



Drivers of copper sensitivity in copepods: A meta-analysis of LC50s

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ABSTRACT

Copper is both an essential trace element and a potent pesticide. The use of copper as an antifoulant has increased in the last decades in line with the expanding aquaculture and shipping industries. In aquatic environments, it also affects non-target taxa. One of which are copepods, which constitute the central link in the marine food web. Despite their ecological importance, there are no systematic reviews of the lethal concentration range and drivers of copper toxicity in this taxon. Here, we combined literature data from 31 peer-reviewed articles recording the Lethal Concentration 50 (LC50) for copper in copepods and the experiments' respective environmental, developmental, and taxonomic parameters. The LC50 is a traditional endpoint for toxicity testing used in standardized toxicity testing and many ecological studies. In total, we were able to extract 166 LC50 entries. The variability in the metadata allowed for a general analysis of the drivers of copper sensitivity in copepods. Using a generalized additive modeling approach, we find that temperature increases copper toxicity when above approximately 25 °C. Counter to our expectations; salinity does not influence copper sensitivity across copepod species. Unsurprisingly, nauplii are more susceptible to copper exposure than adult copepods, and benthos-associated harpacticoids are less sensitive to copper than pelagic calanoids. Our final model can predict sensible specific-specific copper concentrations for future experiments, thus giving an informed analytical approach to range testing in future dose-response experiments. Our model can also potentially improve ecological risk assessment by accounting for environmental differences. The approach can be applied to other toxicants and taxa, which may reveal underlying patterns otherwise obscured by taxonomic and experimental variability.

1. Introduction

Copper, while being an essential element, is a potent toxicant at higher concentrations (Eisler, 1998). It is an active component in many marine antifouling paints that prevents the biofouling of net pens and ship hulls. Both traditional and organic agriculture also apply copper as a pesticide (Eisler, 1998). Other sources of copper to aquatic environments include, besides others, runoff from stormwater events (Pennington and Webster-Brown, 2008), industry (Samecka-Cymerman and Kempers, 2004), and mining activities (Mayes et al., 2010).

Copper use has increased in the last decades in line with the expanding aquaculture and shipping industries. In addition to anti-fouling effects on target organisms, copper negatively affects non-target species in aquatic ecosystems, ranging from phytoplankton to crustaceans (de Oliveira-Filho et al., 2004; Greenfield, 1942; Harrison et al., 1977; Karlsson et al., 2010; Sanders et al., 1981).

Measured dissolved copper concentrations in aquatic habitats vary in time and space but often range from 0.069 to 16.0 $\mu\text{g L}^{-1}$, with the highest values found in marinas and harbors (Hall and Anderson, 1999). High copper concentrations of up to 457 mg Cu kg^{-1} often accumulate in the sediments of harbor and marina areas (e.g., Choi et al., 2006; Schiff et al., 2016; Wang et al., 2002) and in the close vicinity of aquaculture facilities (Dean et al., 2007).

Jones and Bolam (2007) found that on average 27% of the total dissolved fraction in seawater is made of labile copper and thus likely readily bioavailable. Copper toxicity is mainly exerted by free copper ions (Cu^{2+}), which affect organisms by forming reactive oxygen species, which causes damage to proteins, lipids, and DNA (Stohs, 1995). It can ultimately lead to apoptotic cell death (Rhee et al., 2013). Cupric ions also compete with other cations at ion channels and can interfere with osmoregulation and enzyme activity (Wood et al., 2012). These modes of action can ultimately increase mortality, but already sublethal copper

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concentrations can affect several endpoints in crustaceans, including interference with mating behavior (Krång and Ekerholm, 2006) and osmoregulation capacity (Bambang et al., 1995). In planktonic copepods, even low copper concentrations can delay maturation (Lode et al., 2018) and reduce development rates (Kwok et al., 2008), potentially affecting population dynamics and size.

Physico-chemical factors like salinity or temperature may affect the toxicity of contaminants (Heugens et al., 2001), for example by altering Cu speciation or the physiology of organisms (Flemming and Trevors, 1989). The effect of toxicants also depends on biotic factors such as the developmental stage of the exposed individuals (e.g., Bambang et al., 1995; Jager et al., 2016; Haque et al., 2018), the presence of predators (e.g., Lode et al., 2018; Relyea and Hoverman, 2008; Qin et al., 2011) and food availability (e.g., Chandini, 1988).

