups of physiologically important proteins (Wang, Rainbow 2006). Cd also displaces the essential metals in metalloenzymes (Vallee, Ulmer 1972).

Beside uncoupling oxidative phosphorylation, PCP stimulates the citric acid cycle and respiratory chain enzymatic activity, while it inhibits the glycolytic enzymes (Boström, Johansson 1972). This results in a hypermetabolic state and depletion of energy stores (Holmberg et al. 1972).

Since increased sensitivity for single species at elevated temperatures is indicated, a difference between high and low temperature SSDs is expected. Results of this study may be used to make predictions towards other compounds for which an effect of increased sensitivity is anticipated at higher temperature based on individual studies. These include other heavy metals and pesticides such as chlorinated hydrocarbons (Cairns Jr., Heath & Parker 1975, Heugens et al. 2001). Based on the results of this study, the need for temperature specific environmental quality standards for pollutants in the environment through temperature dependent SSDs will be determined. Finally, results in this study should contribute to a reliable risk assessment for variable environmental conditions.

Methods

Compound selection

The keywords 'toxicity', 'temperature' and 'interaction' were used to search online libraries (Scopus, Web of Science, and University of Utrecht online library) for literature describing acute toxicity tests at multiple temperatures. Information

about the compound, the species, type of test, measured endpoint, test temperatures, and found correlation were reported in a database.

To study the extent of potentially increased toxicity at higher temperature, 2 well-studied compounds were selected for analysis for which temperature dependent sensitivity was indicated. Cadmium (Cd) was selected as a representative to the group of heavy metals, and pentachlorophenol (PCP) to the group of organic pollutants. This choice was based on the quantity and quality (e.g. used toxicity endpoints) of the available data; For 9 out of 11 found studies increased sensitivity for Cd toxicity was indicated on the individual species level at elevated temperature. In addition, the review of Heugens reported that 15 out of 17 reviewed Cd toxicity studies showed a positive correlation for joint effects of Cd toxicity and temperature on mortality (Heugens et al. 2001). PCP has been studied less extensively, but appeared one of the best studied among the organochlorine compounds. Also for PCP, most studies indicated increased sensitivity at elevated temperatures. Since no good review exist for Cd, a summary of our literature search results is presented in table 1.

Both Cd and PCP are omnipresent in the aquatic environment due to their extensive use in past and present (UNEP 2008, Euro Chlor 1999). Cd is a heavy metal that is mainly found in batteries, but also used for cadmium pigment, coatings and plating. PCP is an organochlorine compound generally used as a biocidal agent. Both Cd and PCP has been designated as a "priority toxic pollutant" by the United States Environmental Protection Agency (USEPA) under the guidance of the Clean Water Act in 1972 (US EPA 1972). Similarly in the EU, both Cd and PCP are also on the list of "priority substances", and to Cd is even referred as a "priority hazardous substance" (European Commission 2006). Current emissions are declining in developed countries due to imposed restrictions and bans in production and use, but both compounds remain omnipresent (UNEP 2008, Euro Chlor 1999).

Compound databases

For the selected compounds (Cd and PCP), toxicity data and corresponding test conditions were gathered. In contrast to the search that was performed for compound selection, also studies that performed toxicity tests at a single test temperature were included.

SSDs for derivation of environmental quality standards are generally based on chronic NOEC data. NOECs were not used in this study as they depend strongly on the selected test concentrations and the applied statistical analysis (Laskowski 1995, Crane, Newman 2000, Jager, Heugens & Kooijman 2006). Instead the well defined 50% lethal concentration (LC50) was used as a toxicity endpoint. Since LC50 values are a result of concentration and exposure time, only one test duration was selected. 96h tests were selected because most data was available at this test duration.

To gather 96h-LC50 data, a query was performed in the online database of the US Environmental Protection Agency ECOTOX (www.epa.gov/ecotox/).

Table 1. Overview of studies that report sensitivity change towards PCP at multiple temperatures.

Species scientific name	Species common name	Sensitivity change	Reference(s)
<i>Chironomus riparius</i>	Midge	+	(Fisher 1991)
<i>Corbicula fluminea</i>	Asian clam	+	(Johnson, McMahon 1998)
<i>Dreissena polymorpha</i>	Zebra mussel	+	(Johnson, McMahon 1998, Fisher et al. 1999)
<i>Heptacarpus futilirostris</i>	Toy shrimp	+	(Hori, Tateishi & Yamada 2002)
<i>Onchorhynchus mykiss</i>	Rainbow trout	+ (at low concentrations) - (at high concentrations)	(Hodson, Blunt 1981)
<i>Pisidium amnicum</i>	Freshwater clam	+	(Heinonen, Kukkonen & Holopainen 2001)
<i>Penaeus japonicus</i>	Kuruma prawn	+	(Hori, Tateishi & Yamada 2002)

Additionally, online libraries (Scopus, Web of Science, University of Utrecht online library) were searched for toxicity data not appearing in the ECOTOX database. Keywords used were 'LC50', '96h' and 'cadmium'/'Cd' resp. 'pentachlorophenol'/'PCP'.

Salts of the selected compounds with non-toxic counter ions were included in the dataset, assuming that it would not give additional effects. A detailed list of compounds including CAS numbers can be found in Appendix A. Only lab-tests were selected because in field tests the experimental conditions are not adequately under control.

Furthermore, only tests with information on experimental temperatures were selected. When a small range (<3°C) of experimental temperature was reported instead of an exact value, the mean temperature value was used; tests with larger ranges were excluded from the analysis. To standardize the database, all LC50 results were converted to M (mol·L⁻¹) Cd²⁺ resp. PCP, excluding the associated salts.

When doubt arose about the quality of the data (for instance, when intraspecies differences were higher than a factor 5 or when extreme values were reported), the tests in the database were revised using original articles in order to construct a reliable dataset. Since toxicity of Cd and PCP also depends on pH, water hardness and salinity (UNEP 2008) results were reviewed when pH was <6 or >8, hardness was <50 or >200 mg CaCO₃, or when saltwater species were tested in freshwater systems. Tests with effects on LC50 values due to these extreme environmental conditions were excluded. Early Life Stage tests were excluded because this would hamper a correct comparison between species. Also tests with a possible confounding objective (for instance, mixture effect of chemicals, effect of poultry litter addition) were removed from the database. Furthermore, reports of study controls were sometimes coded as 'insufficient' in the US EPA database although the criterion for this classification was unclear. No exclusions were made based on this control classification alone, but data was checked if results deviated from comparable species and excluded when errors were suspected. Finally, some tests did not report an exact LC50 value but only an upper or lower concentration limit instead, indicating that experimental concentrations were outside the range to determine an exact LC50 value for this species. Results were allowed when indicated LC50 values were relatively close to detection limits (for Cd, ≤1.8·10⁻⁶ M or 200 µg/L) or relatively high and hence the most likely the result of solubility issues (for Cd: ≥1.3·10⁻⁵ M or 1500 µg/L, PCP: ≥7.5·10⁻⁷ M or 200 µg/L).

Data analysis

Statistical program R (The R Foundation for Statistical Computing 2009) version 2.10.0 was used for the analysis of the data. The 96h-LC50 data varied among species with differences in several orders of magnitudes. Therefore, the toxicity data was log₁₀-transformed before analysis.

By creating (box)plots, data was visually inspected for differences in median and range of LC50 values between original test chemical by CAS number (with or without an associated salt), chemical analysis (measured/nominal), aquatic living environments (salt/freshwater), LC50 value reporting method (as active ingredient only/dissolved fraction only/total concentration including non-dissolved fraction/formulation including associated salts), compound used to report LC50, and exposure method (static/renewal/flow-through). LC50 values were also scatter plotted against test temperature, pH, salinity, hardness, alkalinity and dissolved oxygen to check for overall trends and outliers in the data. Data was also checked for an equal distribution of temperature among different experimental conditions. Box- and scatter plots of the final dataset can be found in Appendix B and C.

A regression line was fitted to the scatter plot of individual LC50 test results plotted against test temperature in order to detect an overall trend in the toxicity sensitivity at different temperature. The same was done to a scatter plot of species geometric mean LC50- and temperature values.

For the three main groups of organisms (mollusks, crustaceans and fish), the relation between LC50 results and test temperatures were also examined separately.

Construction of SSDs

In the present study toxicity data was assumed to be distributed log normally in the SSDs (Wagner, Løkke 1991).

For derivation of temperature-specific SSDs, the median test temperature of all LC50 tests was used to bin the results in a high and low temperature group. A geometric mean LC50 (GM-LC50) value for every species was calculated in each temperature group, along with the standard deviation of the log₁₀-transformed LC50 values for this species. Species GM-LC50 values were sorted ascending and logarithmic transformed. The log transformed data was plotted against the plotting position p_i for each species (i) described by Equation (1);

$$1) p_i = \frac{i-0.5}{n}$$

where n is the total number of species and i is the index of the species.

By using all data, differences between the groups could also be the result of taxonomic differences between them and not only the test temperature. To eliminate this problem an additional analysis was performed with data that was taxonomically equal in both groups. For both compounds species were selected from the databases that were tested both above and below the median test temperature. SSDs were plotted using the approach described above.

Comparison of sensitivity between high- and low temperature tests

To determine the sensitivity of the high- and low temperature groups, the compound- and temperature specific HC₅ and HC₅₀ were computed. The concentration where $p\%$ of the species are exposed above their LC50 (HC _{p}), was calculated using Equation (2) according to the method described by Aldenberg (Aldenberg, Jaworska 2000);

$$2) \log(\text{HC}_p) = X_m - k_s \cdot S_m$$

X_m and S_m are the mean and standard deviation of log-transformed toxicity data that describe the SSD. k_s is an extrapolation factor which depends on the cumulative distribution function, sample size (n) and the required confidence level of the HC _{p} . For the median HC₅ and at a sample size of 100, k_s is equal to 1.6498, while for the HC₅₀, k_s is zero (Aldenberg, Jaworska 2000). For 2-sided 90% confidence intervals, a k_s of 1.9265 and 1.4143 are required to calculate respectively upper and lower estimate levels of the HC₅ at a sample size of 100 and a normal distribution. For the HC₅₀, these values are 0.1660 and -0.1660 respectively, under the same conditions (Aldenberg, Jaworska 2000).

Statistical differences between the HC₅₀ of high- and low temperature groups were determined using a Welch Two sample t-test assuming a normal distribution (Welch 1947). In addition, the Wilcoxon test (Wilcoxon 1945) was conducted to determine the differences between the HC₅₀ without the assumption that the data is normally distributed. In the Wilcoxon test the null hypothesis is that the distributions of the two groups differ by a location shift of zero and the alternative is that they differ by some other location shift. A two-sample Kolmogorov-Smirnov test is performed to determine whether or not the data from the two groups are samples from the same continuous distribution.

To reflect the sensitivity difference between the groups, a low-/high temperature sensitivity (low/high) ratio was calculated of the determined HC₅ and HC₅₀ values. A low/high ratio >1 would indicate increased sensitivity at higher temperature, whereas a ratio <1 would suggest that sensitivity is increased for species in the low temperature group.

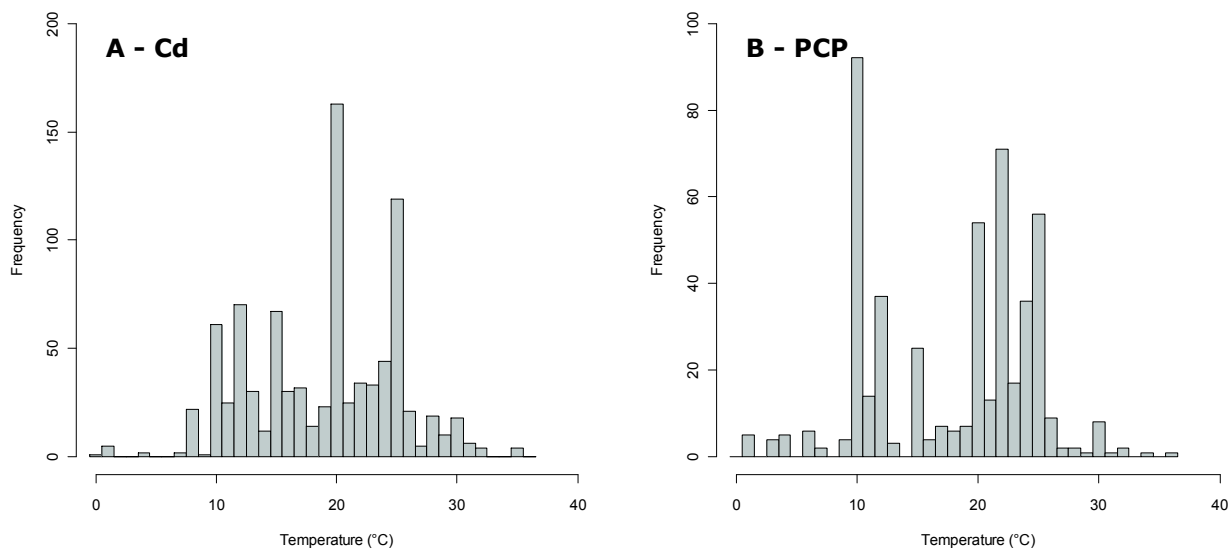


Fig. 1A and 1B. Amount of tests present in the database for each compound, sorted per reported test temperature interval of 1°C. A) Cadmium (Cd). B) Pentachlorophenol (PCP).

Results

Database composition

The frequency of conducted tests for a range of temperature intervals of 1°C is presented in fig. 1. Some test temperatures occurred more frequent than others; peaks were around 10°C, 20°C and 25°C.

For Cd, the final database consisted of a total amount of 284 species. 110 species were present in the PCP database. When Cd LC50 values were scatter plotted against test temperature (fig. 2A), a simple linear regression line indicated a statistically significant decreasing sensitivity trend with increasing temperature ($p < 0.0001$). When the Cd data was summarized by calculating the GM-LC50 per species, a reverse trend was observed (fig. 2B); sensitivity increases slightly at higher temperatures ($p = 0.12$). However, correlations were weak (Adjusted R^2 0.042 and 0.005 respectively).

For PCP (fig. 2C and 2D), no significant trend could be observed taking individual LC50 results (Adjusted $R^2 = -0.002$, $p = 0.97$), but a regression line on GM-LC50 results showed a slight increase in sensitivity when temperature increases although this was also not significant (Adjusted $R^2 = 0.006$, $p = 0.12$). The data distribution of test conditions other than temperature is presented in Appendix B and C.

Data was also plotted separately for the three main species groups –mollusks, crustaceans and fish (fig. 3). At higher temperature, mollusks appeared to be more sensitive towards both Cd (Adjusted R^2 0.098, $p = 0.019$) and PCP (Adjusted R^2 0.031, $p = 0.009$). Crustaceans were more sensitive towards PCP at higher temperature (Adjusted R^2 0.104, $p = 0.006$), but no temperature effect was present for Cd (Adjusted R^2 -0.003, $p = 0.81$). For fish, it was found that for both compounds sensitivity was significant decreased at higher temperatures ($p < 0.0001$, both compounds). Correlations appeared to be relatively strong for fish (Adjusted R^2 0.480 for Cd, and 0.141 for PCP).

Temperature-specific SSDs for all species

The data was divided in a high and low temperature group based on the median temperature of all tests. The median test temperature for both Cd and PCP tests was 20°C. Tests performed at this temperature were assigned to the lower

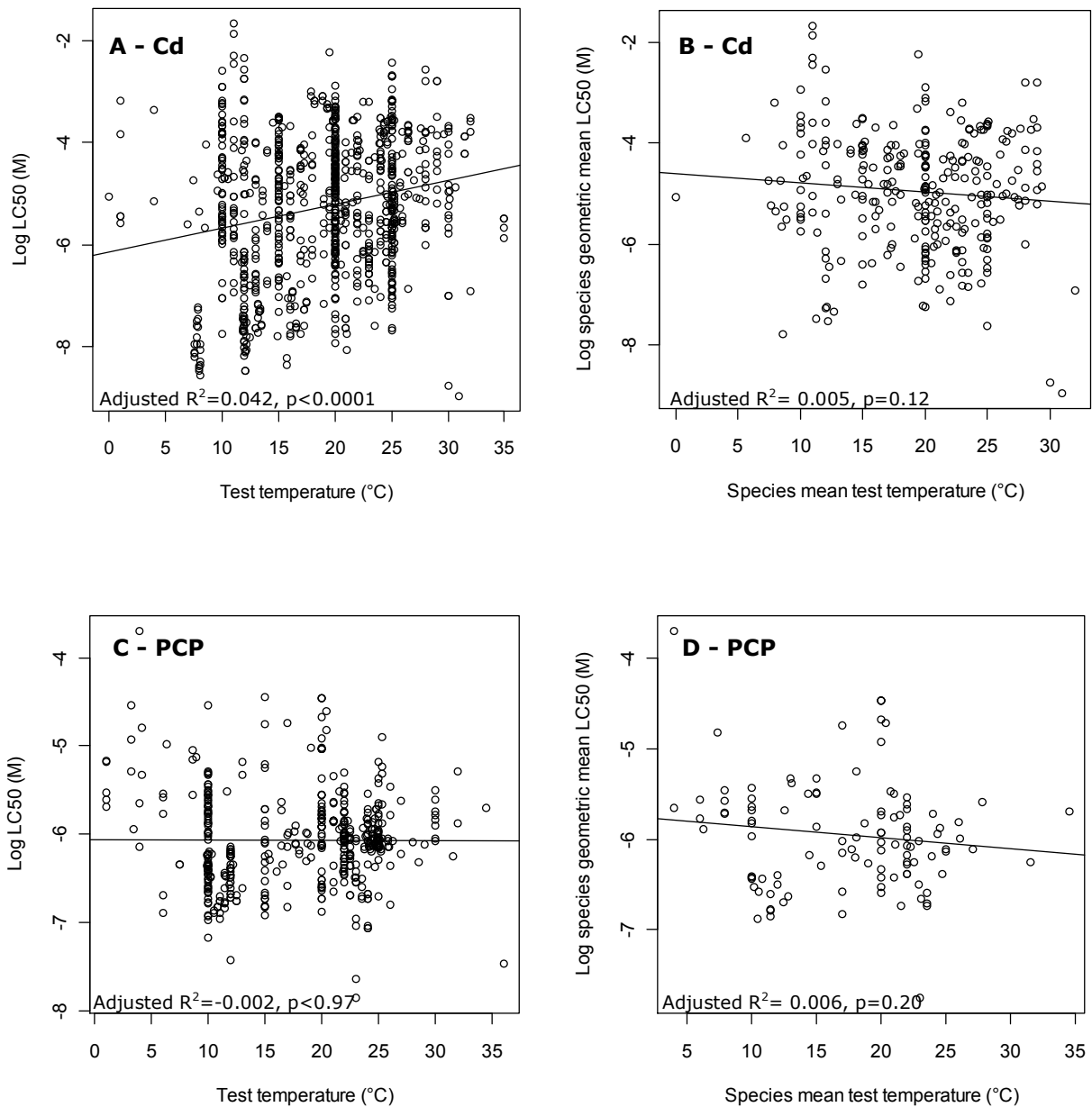


Fig. 2 (A-D). Scatter plots for toxicity data (log 96h-LC50) of cadmium (Cd, panels A+B) and pentachlorophenol (PCP, panels C+D), sorted by reported test temperature. Trends are indicated by regression lines, the adjusted R^2 and statistical significance for the lines (slope \neq 0) are presented in each graph. A+C) All individual LC50 values used in analysis; every data point represents the result of one test. B+D) Species geometric mean LC50 values; for every species the geometric mean LC50 and mean test temperature were calculated taking all tests for one particular species together. One data point represents the combined results for one species.

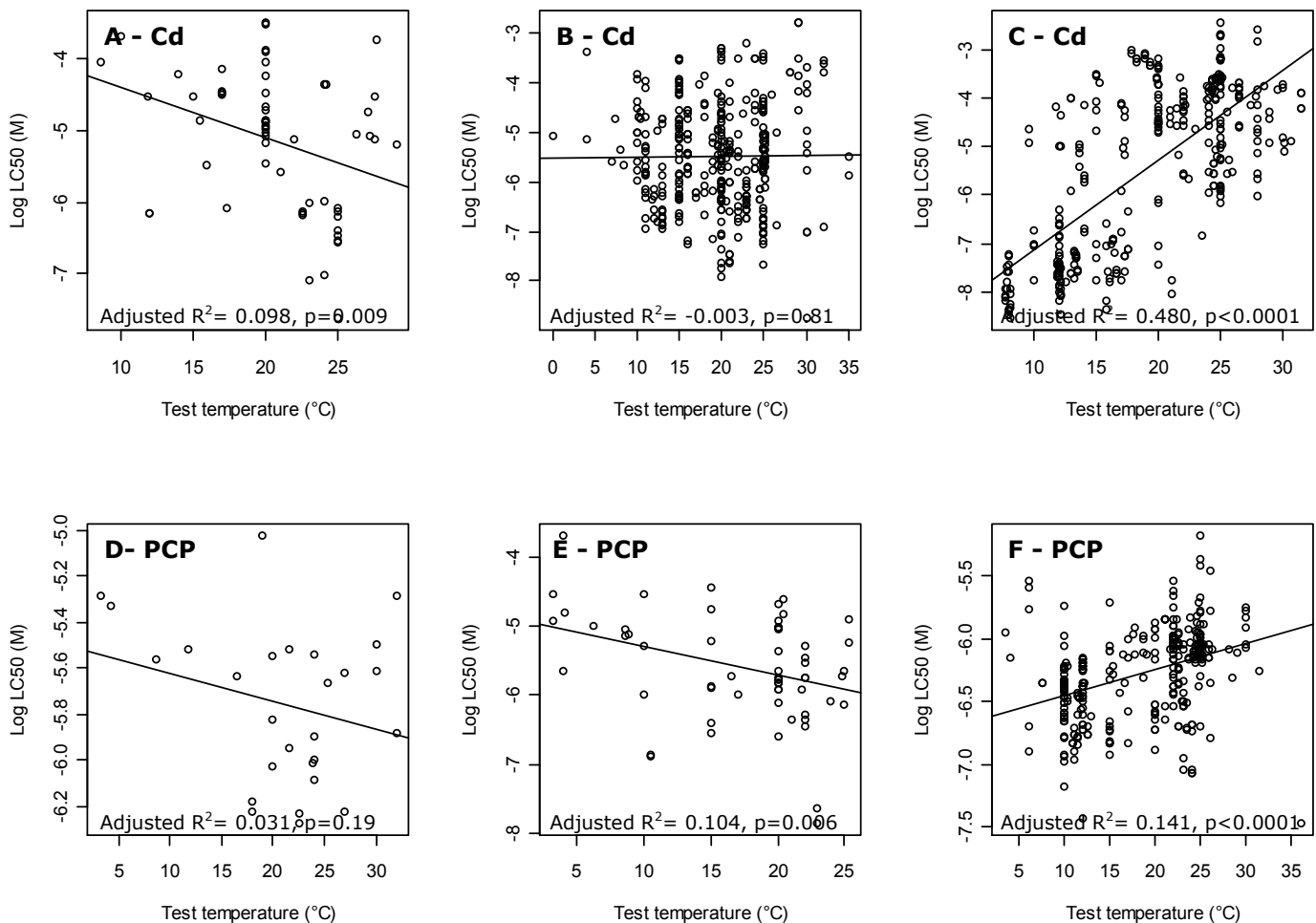


Fig. 3 (A-F). Scatter plots of toxicity data (log 96h-LC50) for cadmium (Cd, panels A-C) and pentachlorophenol (PCP, panels D-F) for 3 species groups, sorted by reported test temperature. Trends are indicated by regression lines, adjusted R^2 and statistical significance are presented in each graph. A+D) Mollusks. B+E) Crustaceans. C+F) Fish.

temperature group; as a consequence the lower temperature group holds more data than the higher group. The summary values of the used tests, the test conditions and species taxonomic composition of the groups are presented in table 2. In the Cd low temperature group, the most sensitive species were fish in the trout family (*Salmonidae*). Trout were also the most sensitive for PCP in the low temperature group, although also other fish (for instance the cyprinoid *Rutilus rutilus*) and a crustacean (*Parastenocaris germanica*) belonged to the most sensitive group. Above 20°C, most sensitive towards Cd were an amphibian tadpole (*Bufo maculatus*), a crustacean (*Rhithropanopeus harrisi*) and a mollusk (*Villorita cyprinoides cochi*). For PCP, the crustacean *Hyalella azteca*, and several fish species (*Lagodon rhomboids*, *Cirrhinus mrigala*, *Notopterus notopterus*) were most sensitive. The SSDs for Cd and PCP are plotted in figure 4A and B. Standard deviations for species GM-LC50 are displayed when multiple data are available for a single species (horizontal error bars). Dashed vertical lines mark the HC₅₀ values of both groups. In the Cd graph, the SSD of the high temperature group (red) is located left of the low temperature SSD (blue), indicating increased sensitivity towards Cd in the high temperature group.