The ongoing climate crisis increases marine temperature, lowers the pH, and influences the salinity, especially in coastal areas (Pörtner et al., 2019). Any related alteration of the chemical and physical environment will inevitably affect the toxicity of pollutants (Schiedek et al., 2007), and often in different directions. The interactions between multiple environmental stressors can range from antagonistic to additive to synergistic (Côté et al., 2016; Gunderson et al., 2016; Przeslawski et al., 2015; Tekin et al., 2020).

Despite the dependency of toxicity on the environment, standard toxicological tests of new chemicals are conducted under very controlled conditions, and each contaminant is tested one at a time on one or a few model organisms. While such an approach increases comparability, it limits predictions of the effects of chemicals in nature where contaminants act in a multi-stress environment (Holmstrup et al., 2010). The Lethal Concentration 50 (LC50) is a traditional metric to determine the toxicity of contaminants for organisms. Its applicability and shortcomings have been discussed before (Kooijman, 1996; Jager et al., 2006). However, the ease of performing LC50 experiments and the already large amount of available LC50 data made it a standard risk assessment tool, although today process-based analysis of lethality experiments should be done instead (Jager et al., 2011a; Kulkarni et al., 2013). In addition, many more studies report the LC50 and explore the interactive effects of toxicants with other environmental parameters but do not necessarily adhere to standard protocols such as the OECD guidelines.

Here we use a meta-analysis to make use of the large number of studies and their variability in species and experimental conditions to explore toxicity patterns within and across species, and their interaction with other environmental parameters differences in experimental conditions in LC50 experiments in copepods. We focus on copepods are the most numerous crustaceans on earth and have a crucial role in the marine food web by linking lower and higher trophic levels (Turner, 2004). The three dominant orders of free-living copepods are calanoids, cyclopoids, and harpacticoids. The first two orders include mostly free-swimming pelagic animals, while the latter consists primarily of benthic and demersal species. They thus represent two distinct aquatic habitats, with potentially different exposure levels to contaminants as benthic sediments are often a sink of pollutants. Copepods are increasingly used in ecotoxicological studies (Raisuddin et al., 2007; Kulkarni et al., 2013). However, despite their ecological importance and the reality of copper exposure in nature, there are no systematic reviews of the lethal concentration range and drivers of copper toxicity in this group. Therefore, we focused our analysis on copper and its influence on acute toxicity on copepods. We excluded sublethal endpoints such as development or reproduction, as less data is available and studies are harder to compare.

2. Methods

2.1. Literature search

We searched the Web of Science (Core Collection) for articles that contain copepod values for Copper LC50 using the terms “Copepod”

AND “LC50” AND “copper” (search performed on the 2020/06/08), and also included copepod LC50 values from the Envirotox database (Connors et al., 2019). If available, we included the accompanying experimental conditions (temperature, pH, salinity, group size (i.e., the number of animals in each experimental container), volume, copper source, number of experimental replicates, secondary treatments, stage, body size, test duration). If any of the articles reported LC50 values from studies that did not appear in our original search, we also tried to acquire these sources. The Web of Science search returned 22 articles with 169 unique LC50 entries. In addition, these articles cited nine more that yielded 40 more entries.

In total, 15 studies were obtained from the Envirotox database for copepods (v. 1.3.0, Connors et al., 2019), however, we could not access three of them (Gentile and Cardin, 1982; Lussier and Cardin, 1985; Menasria and Pavillon, 1994), despite help from the University of Oslo Library. The Envirotox database reports only the lowest value from a study, but the articles reported 16 more LC50 values that we included in our database. To make use of the variance in the data, we extracted any other LC50 values from the listed articles if we could access them and reported their respective details about the acute toxicity test procedures and environmental conditions. This resulted in 43 LC50 values for the Envirotox database. We found a discrepancy between values in the article and the Envirotox database for one article. We thus changed two entries for the experimental duration from 96 to 24 h for *Acartia tonsa* and *Calanus plumchrus* from Reeve et al. (1976).

In case LC50 values were only presented in figures, we used Fiji (ImageJ) (Schindelin et al., 2012) to digitize them manually. The individual LC50 data points are shown in Fig. 1A. The average LC50 values for each species and each order are available in Tables A.1 and A.2, and the distribution of LC50 for the different groups is shown in Fig. A.1.

Overall our literature search returned LC50 values for brackish and marine species. This most likely reflects a general trend that toxicity testing with copepods is done with marine species. In freshwater, testing on crustaceans is mainly conducted on cladocerans such as *Daphnia*. By focusing on one search engine, we might have missed some more studies on freshwater species. To keep the literature search reproducible, we opted to exclude other studies that were not detected initially.

2.2. Data censoring

We excluded the study from the statistical analysis when we could not obtain the original articles. Therefore, after an initial screening, we focused on the most commonly reported parameters listed in Table 1 due to their potential relevance for the LC50. An overview of the relationships of the reported LC50 with all available environmental parameter values is represented in Fig. A.2.

Considering each study's availability of environmental information, we restricted our statistical analyses to instances where copper was administered through water, not via dietary uptake. While we initially also included pH due to its well-known effects on copper speciation (Flemming and Trevors, 1989) and subsequently on the LC50 (Grosell et al., 2002), we excluded it from the analysis because of the lack of data reporting in many studies. Also, pH is likely to be correlated with salinity, which renders a statistical interpretation problematic due to collinearity. Due to a relationship between exposure time and temperature in the data, we restricted the dataset further to only include LC50 values from experiments lasting up to 96 h. We also removed data points where a second toxicant was administered simultaneously and entries for the only two cyclopoid species to have a more balanced dataset when comparing taxonomic orders. Some papers did not explicitly state the exposed life stage and some environmental parameters. Therefore, we removed these in our statistical analysis. Before the analysis, we ln-transformed the LC50 values.

After restricting the data, we were left with in total 31 articles and 166 LC50 values from 19 different species. Of these the Web of Science search contributed 16 articles with 111 unique LC50 entries, and 7

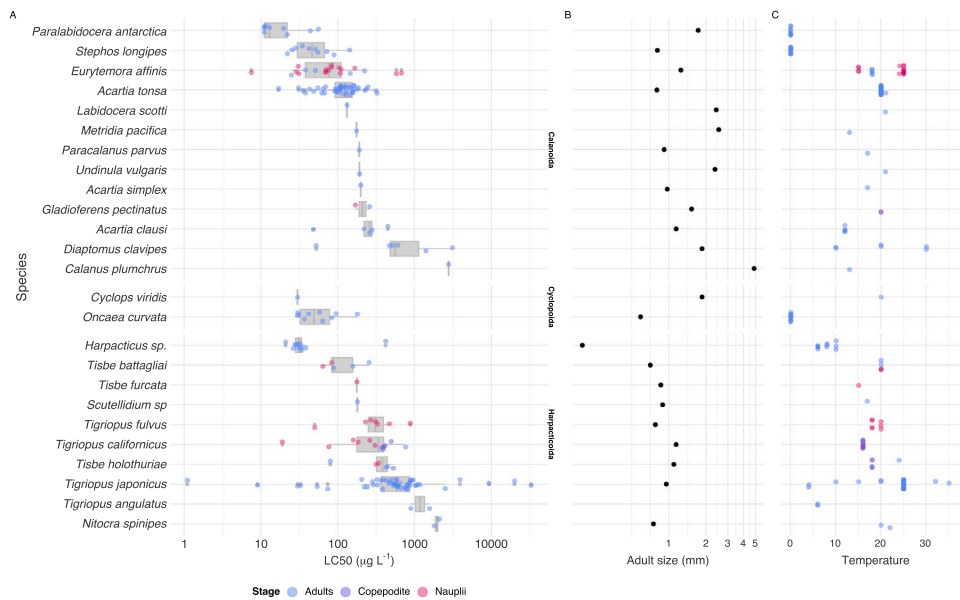


Fig. 1. A) Overview of the available LC50 for the uncensored dataset, with points indicating single entries color-coded by development stage and the boxplot showing median, 25% and 75% quantiles, B) reported body sizes of the different species, and C) the temperatures used in the experiments.

Table 1
Tested environmental drivers with the corresponding hypotheses of their LC50 effect.