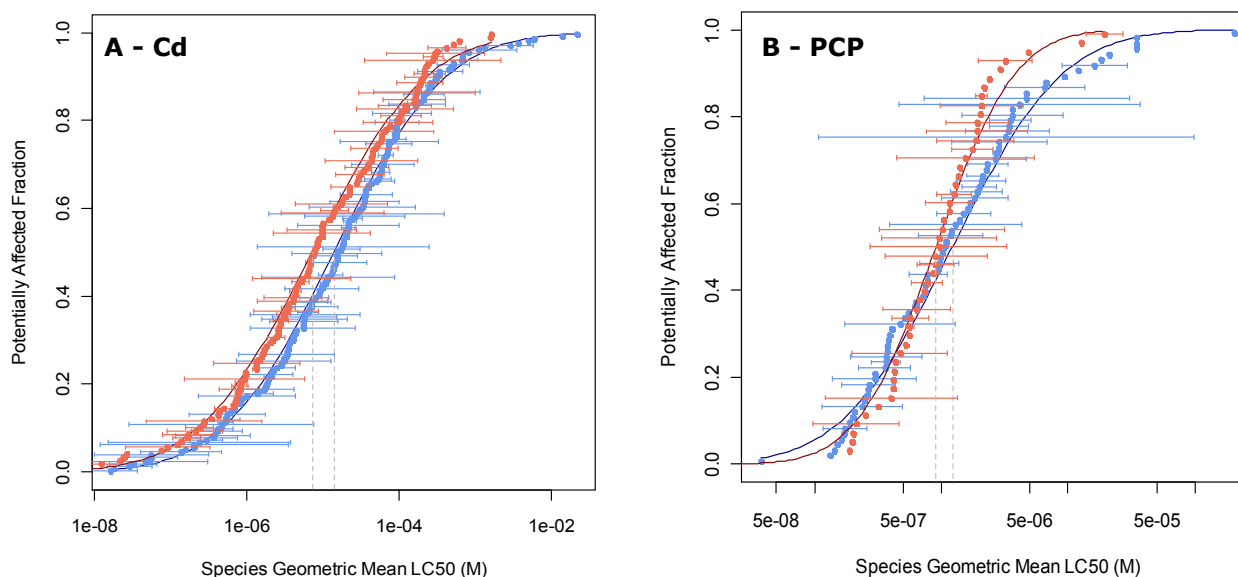


Fig. 4A and 4B. All species - temperature specific species sensitivity distributions (SSD) of log LC50 data for cadmium (Cd, panel A) and pentachlorophenol (PCP, panel B). The blue points correspond to the SSDs (cumulative normal distributions) at low test temperatures ($\leq 20^{\circ}\text{C}$); the red lines at high temperatures ($> 20^{\circ}$). Each point represents the toxicity (96h-LC50) data of one species. Horizontal lines represent standard deviations in data of a species when a geometric mean species LC50 value was derived of multiple tests for this species. For each temperature specific SSD, (mostly) different test species have been used. Dashed vertical lines mark the log concentration where 50% of the species are potentially affected (HC_{50}). Note that scales on the x-axes are different for PCP than for Cd.

A) Cd. 188 species for the low temperature SSD, $\text{HC}_{50} = 1.40\text{e-}05$. 135 species for the high temperature SSD, $\text{HC}_{50} = 7.21\text{e-}06$. B) PCP. 79 species for the low temperature SSD, $\text{HC}_{50} = 1.22\text{e-}06$. 49 species for the high temperature SSD, $\text{HC}_{50} = 9.05\text{e-}07$.

Although the SSD slope of the PCP high temperature group is steeper, no significant difference in the SSDs between high and low temperature PCP tests can be found regarding the location of the temperature dependent SSDs for PCP. The HC_5 and HC_{50} values, along with their corresponding 90% two-sided confidence levels determined according to Aldenberg (Aldenberg, Jaworska 2000), are listed in table 3. HC_5 and HC_{50} values for the high temperature Cd group are ~ 2 times lower, indicating an increased sensitivity at higher temperatures. For PCP, the HC_5 is higher at temperatures $\leq 20^{\circ}\text{C}$ while the HC_{50} is higher in the $> 20^{\circ}\text{C}$ group (low/high ratio 0.65 and 1.35 for respectively HC_5 and HC_{50}). The statistical difference between the mean species LC50 values for low and high temperature SSDs, determined by a Welch two-sample t-test was statistically significant for Cd ($p=0.03$). The Kolmogorov-Smirnov and Wilcoxon tests confirmed that the SSDs differed between the temperature groups, although the differences were borderline significant according to these tests. For PCP, none of statistical tests indicated a significant difference between the two groups (table 3).

Temperature-specific SSDs using the same set of species

Using the median experimental temperature (20°C), species were selected with one or more tests performed both at $< 20^{\circ}\text{C}$ and $> 20^{\circ}\text{C}$. For Cd, a total of 20 species were selected; for PCP 12 species comply with this condition. In table 4,

Table 2. Test- and species composition of the data used to construct the temperature specific SSDs for Cd and PCP, taking all test species into account.

Variable	Cd		PCP	
	≤20°C	>20°C	≤20°C	>20°C
Mean of log transformed LC50 data (± SD)	-4.86 ± 1.15	-5.14 ± 1.17	-5.91 ± 0.68	-6.04 ± 0.49
Tests (N)	555	346	273	222
Species (N)	188	135	79	49
Phyla (N species/phylum)				
Algae	1 (0.5%)	0	0	0
Annelida	23 (12.2%)	2 (1.5%)	15 (19.0%)	3 (6.1%)
Arthropoda	95 (50.5%)	53 (39.3%)	19 (24.0%)	9 (12.2%)
Ascomycota	0	0	0	1 (2.0%)
Chordata	35 (18.6%)	50 (37.0%)	34 (43.0%)	31 (63.3%)
Cnidaria	4	2 (1.5%)	0	0
Ectoprocta	0	3 (2.2%)	0	0
Echinodermata	2 (1.1%)	0	1 (1.3%)	0
Magnoliophyta	0	1 (0.7%)	0	0
Mollusca	20 (10.6%)	20 (15.8%)	5 (6.3%)	5 (10.2%)
Nemata	3 (1.6%)	1 (0.7%)	4 (5.1%)	0
Platyhelminthes	4 (2.1%)	3 (2.2%)	1 (1.3%)	0
Water type (N tests)				
Fresh	337 (60.7%)	174 (50.3%)	221 (81.0%)	204 (91.9%)
Salt (incl brackish water)	218 (39.3%)	172 (49.7%)	52 (19.0%)	18 (8.1%)
Exposure method (N tests)				
Static	288 (51.9%)	243 (70.2%)	109 (40.0%)	70 (31.5%)
Renewal	139 (25.0%)	70 (20.2%)	97 (35.5%)	38 (17.1%)
Flow-through	128 (23.1%)	33 (9.6%)	67 (24.5%)	114 (51.4%)
Temperature (mean, °C)	15.11 ± 4.25	25.05 ± 2.75	12.09 ± 4.69	24.97 ± 2.43
pH (mean)*	7.45 ± 0.53	7.64 ± 0.51	7.34 ± 0.53	7.65 ± 0.45
Salinity (mean, ‰)*	20.20 ± 13.15	19.09 ± 15.86	9.31 ± 10.00	8.50 ± 11.60
Dissolved Oxygen (mean, mg/L)*	7.74 ± 1.74	6.73 ± 1.47	6.73 ± 2.11	6.26 ± 1.32
Hardness (mean, mg/L CaCO ₃)*	105 ± 99	123 ± 92	113 ± 82	108 ± 75
Alkalinity (mean, mg/L CaCO ₃)*	66 ± 66	112 ± 91	129 ± 33	127 ± 70
Organic carbon (mean, mg/L)*	4.23 ± 2.50	2.32 ± 1.18	n.c.	n.c.

*Only tests included where parameters were reported

Table 3. Compound- and temperature group specific values of HC₅ and HC₅₀ (M) along with their 90% 2-sided confidence intervals, derived using the temperature specific SSDs for all test species. Difference between the high- and low temperature groups are shown by a HC₅- resp. HC₅₀- low/high temperature sensitivity ratio. Statistical significance between the average GM-LC50 values of the groups are computed using the Welch t-test, Kolmogorov-Smirnov test and Wilcoxon test (intra-species differences not taken into account).

Compound	Temperature group	Mean HC ₅ (M)	HC ₅ 90% C.I.	HC ₅ ratio	Mean HC ₅₀ (M)	HC ₅₀ 90% C.I.	HC ₅₀ ratio	P-value (1)	P-value (2)	P-value (3)
Cd	≤20°C	1.81e-07	8.62E-8 - 3.33E-7	2,11	1.40e-05	9.01E-6 - 2.16E-5	1.94	0.03	0.07	0.05
	>20°C	8.56e-08	4.02E-8 - 1.60E-7		7.21e-06	4.61E-6 - 1.13E-5				
PCP	≤20°C	9.18e-08	5.89E-8 - 1.32E-7	0.65	1.22e-06	9.41E-7 - 1.59E-6	1.35	0.21	0.15	0.49
	>20°C	1.41e-07	1.03E-7 - 1.84E-7		9.05e-07	7.50E-7 - 1.09E-6				

(1) Welch two sample t-test

(2) Two-sample Kolmogorov-Smirnov test

(3) Wilcoxon rank sum test with continuity correction

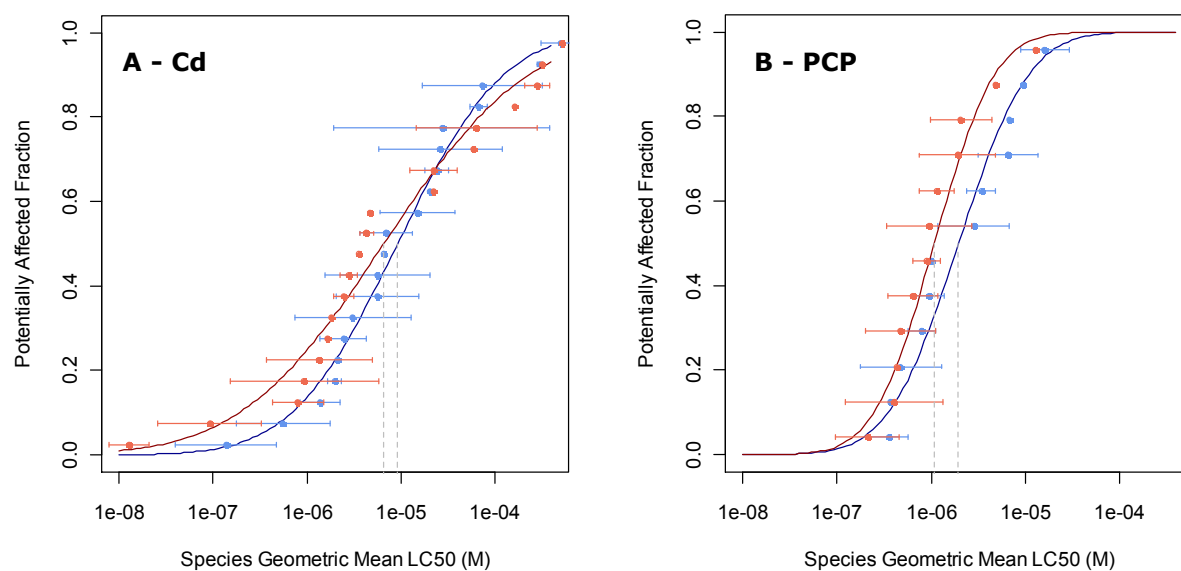


Fig. 5A and 5B. Equal species- temperature specific Species Sensitivity Distributions (SSDs) for cadmium (Cd, panel A) and pentachlorophenol (PCP, panel B). The blue points correspond to the SSDs (cumulative normal distributions) at low test temperatures ($\leq 20^{\circ}\text{C}$); the red lines at high temperatures ($>20^{\circ}$). Each point represents the toxicity (96h-LC₅₀) data of one species. Horizontal lines represent standard deviations in data of a species when a geometric mean species LC₅₀ value was derived of multiple tests for this species. For each compound, the high- and low temperature SSD represent the same set of species tested both at $\leq 20^{\circ}\text{C}$ and $>20^{\circ}\text{C}$. Dashed vertical lines mark the concentration where 50% of the species are potentially affected (HC₅₀). A) Cd, 20 species. Low temperature HC₅₀ = $9.24\text{e-}06$, high temperature HC₅₀ = $6.58\text{e-}06$. B) PCP, 12 species. Low temperature HC₅₀ = $1.96\text{e-}06$, high temperature HC₅₀ = $1.09\text{e-}06$.

the taxonomic composition and test conditions are presented for the low and high temperature groups. The corresponding SSDs are displayed in figure 5A and 7B. Sensitivity towards Cd was increased only in the lower tail of the SSD, thus for the most sensitive species of the group. The most sensitive species were the striped bash (*Morone saxatilis*) and the scud *Hyalella azteca*, which were both more sensitive at a higher temperature. For PCP, Asiatic knifefish (*Notopterus notopterus*) was the most sensitive species in both groups. Overall, a (non-significant) increased sensitivity towards PCP was found $>20^{\circ}\text{C}$. In table 5, the HC₅ and HC₅₀ are presented, which were computed using the temperature-specific SSDs. Especially the Cd HC₅ value of the high temperature group is higher than of the low temperature group (about 5 times), which is especially a result of large increase in sensitivity at higher temperature towards Cd for the two most sensitive species.