Environmental factor	Expected effects
Temperature	Higher temperatures decrease the LC50 (Kwok and Leung, 2005), i.e. increases the toxicity of copper, due to raised metabolic activity (Vidal, 1980; Isla, 2004).
Salinity	Higher salinity increases the LC50, i.e. reduces the toxicity of copper. (Pinho and Bianchini, 2010; Kwok and Leung, 2005) because copper reacts with free anions in the water rather than forming reactive oxygen species that could harm the animals.
Duration	Longer exposure duration will decrease the LC50 as the absorbed dose should increase with exposure time (Kooijman, 1996).
Taxonomy	Harpacticoids have higher LC50s compared to calanoids, because harpacticoid species are mostly benthic and littoral species and thus adapted to higher abiotic stress and potential contaminant levels, whereas pelagic calanoids live in a more stable environment and likely encounter fewer contaminant hot spots (Holan et al., 2016).
Developmental stage	Nauplii stages are more affected by contaminants than adults, because their body is undergoing more substantial structural changes with copper sensitive processes of DNA replication and protein synthesis. This especially applies to metamorphosis from nauplii to copepodite. Moreover, the higher surface to volume ratio should make smaller stages more prone to copper uptake by diffusion, but see Kulkarni et al. (2013).

articles that were cited in these studies contributed 31 more entries. The Envirotox database contributed 8 articles with 24 unique LC50 entries to the final dataset.

2.3. Body size

To test how much body size influences the sensitivity to copper, we added species specific adult (total) body length estimates from either the source article if available or from other published articles. As sizes were rarely reported, most calanoid lengths were taken from Brun et al. (2016, 2017), while harpacticoid lengths came from other publications. All sources are specified in the uncensored datafile in the electronic appendix. If only maximum and minimum values were reported we used

the average length.

2.4. Statistical analysis

To test which factors affect the LC50 in copepods, we used a generalized additive model approach (GAM) based on the mgcv-package (Wood, 2011) in R (R Core Team, 2021). GAMs can model any response shape, making them ideal for situations without fixed prior assumptions.

We specified four different models. We started with a full model with developmental stage, taxonomic order, and copper source included as factors, exposure duration (D) as linear term, and temperature (T) and salinity (S) included as smooth functions using thin plate regression splines. The model allowed for separate smooth functions for temperature and salinity for each taxonomic order.

Based on a visual inspection of the raw data, we did not include the experimental matrix, light conditions, and pH as terms in the final model (Fig. A.2). These factors had no apparent relationship with LC50 or were not reported in many articles (Fig. A.2). pH can influence heavy metal toxicity (Fitzer et al., 2013). However, in the collected papers, it was a) often not reported, b) changed throughout the experiments, or c) correlated with other factors such as salinity and temperature, complicating any statistical analysis.

In the other models, we removed the interaction of order with either temperature, salinity or both. To account for differences in the number of experimental replicates between the studies, we included the replicate number as a weight in the model (replicate number divided by the average number of replicates). To account for multiple studies of the same species and the fact that there might be inherent differences between the species, we included species as a random factor. To reduce the influence of studies with several values, we also added the Article ID as a random factor. We implemented random factors in all cases as random smoothers (Wood, 2011). The following competing models were tested:

$$\begin{aligned}
 M1: \ln(\text{LC50}) &= \text{Stage} + \text{Order} + \text{Chemical} + s(\text{D}) + s(\text{T}, \text{ by} = \text{Order}) + s(\text{S}, \text{ by} = \text{Order}) + s(\text{Species}, \text{ bs} = \text{"re"}) + s(\text{ArticleID}, \text{ bs} = \text{"re"}) \\
 M2: \ln(\text{LC50}) &= \text{Stage} + \text{Order} + \text{Chemical} + s(\text{D}) + s(\text{T}) + s(\text{S}, \text{ by} = \text{Order}) + s(\text{Species}, \text{ bs} = \text{"re"}) + s(\text{ArticleID}, \text{ bs} = \text{"re"}) \\
 M3: \ln(\text{LC50}) &= \text{Stage} + \text{Order} + \text{Chemical} + s(\text{D}) + s(\text{T}, \text{ by} = \text{Order}) + s(\text{S}) + s(\text{Species}, \text{ bs} = \text{"re"}) + s(\text{ArticleID}, \text{ bs} = \text{"re"})
 \end{aligned}$$

$$M4: \ln(LC50)=\text{Stage}+\text{Order}+\text{Chemical}+s(D)+s(T)+s(S)+s(\text{Species}, \text{bs}=\text{"re"})+s(\text{ArticleID}, \text{bs}=\text{"re"})$$

We allowed for the complete removal of model terms using the "select"-function of mgcv (Wood, 2011), during the model fitting process based on restricted maximum likelihood. The best model was chosen based on AICc.