Differences between the SSDs were not statistically significant at the HC₅₀ level but the HC₅ 90% two sided confidence intervals of the Cd-low and Cd-high data did not overlap (table 3).

Discussion

In this study, the effect of temperature on species sensitivities to toxicity of Cd and PCP was studied by comparing SSDs constructed for high- ($>20^{\circ}\text{C}$) and low ($\leq 20^{\circ}\text{C}$) test temperatures. Two model compounds were used; the metal Cd and the organic pollutant PCP.

For Cd, it was shown that both the mean sensitivity of all species (HC₅₀) as well

Table 4. Test- and species composition of the temperature specific SSDs for Cd and PCP. For each compound, the high- and low temperature SSD consist of the same set of species.

Variable		Cd		PCP	
		≤20°C	>20°C	≤20°C	>20°C
Mean of log transformed LC50 data (± SD)		-5.03 ± 0.88	-5.18 ± 1.20	-5.71 ± 0.58	-5.96 ± 0.50
Tests (N)		104	98	46	133
Species (N)		20	20	12	12
Phyla (N species/phylum)	Annelida	2 (10.0%)	2 (10.0%)	0	0
	Arthropoda	12 (60.0%)	12 (60.0%)	5 (41.7%)	5 (41.7%)
	Chordata	6 (30.0%)	6 (30.0%)	5 (41.7%)	5 (41.7%)
	Mollusca	0	0	2 (16.7%)	2 (16.7%)
Water type (N tests)	Fresh	39 (37.5%)	45 (45.9%)	45 (97.8%)	132 (99.2%)
	Salt (incl brackish water)	65 (62.5%)	53 (54.1%)	1 (2.2%)	1 (0.8%)
Exposure method (N tests)	Static	89 (85.6%)	90 (91.8%)	9 (19.6%)	28 (21.1%)
	Renewal	12 (11.5 %)	4 (4.1%)	3 (6.5%)	6 (4.5%)
	Flow-through	3 (2.9%)	4 (4.1%)	34 (73.9%)	99 (74.4%)
Temperature (mean, °C)		16.90 ± 3.59	24.64 ± 2.80	13.75 ± 5.85	24.00 ± 2.14
pH (mean)*		7.62 ± 0.46	7.60 ± 0.43	7.74 ± 0.29	7.65 ± 0.35
Salinity (mean, ‰)*		18.97 ± 13.23	11.27 ± 11.03	n.c.	n.c.
Dissolved Oxygen (mean, mg/L)*		6.40 ± 1.71	6.73 ± 1.29	5.91 ± 1.03	6.28 ± 1.19
Hardness (mean, mg/L CaCO ₃)*		92 ± 104	139 ± 122	158 ± 64	87.5 ± 73
Alkalinity (mean, mg/L CaCO ₃)*		55 ± 21	84 ± 98	138 ± 29	130 ± 77
Organic carbon (mean, mg/L)*		4.23 ± 3.33	2.32 ± 1.32	n.c.	n.c.
*Only tests included where parameters were reported					

as the sensitivity of the 5% most sensitive species (HC₅) was increased by a factor two in the high temperature group (>20°C) compared to the low temperature group (≤20°C) (table 3). When an equal set of 20 species was used to construct high- and low temperature SSDs, the increased sensitivity at higher temperature was especially visible in the lower tail of the SSDs, but not in the central part of the SSDs. This resulted in a sensitivity of 5 times higher at the HC₅. For PCP, the results differed between the two approaches for SSDs. When using all data, the sensitivity at the HC₅ of the high temperature was lower compared to the high temperature group (low/high ratio =0.65) but a factor 1.35 higher at the HC₅₀ level. The sensitivity was increased with a factor 1.32 at the HC₅ and 1.80 at the HC₅₀ for high temperatures, when using the same species set for high and low temperature SSDs. However, these differences are not statistically significant.

Quality of the data and SSDs

The major part of the database was extracted from the US EPA ECOTOX database. As a result, there was little control on the selection of the studies. Therefore, papers were reviewed when a quality check was necessary; In this way, potential errors were removed as much as possible.

In this study, species-specific temperature tolerance ranges were not included in the analysis. It was assumed that toxicant mortality would not result because of thermal-induced stress only. This assumption only holds when negative controls were tested at the same temperature as the positive controls. When this is not the case, thermal induced stress (not related to toxicity) might occur. However, not for all controls of the used studies from the ECOTOX database were classified as being sufficient. Criteria for this classification are not elaborated by the US EPA and therefore data was not excluded in the present analysis when the control was labeled 'insufficient' in the US EPA ECOTOX database.

Table 5. Compound- and temperature group specific values of HC₅ and HC₅₀ (M) along with their 90% 2-sided confidence intervals, derived using the temperature specific SSDs consisting of the same set of species. Difference between the high- and low temperature groups are shown by a HC₅- resp. HC₅₀- low/high temperature sensitivity ratio. Statistical significance between the average GM-LC₅₀ values of the groups are computed using the Welch t-test, Kolmogorov-Smirnov test and Wilcoxon test (intra-species differences not taken into account).

Compound	Temperature group	Mean HC ₅ (M)	HC ₅ 90% C.I.	HC ₅ ratio	Mean HC ₅₀ (M)	HC ₅₀ 90% C.I.	HC ₅₀ ratio	P-value (1)	P-value (2)	P-value (3)
Cd	≤20°C	3.31e-07	1.87E-7 – 5.27E-7	4.95	9.24e-06	6.60E-6 – 1.29E-5	1.40	0.66	0.57	0.55
	>20°C	6.96e-08	3.19E-8 - 1.32E-7		6.58e-06	4.16E-6 - 1.04E-5				
PCP	≤20°C	2.15e-07	1.84E-7 – 2.94E-7	1.32	1.96e-06	2.94E-7 – 2.45E-6	1.80	0.26	0.54	0.32
	>20°C	1.63e-07	1.18E-7 – 2.14E-7		1.09e-06	9.00E-7 - 1.32E-6				

(1) Welch two sample t-test

(2) Two-sample Kolmogorov-Smirnov test

(3) Wilcoxon rank sum test with continuity correction

Also, acclimation temperature, -rate and -duration prior to toxicity testing is known to influence test outcome (Cairns Jr., Heath & Parker 1975, Moller, Forbes & Depledge 1994). In our study, it was not possible to test whether acclimation procedures were influencing the results in the SSD as this information was not collected. It is assumed that differences due to acclimation durations and temperature intervals are randomly distributed among different species, and not temperature dependent. Therefore, it is expected that acclimation might have influenced individual LC₅₀ values, but probably not results in the SSDs. However, this is a source of noise, further research will be necessary to study the consequences on the results of the present analysis.

The toxicity tests were usually performed under a variety of test conditions, such as gradients of hardness, pH or other variables such as seasonal differences or different life stages. To construct the SSDs, species GM-LC₅₀ values were calculated when more than one test result was available a certain species. It is assumed that using species GM-LC₅₀ values noise from the different experimental conditions is partially filtered out. The variable test conditions and differences between labs resulted in considerable standard deviations of the species GM-LC₅₀ values. This interspecies variation in LC₅₀ was not included in the analysis, as there are no standardized approaches to include these sources of uncertainty in the analysis of SSDs. For further research, it is recommended to have a closer look into intraspecies differences and their effect on the location and shape of the SSDs.

For Cd, the original test results and the species GM-LC₅₀ scatter plot showed a difference in the slope of the regression lines for the relationship between temperature and toxicity (fig. 2). The LC₅₀ values for all tests combined indicated decreasing sensitivity at higher temperature, whereas a reversed trend was seen when using species GM-LC₅₀ values. This indicates that the interspecies sensitivity is different than the differences within a species tested at different temperatures. Therefore, results need to be analyzed with caution.

It is possible that the difference in trends is due to the taxonomic composition of the database. For instance, a major part of the database exists of fish data. Analysis of fish data only resulted in a strong trend towards decreasing sensitivity at higher temperatures (fig. 3C and 3F). When one value is used per species (e.g. the GM-LC₅₀) the relative amount of fish data is reduced and therefore altering the overall trend. Results are also influenced by other potential differences between taxonomic groups (fig. 3A-F) and their relative contribution to the database. In addition, data may be skewed when multiple tests are performed for relative (un)sensitive species and all single data points are included in the scatter

plot. The use of GM-LC50 will result in the equal importance of every species in the database.

In order to construct temperature dependent SSDs, the data was binned into high and low temperature groups based on the median test temperature (20°C for both compounds). The median test temperature was also the most frequently used test temperature (fig. 1). Tests performed at 20°C were assigned to either the low- or high temperature group. This resulted in an unequal sample size of the two groups.

Another option to assess the influence of temperature on toxicity would be to split the data into three groups and create low-, intermediate- and high temperature groups. However, the intermediate group also showed the highest variability of responses. Therefore, no clear trend resulted from this analysis. Means of low- and high temperature groups of the two- and three temperature group's analyses were comparable. The partition of the data from the intermediate group did not influence the results, but increased the power of the study because each group consisted of more tests.

SSDs were plotted with the assumption that other factors that could possibly influence toxicity (like pH, salinity or exposure type) were not correlated to test temperature. Therefore, factors are assumed to be equally distributed among the high and low temperature groups and would not influence temperature dependent SSDs. However, it resulted (by Welch two-sample t-tests) that this was not true for some test conditions, a sign that the data was not homogeneously distributed (Appendix B and C, fig. 2). This would not be a problem; however, some of these factors and test conditions were also related to toxicity- exposure methodology, parent compound, and measurement methodology. Also, temperature could have influenced exposure levels and oxygen concentration. These differences were hampering the comparison between the high- and low temperature groups. Also a different taxonomic composition was of influence on the results.

The frequency of exposure methodology was unequally distributed among the temperature groups, especially for Cd test. For example, 9.6% of the tests conducted >20°C existed of flow-through exposure tests, while in the low temperature this was 23.1% (table 2). For Cd, the use of flow-through exposure methodology ($7.59 \cdot 10^{-7}$ M) resulted in significant higher toxicity than of static- and renewal tests ($9.12 \cdot 10^{-6}$ M, $p < 0.0001$) (Appendix B, table 1B and fig.1B). This is because in flow-through tests, the contaminant concentration in the exposure medium is held constant by continually renewal of new test water. When the medium is not replenished, the concentration of the toxicant will gradually decrease due to uptake by the organism, sorption to the test system or degradation. In static- or (daily) renewal tests, exposure will thus be lower while using the same toxicant test concentration.

Without any explanation, it was also found that test temperature differed significantly between the exposure method groups ($p < 0.0001$). Mean test temperature of flow-through tests was 15.6°C compared to 19.7°C of the other exposure methods (Appendix B, table 1B and fig. 2B).

It also appeared that when concentration was reported as the measured concentration instead of the nominal concentration (calculated for dilution series) the toxicity was found higher ($2.04 \cdot 10^{-7}$ M resp. $1.02 \cdot 10^{-5}$ M, $p < 0.0001$) (Appendix B, table 1C and fig.1C). This could be a result of impurity, uptake or sorption to the test system of the substance. Mean test temperatures were 17.1°C resp. 20.0°C ($p < 0.0001$) (Appendix B, table 1C and fig. 2B).

Furthermore, for one of the Cd parent compounds, Cd nitrate, a lower toxicity was found ($1.70 \cdot 10^{-5}$ M) than for other Cd compounds ($5.25 \cdot 10^{-6}$ M) (Appendix B, table 1A and fig. 1A). This difference was statistically significant ($p = 0.02$). A possible explanation is that NO_3^- is a potential less toxic counter ionor that Cd nitrate tested species may be more sensitive. Just a small amount of tests with Cd nitrate were included in the analysis (39 out of 901 tests), increasing the

probability that the difference is coincidental. Mean test temperature of these tests was 22.3°C resp. 18.8°C, a significant difference ($p=0.0002$) (Appendix B, table 1A and fig. 2A). For PCP, no significant differences in toxicity and for the temperature groups were observed.

Despite the differences, the observed relations between test conditions and toxicity could not explain the difference between the SSDs of the temperature groups. While toxicity for flow-through test, measured test and non-Cd nitrate compounds was higher, the mean test temperature was significantly lower. This implies that the temperature effect on the SSDs reflected here might be an underestimation of the real effect of temperature induced Cd toxicity on species sensitivity. For further research it is recommended to further standardize test protocols.

Water solubility of toxicants generally increases at higher temperatures, while sorption to organic matter and the test system decreases. For that reason, temperature could have interfered with exposure levels, especially in studies reporting concentrations as nominal and thereby using static test systems.

Also the solubility of oxygen is altered and decreases with increasing temperature. Indeed, it was found that among our tests a lower dissolved oxygen concentration was reported for tests conducted in the higher temperature groups. Dissolved oxygen was respectively ~ 1 mg/L and ~ 0.5 mg/L lower in the high temperature exposure group of Cd and PCP. This regular decrease in dissolved oxygen concentration can be tolerated by most aerobic aquatic organisms (Cairns Jr., Heath & Parker 1975). Dissolved oxygen decreases at a constant temperature showed to have little effect on toxicity unless these decreases were severe (Lloyd, Herbert 1962). However, metabolic rates are higher at high temperature, resulting in a higher oxygen demand. Low oxygen availability could stress aquatic organisms and this might mediate the toxic effects (Cairns Jr., Heath & Parker 1975). Since many toxicants including Cd and PCP, interfere with metabolic oxygen demand, the combined effects are expected to increase toxicant sensitivity at elevated temperatures (Heugens et al. 2001).

Another important factor of influence is the difference between the groups resulting from species composition. It is difficult to distinguish between effects on the SSD from the test temperature and the species taxonomic origin or common living environment. The data consist of a broad spectrum of test species, where data on toxicity and experimental temperature was available.

With regard to the normal living conditions, no differences between different climatic zones were made, nor differences between salt- and freshwater species. However, sensitivities between these groups might not be comparable. For instance, freshwater species tended to be more sensitive towards ammonia and metal compounds (Wheeler et al. 2002). In contrast, for pesticide and narcotic compounds such as PCP, saltwater species tended to be more sensitive. Also for some compounds, differences in sensitivity between climatic zones were found (see later on in this section).

Concerning the taxonomic composition, some differences may also have influenced the results. For instance, 12.2% of the species in the Cd-low temperature SSD belonged to the phylum Annelida, whereas in the Cd-high temperature SSD this was only 1.5%. In general, it was found that these species were less sensitive toward Cd (Appendix B, fig. 1H). Also, in the Cd low temperature group, over 50% of the species were of the phylum Arthropoda compared to 35% in high low temperature group (table 2). Arthropods were on average a sensitive group (Appendix B fig. 1H). Also, 8 times more species of the phylum Annelida were present in the low temperature group (table 2), while these were in general less sensitive towards Cd (Appendix B, fig. 1H). Thus, the low temperature group existed of a greater quantity of more sensitive arthropods and less sensitive annelids. The high temperature group had more chordates and

mollusks. Fish (an important chordate group) appeared less sensitive at high temperature while mollusks tend to be more sensitive (fig. 3).

Similar for PCP, species composition may also have affected the results in table 3. The species belonging to the phylum Chordata were in general more sensitive than arthropods, annelids and mollusks (Appendix C, fig. 1H). In the low temperature group 43% of the species were chordates, compared to 63% in the high temperature group (table 2). Furthermore, more annelids and arthropods were found in the low temperature SSD, although some arthropods tend to be more sensitive at higher temperature (crustaceans, fig 3).

The fact that the taxonomic distribution and sensitivity is different between the groups makes a correct comparison difficult. It may have a role in differences in sensitivity of the temperature groups and might camouflage temperature effects.

Differences due to taxonomic composition, however, are not present when using the same species for each SSD. This comparison minimized the taxonomic noise although species choice becomes more important with this approach. Since less species will be included in the SSD, much depends on the sensitivity of the selected species and how well they represent their taxonomic group. The species compositions of datasets used are important to explain the found effects of species distributions (Wheeler et al. 2002).

Furthermore, not much is known about the sensitivity of endangered species, especially those endemic in the tropics, as they are generally not used for toxicity testing (Daam et al. 2009). Some studies were included in our analysis for which it was emphasized that tropical endangered species were tested for PCP (Dwyer et al. 1999, Sappington et al. 2001, Dwyer et al. 2005). For Cd, this was only one study (Buhl 1997) although other studies also used endangered species such as bull trout (Stratus Consulting Incorporated 1999). It is unknown in which extent the SSDs are influenced by these studies, or whether more of such site-specific species should be included.

Temperature-toxicity effects for the chosen compounds

Reviews on results for individual species (Cairns Jr., Heath & Parker 1975, Heugens et al. 2001, McLusky, Bryant & Campbell 1986) suggest that when data of many species would be combined into a SSD and compared, increased overall sensitivity could be anticipated. This assumption was largely based on temperature-effects studies where some toxicants exhibit a positive correlation of toxicity with increased temperature. The commonly known Q10 concept (doubling of metabolism with 10°C increase) has been used to support this (for instance, in Cairns Jr., Heath & Parker 1975). Increased metabolism may help explain increased uptake and distribution of toxicants, culminating in toxic responses, for a single species exposed to an array of temperatures. The effect of any toxicant that interferes with metabolic functions (such as PCP and Cd) may thus be potentiated by increased temperatures. Tissue anoxia might occur earlier and at lower concentrations when the organism is exposed to both high temperatures and toxicants (Cairns Jr., Heath & Parker 1975).

The extent of this effect of the sensitivity of a group of species, however, could not be predicted based on a comparison of results of studies on single species and temperature effect. The change in sensitivity of individual species varied among species and different tests. For some species, a decrease in sensitivity was found, for others, sensitivity increased considerable at increasing temperature. The interactions found for single species might be dependent on physiological tolerance to changing temperatures which might have played a role for toxicant sensitivity.

In the present study it was found that sensitivity differences between high and low temperature tested organisms existed. However, these differences on the multispecies level could not be generalized. The highest (and only statistically significant) result was for sensitivity towards Cd, which was twice as high for

species tested at $>20^{\circ}\text{C}$ in comparison to species tested at $\leq 20^{\circ}\text{C}$. When assuming that metabolism and toxicity are related to each other on a 1-to-1 base, this is in concordance with the Q10 principle. Between other temperature groups differences between the high- and low temperature groups were smaller and non-significant.

It is indicated that the increase of overall sensitivity at higher temperature cannot be predicted by several individual tests that were performed for some species. Furthermore, it is indicated that sensitivities on the individual species level might be smaller than the inter-species variation in a certain temperature range.

A study publication bias might also exist; more papers reporting on (positive) effects have been published in comparison to papers reporting no specific effects. Evidence for publication bias has been shown in many studies (Stern, Simes 1997). If studies with a positive effect on the individual species level are more likely to be published, then a review limited only to responses on the individual species level would also give a more positive effect of than a review based on all test not restricted to published specific research to temperature effects.

In the temperature specific SSDs of all species, noise has been introduced by using two groups that were fundamentally different in their taxonomic composition. However, also when the same sets of species were used to create temperature-specific SSDs for Cd and PCP, no statistical significant difference for overall sensitivity was found, although the HC_5 of Cd differed by a factor 5. Since the same set of species was used, noise due to interspecies variation was excluded. It appeared that not for all species, increased temperature led to a change in sensitivity.

Difference in response differed greatly between taxonomic groups (fig. 3). Especially fish sensitivity decreased significantly at higher temperatures. For copper, it was stated that decreased fish sensitivity might be associated with reduced resistance mechanisms at low temperature. These mechanisms include decreased enzyme production and enzymatic activity, decreased transport through membranes and elimination rates, and decreased mucus production (Sprague 1985, Perschbacher 2005). It is unknown whether these explanations may also apply to other compounds such as PCP and Cd.

The fact that fish are more sensitive at low temperature than mollusks and crustaceans (fig. 3) is also remarkable considering their body size. For fish, the volume-to-surface ratio is bigger due to their size; an internal dose will therefore be expected to be not reached as quickly as for small organisms. On the other hand, smaller organisms may therefore become more sensitive at increasing temperatures. Higher internal exposure at elevated temperatures might occur because increased diffusion or active uptake, but the same processes increase also detoxification and elimination. Furthermore, a higher temperature increases metabolic rates. As a result, the rate of detoxification is also increased, which may explain why no strong differences between the high- and low SSDs.

However, a positive correlation was found for accumulation and elevated temperatures for most of the studies (13 out of 15) that reported on accumulations of metals in aquatic organisms (Sokolova, Lannig 2008). This indicates that detoxification mechanisms and elimination rates are not sufficient to deal with exposures at elevated temperatures.

The fact that both compounds act on cellular respiration cannot explain why greater differences were found for temperature specific SSDs of Cd than for PCP. Interference with respiratory systems can also contribute to the toxic effects of pollutants. Some metals, like zinc, induce gill damage (Cairns Jr., Heath & Parker 1975). This could potentiate toxic effects at higher temperature due to the higher metabolic demand and thus the need for increased respiration. However, Cd is not associated with gill damage although Cd accumulation in the gills of rainbow trout was found 2-4 times higher than in the rest of the body (Kamunde 2009).

For PCP, severe gill damage was reported by a study to the grass shrimp (*Palaemonetes pugio*) (Rao, Doughtie 1984).

It remains unclear which specific mechanism is responsible for the effect of an increasing sensitivity at higher temperature and can explain the difference in effects between PCP and Cd.

Implications for risk management

Environmental quality standards are derived using mostly test data for species from temperate climatic regions tested at temperatures which are below 20°C in general. For tropical ecosystems and in temperate regions during summer, temperatures of water bodies often exceed this temperature. Also a significant rise is possible due to thermal discharges. To date, several studies have reviewed the impact of climatic change (and especially, global warming) to toxicant sensitivity. They indicate increased sensitivity to toxicants due to the expected (effects of) rise in temperature and other climatic conditions (Sokolova, Lannig 2008, Schiedek et al. 2007, Noyes et al. 2009, Kim et al. 2009, Brian et al. 2008). This stresses the need to integrate temperature potentiated toxicity into existing models used for derivation of safety levels.

Taking the two compounds as an example, the current study aimed to assess whether possible differences between temperature groups would lead to the indication to apply an extrapolation factor in order to protect species living at unnaturally higher temperatures.

The current study showed that the Cd HC₅ value is increased twofold when the temperature is raised with 10°C (mean temperature of the low and high group are respectively 14.5°C and 24.5°C). Based on all species, it is indicated that species living in an environment at temperatures higher than 20°C are twice as sensitive towards Cd as species in lower temperature water bodies. Thus, it is indicated that if a safety factor of 2 would be applied, environmental quality standards for temperature regions would also be protective to warmer aquatic environments. Since species from all climatic regions were included, it may apply to both (sub)tropical regions and also to temperate regions at seasonal temperature peaks. Furthermore, for risk assessment of environments in which thermal discharges occur (De Vries et al. 2008), this factor can be taken into account.

Results for PCP indicated no significant effect on temperature rise thus current standards are expected to be sufficient to protect species at higher temperatures. However, the sensitivity was up to a factor 1.80 higher in the high temperature group. However, to assure protection for species at elevated water temperature, it is suggested that also a safety factor of 2 may be applied to environmental quality standards for PCP.

In the Netherlands, the maximum permissible concentration (MPC) in the aquatic environment for PCP is set on 4 µg/L ($1.50 \cdot 10^{-8}$ M). For Cd, MPC for total Cd is 2 µg/L ($1.78 \cdot 10^{-8}$ M) (RIVM 2009).

If we compare the HC₅ derived in this study to environmental quality standards, we see that the Dutch Cd MPC compared to the Cd HC₅ derived in this study, is a factor 10.2 lower for species <20°C and a factor 4.8 lower for species >20°C. For PCP, the difference for low and high temperature species is respectively a factor 6.1 and 9.4 lower. The SSDs used in this study do not fully fulfill the requirements for derivation of environmental quality standards in the EU (European Chemicals Bureau 2003). Not all prescribed phyla are represented (table 6). Also, instead of NOEC data, LC50 data was used as endpoint. Thus, a certain extrapolation factor between NOEC and LC50 should be taken into account. Within a species, it was found that for pesticides an acute LC₅₀ value can be divided by 10 to extrapolate to chronic NOEC data (Jager, Rikken & Van der Poel 1997, Margni et al. 2002). If applying this extrapolation factor to the data, then values are found in the same order of magnitude. However, the MPC is

Table 6. Comparison between SSD requirements for the use of derivation of legislative environmental quality standards in the EU, and the data used for the SSDs prepared in the current study.