Body size is one of the most important traits of an organism because it links to several life-history processes through physical, physiological and other constraints (Andersen et al., 2016). We formulated an additional model (m5) to test whether size differences between species could explain the data's variation. Here we replaced the random factor "species" of the best model with a linear term representing "adult body size" of the different species.

To check whether size and species explain the same variation in the data, we conducted a variance analysis by first establishing a model with size and species included as terms (m6), and the other terms as included in the best previous model (m4). We then formulated two additional models, where we kept either size (m7) or species (m8). To compare the explained deviances, we used the smoothing parameters of the model with both terms (m6). As a final model, we also formulated a null model with no explanatory terms ($y \sim 1$) (m9). The proportion of deviance explained (D_{exp}) by size was calculated with $P_{Size} = (D_{exp,Species} - D_{exp,both}) / D_{exp-null}$ and the proportion explained by species using $P_{Species} = (D_{exp,Size} - D_{exp,both}) / D_{exp-null}$.

To determine how much the addition of size can explain of the total deviance, we compared the explained deviances of a model with (m10) and without size (but not species, m11) where we fix the smoothing parameters to the model with factor size.

3. Results

Model selection revealed that two models explained similar amounts of information in the data: a model without any interactions and one with an interaction between salinity and taxonomic order (Table A.3). From these, we chose the simpler one to avoid over-parameterization and because salinities were not evenly distributed among the two orders. The model summary for both parametric and smooth functions are presented in Table 2.

The partial effects of temperature and salinity on the LC50 are depicted in Fig. 2. Increasing temperature reduces the LC50 value, but this effect starts only above approximately 25 °C. At lower values, temperature has little influence on the LC50 than the other factors. The response was also similar between calanoids and harpacticoids. Salinity seemingly influences LC50 less than temperature, and the factor was barely retained in the model (Fig. 2B). The developmental stage affects the LC50, with nauplii being more vulnerable to copper toxicity than adults (Fig. 3A). The LC50 was 70% different between nauplii and

Table 2
Summary statistics of the best generalized additive model (M1).

	Estimate	Std. Error	t value	Pr (> t)
Model without Bodysize M1 (Intercept)	5.751	0.907	6.34	0
Stage: Copepodite	0.168	0.393	0.426	0.671
Stage: Nauplii	-0.776	0.286	-2.711	0.008
Salt: Cu nitrate	-0.343	0.88	-0.389	0.698
Salt: Cu pyrithione	-3.077	0.949	-3.243	0.002
Salt: Cu sulfate	-0.162	0.732	-0.221	0.825
Salt: Cu	-1.609	1.111	-1.449	0.15
Salt: Cu(I)chloride	0.463	0.98	0.472	0.637
Salt: Cu(II)chloride	-0.146	0.827	-0.176	0.861
Duration (h)	-0.004	0.006	-0.772	0.441
Taxonomic order: Harpacticoida	0.763	0.543	1.405	0.163
	edf	Ref.df	F	p-value
s(Temperature)	3.631	4	166.663	0
s(Salinity)	0.55	4	0.777	0.174
s(Species)	7.642	17	9.603	0.049
s(Source article)	16.034	29	3.04	0.121

adults.

We found a weak negative relationship between exposure duration for the first 96 h and the LC50 (Fig. 3B). A closer investigation of the raw data reveals a general decline of the LC50 at longer exposures exceeding 96 h (Fig. A.2G), which was not tested here. We also tested whether the type of copper salt used in the experiment influenced the measured toxicity. Except for copper pyrithione, all copper salts yielded similar LC50 values for copepods when corrected for environmental and taxonomic differences (Fig. 3C).

The phylogeny of the tested copepods also influences the baseline sensitivity to copper (Fig. 3D). Cyclopoid LC50s resemble those of calanoids, but they were omitted since data was only available for two species (Fig. 1, A.1). Harpacticoids are more robust than calanoids, with a percentage difference of 75%, but the confidence intervals of the estimates of both groups overlapped when accounting for species-specific differences. When we substitute species information with their approximate (total) adult length, the difference between calanoids and harpacticoids becomes more pronounced (Fig. 4B). The overall relationship between body size and LC50 is positive, i.e., with bigger sizes comes a higher resistance against copper (Fig. 4A, Table A.4). However, this is mainly driven by one large species (*Calanus plumchrus*). Except for taxonomic order, substituting species with size does not lead to a qualitative difference in the results.