Requirement category	SSD, EU environmental quality standards	SSD, this study
Time span	long-term	96h
Endpoint	NOEC	LC50
Data transformation	logarithmic	logarithmic
Species	<ul style="list-style-type: none"> • fish • a second family in the phylum Chordata • a crustacean; • an insect; • a family in a phylum other than Arthropoda or Chordata • a family in any order of insect or any phylum not already represented; • algae; • higher plants. 	<ul style="list-style-type: none"> Yes Yes Yes Yes Yes Yes Only in Cd $\leq 20^{\circ}\text{C}$ Only in Cd > 20
Sample size	>10 (pref. >15) over 8 taxonomic groups	Yes

exceeded for Cd at high temperature and PCP at low temperature, indicating that current MPCs may not be protective enough to protect 95% of the species at all occurring temperatures.

Extrapolations can be made towards other pollutants of similar characteristics but should be done with care. Toxicity and associated hazards of toxicants can be compound-specific. The individual compounds need to be judged separately whether it is necessary to apply an additional safety factor for altered toxicity at increasing temperature. However, with a precautionary approach it is advisable to introduce the proposed safety factor of 2 when environmental quality standards based on average low temperature data will be applied to waters where temperature rises above 20°C .

Comparison with similar studies

To the extent of our knowledge, SSDs have never been used to assess differences in sensitivity between organisms sorted by their experimental test temperature. Some studies exist that compare the sensitivity between species of different geographic zones, mostly distinguishing between arctic-, temperate- and tropical test species. This is clearly distinct from our study; we did not assign the aquatic test species to a specific geographical background. However, it is expected that the majority of the species in our low temperature group are arctic- and temperate species, whereas in the high temperature group most species are of (sub)tropical origin. Since temperature is probably the most important environmental condition for the classification of the climatic zones, these studies will be used to compare the findings of the current study.

In a study assessing heavy metal sensitivity of marine organisms of the three main climatic regions (tropical-, temperate- and polar zone) no clear pattern for toxicity along the regions was found by use of SSDs (Chapman et al. 2006). For Cd, tropical species were the most sensitive and polar species the least. However, this relation was different for copper, zinc and lead. Polar species were also least sensitive for zinc and lead. For copper, polar species were the most sensitive although this data also showed the highest variation. Tropical species were the least sensitive for copper.

A similar comparison by SSDs was done between tropical and temperate freshwater animal species for 18 chemical substances (Kwok et al. 2007). Various SSD fitting approaches were used to derive HC_{10} values for sensitivity comparison. Results indicated that for some of these chemicals, the relative sensitivities were noticeably different. For most metals, including Cd, temperate

species tended to be more sensitive than their tropical counterparts according to all approaches. However, tropical species were more sensitive for arsenic and zinc. By most SSD approaches, it was found that tropical species were more sensitive for ammonia, phenol, chlordane and chlorpyrifos. Tested tropical and temperate species were in general of equal sensitivity towards PCP.

Using only arthropod species, sensitivities of temperate and tropical species to three pesticides (chlorpyrifos, fenitrothion and carbofuran) were compared (Maltby et al. 2005). For all pesticides, HC₅ values derived by SSDs were smaller for tropical species than for temperate species.

Some comparisons between species of different climatic zones have also been made without using SSDs.

A comparison of coldwater-, temperate- and tropical fish sensitivity towards 6 pesticides was made by a two-way analysis of variance of species mean log LC50 data (Dyer, Belanger & Carr 1997). Only for DDT, tropical fish were significant more sensitive than temperate and coldwater species. For carbaryl, PCP, lindane, malathion and phenol there was no significant difference in sensitivity between tropical and temperate fish species. However, coldwater fish were the least sensitive for all compounds. This was in contradiction with the results in the current study for the sensitivity of fish (fig. 3).

For the fungicide carbendazim and the herbicide linuron, the results of outdoor microcosms studies have been compared between the Netherlands and Thailand (Daam et al. 2009, Daam, Van den Brink & Nogueira 2009). The studies were performed by the same researchers using a comparable system and experimental set-up, although different climate-specific species were used. For both compounds, results of sensitivity were comparable between the two climatic regions, although the most sensitive representative species were of different taxonomic groups.

In general, these studies agree that no predictable, universal pattern for species sensitivity on toxicity can be derived based on climatic zones only. Therefore, results of these studies indicate that temperature might not be the only factor that could explain differences in toxicant sensitivity between the geographical regions.

Kwok (Kwok et al. 2007) proposed that a safety factor of 10 should be applied to protect tropical species if only temperate species data would be available. However, this approach is rather protective, taking into account all effects that have been found using the sensitivity data for all 18 compounds. For most compounds used in their study, a lower or comparable sensitivity was found for tropical species compared to temperate species. They stated that without extrapolation, 90% of the species for 60% of the chemicals would be protected.

Other studies support the initial use of commonly tested aquatic species endemic to coldwater and temperate climates for assessing effects in tropical environments (Dyer, Belanger & Carr 1997, Daam et al. 2009). They indicated that quality standards derived from temperate species data would be sufficiently protective to tropical species.

Heugens applied data of several species derived from reviewed papers on temperature dependent sensitivity to the Arrhenius equation. A rise from 20°C to 30°C caused an increased sensitivity of the reviewed species by a factor 1.7 and 5.6 for respectively 24h-LC50 data and 48h-LC50 data (Heugens et al. 2001).

In general, results of other studies are in concordance with the results in the current study. Differences in sensitivities are usually small, but in order to be protective a (small) safety factor is suggested to assure protection for 95% of the species in waters of all temperatures.

Conclusion

The comparison between temperature-specific SSDs demonstrated the species

sensitivities towards two compounds at different experimental temperatures. Both a general set of all species and an equal set of selected species have been used. The first approach included many species, but taxonomic variation and experimental differences may have interfered with the results. By the second approach, the species that were used for the temperature specific SSDs were equal, thus excluding interference of taxonomic differences between temperature groups.

For both approaches and compounds, relative sensitivity by comparison of HC₅ and HC₅₀ low/high ratios ranged between a factor 0.65 and 2.11. For Cd, the sensitivity increase of the latter factor 2.11 was significant.

Based on the results, it was indicated that a safety factor of minimal 2 should be applied to Cd environmental quality standards when striving to protect 95% of the species at temperatures above 20°C. For PCP, when upholding a precautionary principle it would also be advisable to introduce an extra safety factor of approximately 2 for waters with elevated temperature.

This study indicates that current environmental quality standards may not provide the same level of protection for chemical pollution in waters with elevated temperature when compared with waters with low temperature. By taking relevant environmental conditions such as temperature into account, environmental risk assessment becomes more realistic.

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Appendix A

CAS numbers of compounds, along with their synonyms used in literature

Cd compounds

CAS Number:	7440439
Preferred Name:	Cadmium
Synonyms:	<ul style="list-style-type: none"> • Cadmium
CAS Number:	10108642
Preferred Name:	Cadmium chloride
Synonyms:	<ul style="list-style-type: none"> • Cadmium chloride
CAS Number:	10124364
Preferred Name:	Sulfuric acid, Cadmium salt (1:1)
Synonyms:	<ul style="list-style-type: none"> • Sulfuric acid, Cadmium salt (1:1) • Cadmium sulfate (CdSO₄) • Cadmium sulphate
CAS Number:	10325947
Preferred Name:	Cadmium nitrate
Synonyms:	<ul style="list-style-type: none"> • Cadmium nitrate

PCP compounds

CAS Number:	87865
Preferred Name:	Pentachlorophenol
Synonyms:	<ul style="list-style-type: none"> • Pentachlorophenol • 2,3,4,5,6-Pentachlorophenol • Dowicide EC-7 • PCP • Weedone * • Pentachlorophenate • Santobrite • 2,3,4,5,6-Pentachlorophenate
CAS Number:	131522
Preferred Name:	Pentachlorophenol, Sodium salt
Synonyms:	<ul style="list-style-type: none"> • Pentachlorophenol, Sodium salt • Dowicide G • Dowicide G-ST • Na-pentachlorophenate • PCP-Na • Pentachlorophenate-Na • Sodium pentachlorophenate • Sodium pentachlorophenol • Sodium pentachlorophenolate • Sodium pentachlorophenoxide • Santobrite
CAS Number:	7778736
Preferred Name:	Potassium pentachlorophenate
Synonyms:	<ul style="list-style-type: none"> • Potassium pentachlorophenate

Appendix B Distribution of Cd data

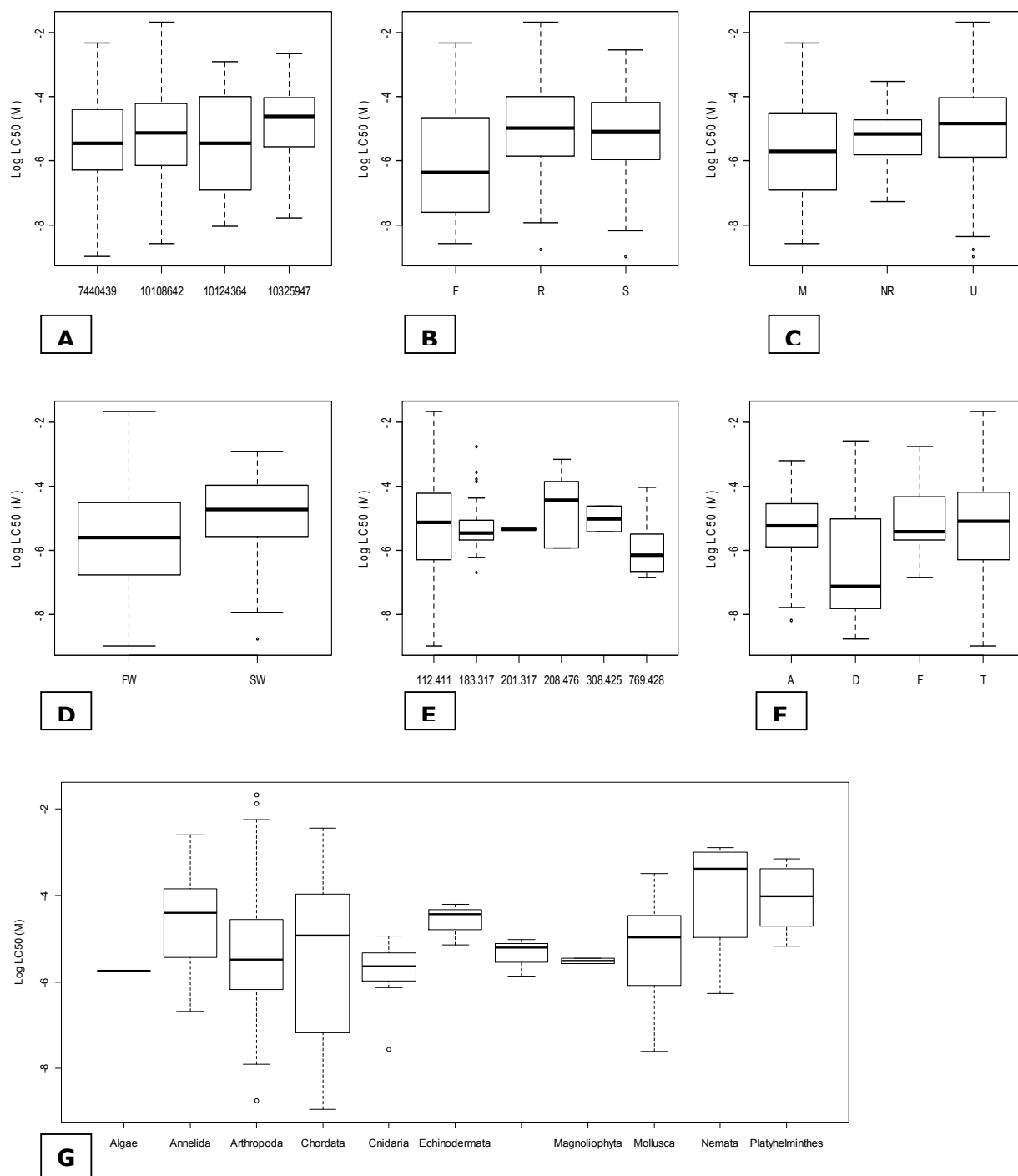


Fig. 1 (panel A-F). Boxplots computed to check the log transformed Cd LC50 data (M) distribution by different components in the database. **A)** Original added test compound. CAS nr.7440439=Cd., 10108642=CdCl₂, 10124364=CdSO₄, 10325947=CdNO₃. **B)** Exposure methodology. F=flow-through, R=daily-renewal, S=static. **C)** Test concentrations determined by measurements (M), nominal by calculation of dilutions (U), or not reported (NR). **D)** Fresh (FW) and saltwater (SW) tests. SW test include tests performed in brackish water. **E)** LC50 values computed as concentration Cd (112.411), CdCl₂ (183.317), CdCl₂·H₂O (201.317), CdSO₄ (208.476), Cd(NO₃)₂·4H₂O (308.425), 3CdSO₄·8H₂O (769.428). **F)** Distribution of data by descriptive for the LC50. A=active component of the compound, D=dissolved fraction, F=formulation, T=total concentration, non-dissolved fraction included. **G)** Species Phylum (missing phylum name=Ectinoprocta)

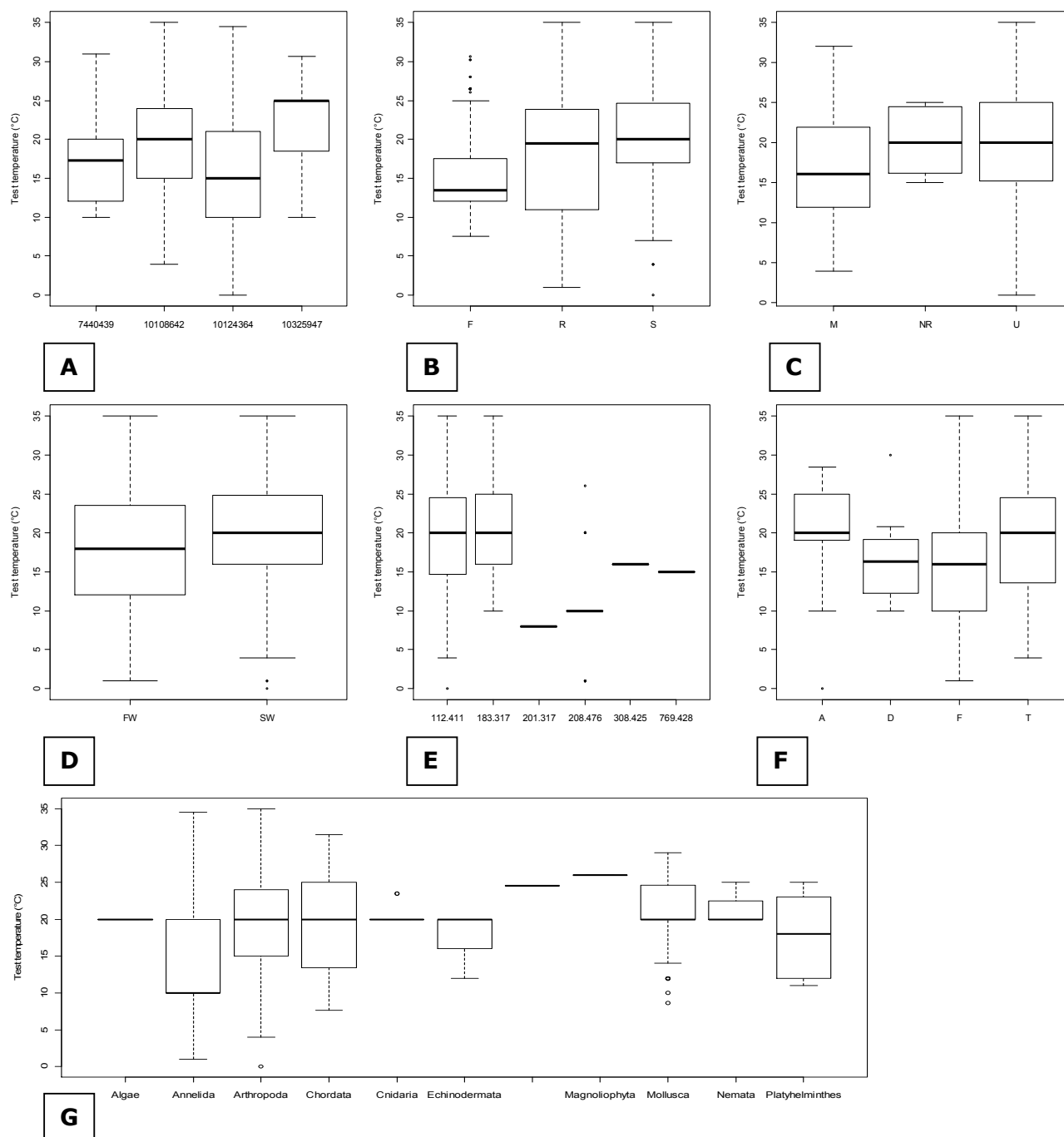


Fig. 2 (panel A-F). Boxplots computed to check the temperature data distribution by different components in the database for Cd. **A)** Original added test compound. CAS nr.7440439=Cd., 10108642=CdCl₂, 10124364=CdSO₄, 10325947=CdNO₃. **B)** Exposure methodology. F=flow-through, R=daily-renewal, S=static. **C)** Test concentrations determined by measurements (M), by calculation of dilutions (U), or not reported (NR). **D)** Fresh (FW) and saltwater (SW) tests. **E)** LC50 values computed as concentration Cd (112.411), CdCl₂ (183.317), CdCl₂·H₂O (201.317), CdSO₄ (208.476), Cd(NO₃)₂·4H₂O (308.425), 3CdSO₄·8H₂O (769.428). **F)** Distribution of data by descriptive for the LC50. A=active component of the compound, D=dissolved fraction, F=formulation, T=total concentration, non-dissolved fraction included. **G)** Species Phylum (missing phylum name=Ectinoprocta)

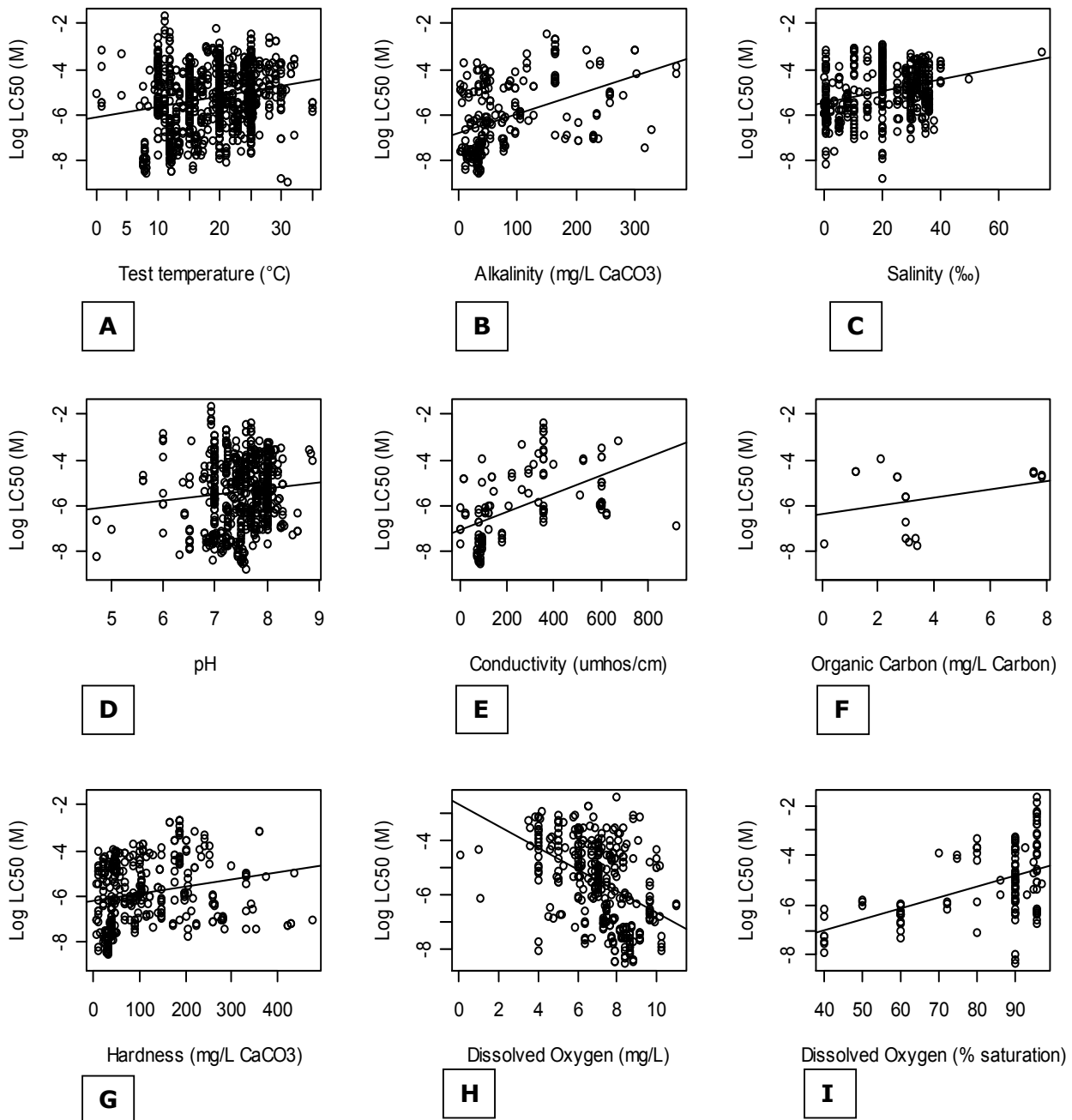


Fig. 3 Plot of Cd LC50 values (M) arranged by different test conditions. **A)** Plot of LC50 values arranged by tested temperature (°C). **B)** LC50 arranged by tested alkalinity (mg/L CaCO₃). **C)** LC50 arranged by tested salinity (‰) (outlier=extreme insensitive crab) **D)** LC50 arranged by tested pH. **E)** Plot of LC50 values arranged by tested Conductivity(umhos/cm). **F)** LC50 arranged by tested Organic Carbon content (mg/L Carbon). **G)** Plot of LC50 values arranged by tested hardness (mg/L CaCO₃). **H)** Dissolved oxygen reported as mg/L O₂. **I)** Dissolved oxygen reported as % O₂ saturation (dependent on temperature)

Table 1 (A-C). Means of unequally distributed Cd data along with results of performed Welch two-sample t-tests. **A)** Differences between Cd nitrate and other compounds. **B)** Differences between flow-through tests and static/renewal tests. **C)** Differences between test with measured- and nominal LC50 concentrations.

A	Cd nitrate	Cd compounds other than Cd nitrate	p-value
Mean LC50 values (log transformed, M)	-4.767695	-5.283472	0.02268
Mean of temperature (°C)	22.28462	18.79380	0.0002589

B	Exposure method: flow through	Exposure method: static or daily-renewal	p-value
Mean LC50 values (log transformed, M)	-6.120004	-5.074824	7.431e-13
Mean of temperature (°C)	15.56584	19.66825	3.593e-14

C	LC50 concentration as measured	LC50 concentration as nominal	p-value
Mean LC50 values (log transformed, M)	-5.688601	-4.987857	6.338e-12
Mean of temperature (°C)	17.07238	19.97220	4.607e-11

Appendix C

Distribution of PCP data

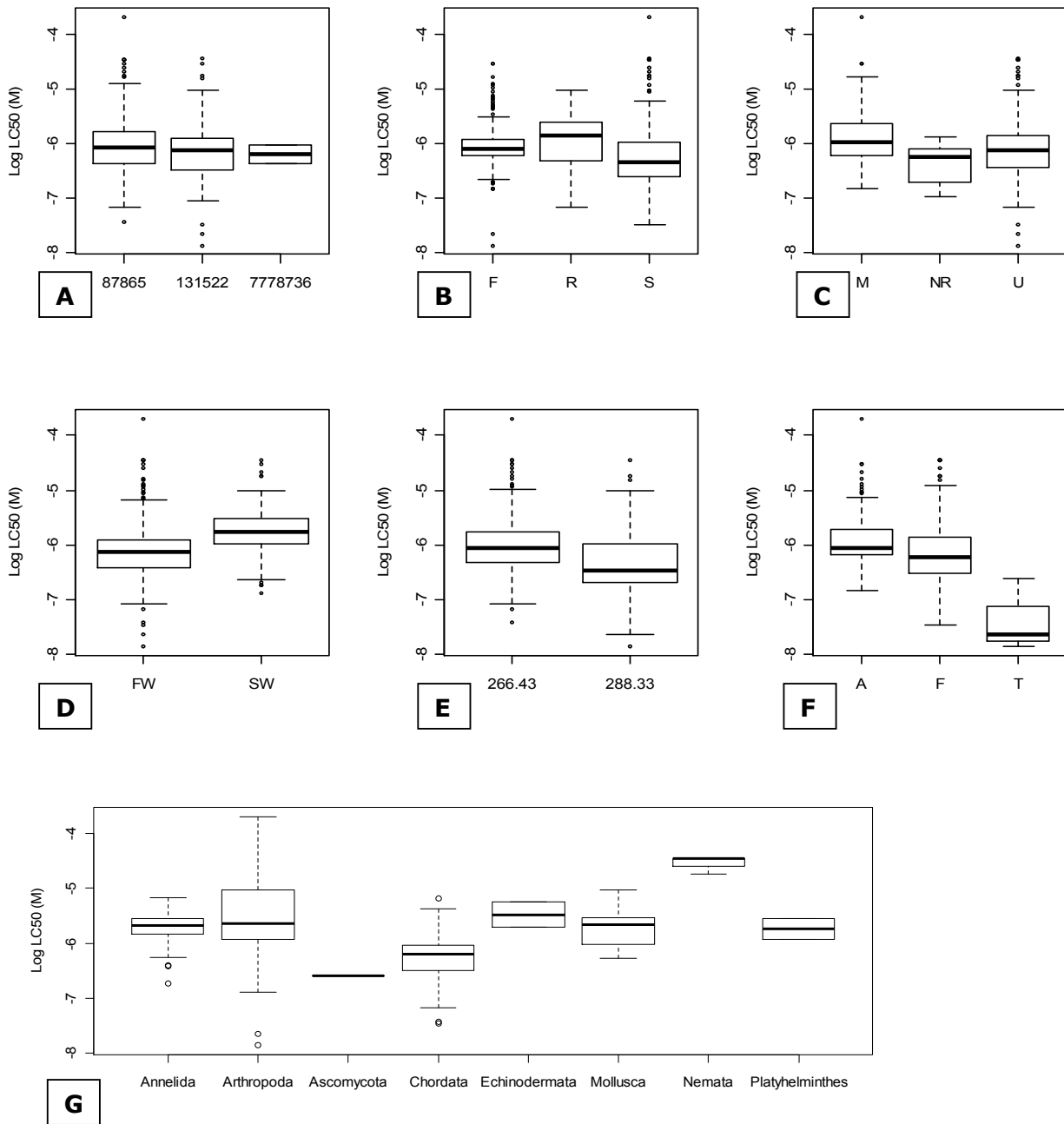


Fig. 1 (panel A-F). Boxplots computed to check the log transformed PCP LC50 data (M) distribution by different components in the database. **A)** Original added test compound. CAS nr. 87865=PCP, 131522=PCP-Na, 7778736=PCP-K. **B)** Exposure methodology. F=flow-through, R=daily-renewal, S=static. **C)** Test concentrations determined by measurements (M), by calculation of dilutions (U), or not reported (NR). **D)** Fresh (FW) and saltwater (SW) species. **E)** LC50 values computed as concentration PCP (266.43) or concentration PCP-Na (288.33). **F)** Descriptive for the LC50. A=active component of the compound, F=formulation, T=total concentration, non-dissolved fraction included. **G)** Species Phylum.

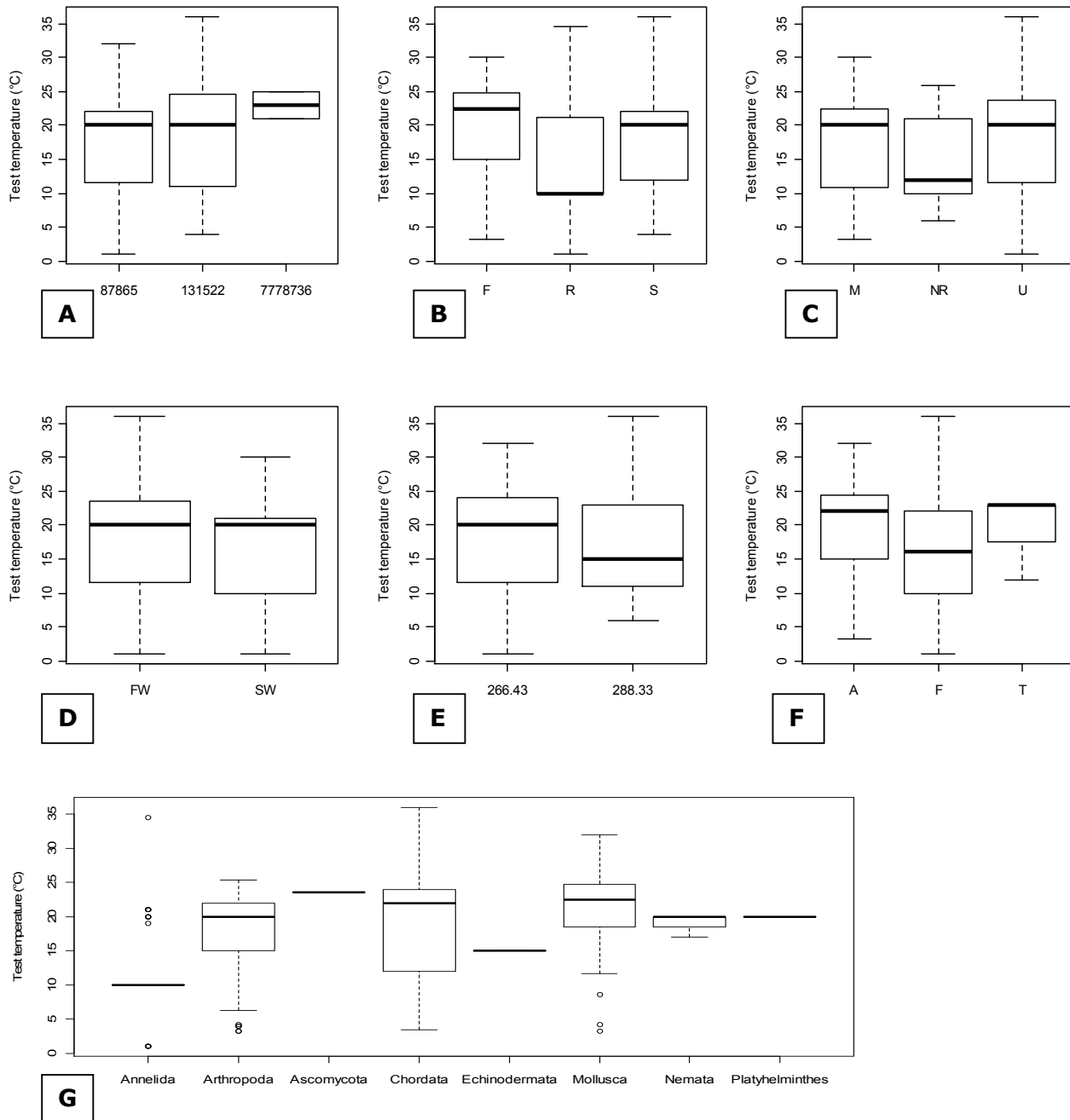


Fig. 2 (panel A-F). Boxplots computed to check the temperature distribution by different components in the database for PCP. **A)** Original added test compound. CAS nr. 87865=PCP, 131522=PCP-Na, 7778736=PCP-K. **B)** Exposure methodology. F=flow-through, R=daily-renewal, S=static. **C)** Test concentrations determined by measurements (M), by calculation of dilutions (U), or not reported (NR). **D)** Fresh (FW) and saltwater (SW) species. **E)** LC50 values computed as concentration PCP (266.43) or concentration PCP-Na (288.33). **F)** Descriptive for the LC50. A=active component of the compound, F=formulation, T=total concentration, non-dissolved fraction included. **G)** Species Phylum.

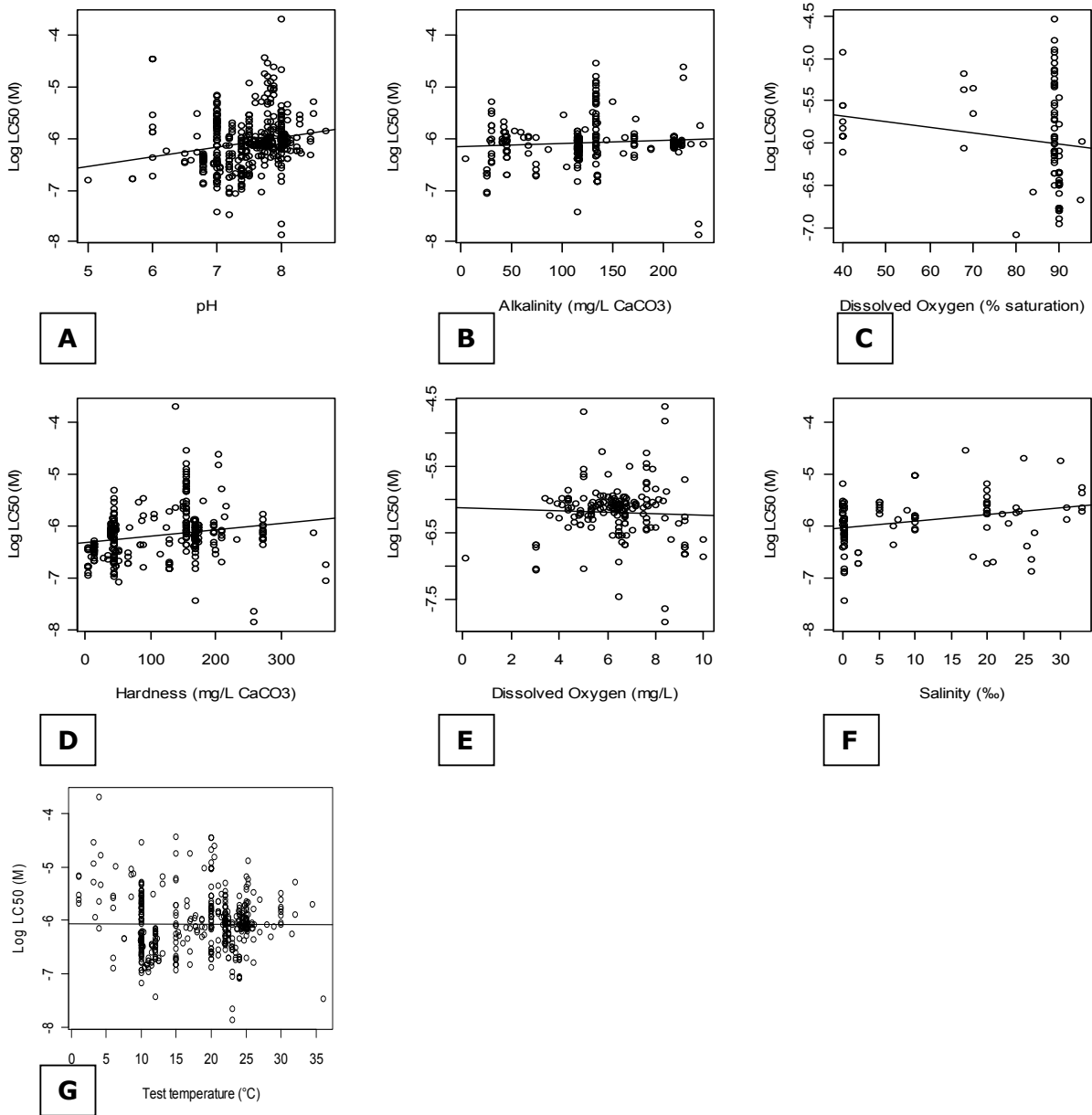


Fig. 3 Plot of PCP LC50 values (M) arranged by different test conditions. **A)** Plot of LC50 values arranged by tested pH. **B)** LC50 arranged by tested alkalinity (mg/L CaCO₃). **C)** LC50 arranged by tested salinity (‰) **D)** Plot of LC50 values arranged by tested hardness (mg/L CaCO₃). **E)** Dissolved oxygen reported as mg/L O₂. **F)** Dissolved oxygen reported as % O₂ saturation (dependent on temperature) **G)** By test temperature (°C).

Table 1 (A-B). Means of unequally distributed PCP data along with results of performed t-tests. **A)** Differences between LC50 values as total concentration and as active component/formulation. **B)** Differences between LC50 values computed as PCP-Na and PCP.

A	LC50 as total concentration	LC50 as active component/formulation	p-value (t-test)
Mean LC50 values (log transformed, M)	-7.370282	-6.061410	0.07688
Mean of temperature (°C)	19.33333	17.86349	0.7277

B	PCP-Na	PCP only	p-value (t-test)
Mean LC50 values (log transformed, M)	-6.316945	-6.024154	9.338e-05
Mean of temperature (°C)	16.91926	18.05713	0.1712