The model with size and a model with species as random factor had the same overall explanatory power (explained deviance for both models was 82%). To test whether there is any overlap in the explained variation, we compared the explained deviances of models with both size and species, and models with only one of them, while fixing the smoothing parameters to the full model. In this analysis, species explains 9% of the variation in the model with both factors, while size explains almost none (less than 0.001%). To see how much size alone contributes to a model or whether other factors explain the variance once species is removed from the model, we also compared a model with size but not species and a model without size. This revealed that the size model parameters explain only 1.7% more deviance than a model without.

4. Discussion

Reported copper LC50s for copepods span five orders of magnitude (Fig. 1). Variation exists within and between species, suggesting that many environmental and intrinsic factors influence the vulnerability of copepods to copper exposure.

4.1. Abiotic factors

Environmental conditions can alter the bioavailability and toxicity of pollutants and heavy metals such as copper (Eisler, 1998). Temperature has the most substantial influence on copper toxicity compared to the other tested factors, with a notably stark increase in copper sensitivity above 25 °C. Braginskiy and Shcherban (1978) found a similar temperature-dependent increase within four different freshwater species (Fig. A.3), and Li et al. (2014) detected a comparable rise in temperature-dependent toxicity in the copepod *Tigriopus japonicus* for dichlorodiphenyltrichloroethane, triphenyltin chloride, copper pyrithione, and Cu. The decline in LC50 could thus indicate that copepods are approaching an upper physiological limit or that most tested species are from temperate regions and, therefore, not adapted to warmer conditions. Unfortunately, there is a lack of short-exposure studies at high and low temperatures close to or exceeding the optimal thermal range (Fig. 2). We encourage more studies at high temperatures to verify the trends we detect in this meta-analysis to remedy the data gap.

A temperature rise can elevate toxicity due to several reasons (reviewed in Cairns et al., 1975; Sokolova and Lannig, 2008), including an increased metabolic rate (Clarke and Fraser, 2004), increased toxicant uptake rates (reviewed in Sokolova and Lannig, 2008), reduced uptake of food and oxygen to meet the increased energy demands

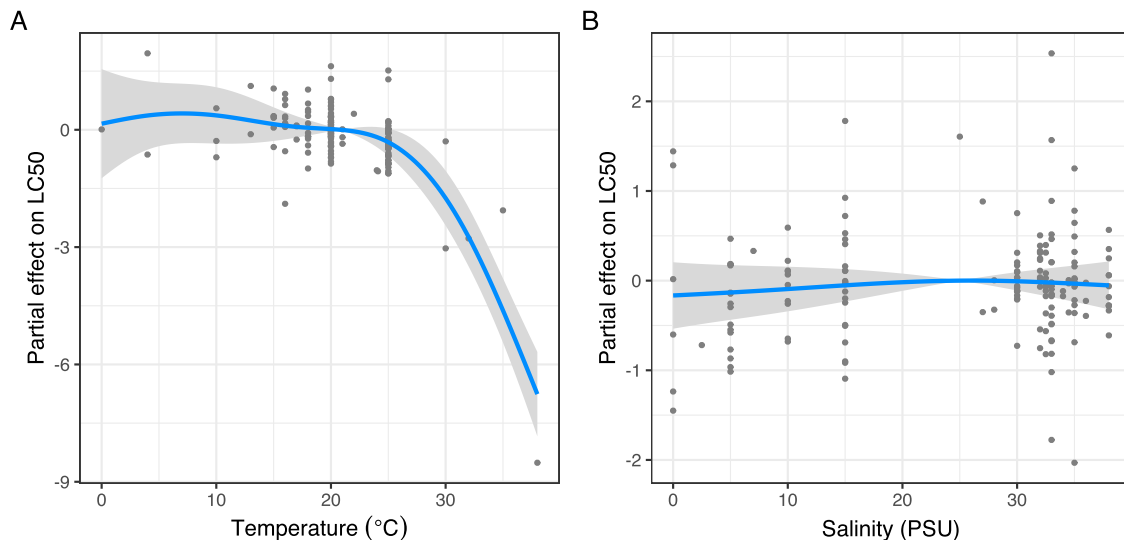


Fig. 2. Partial effects (i.e., the effects on the expected value by moving away from the average value) \pm 95% confidence interval for the smooth terms for temperature and salinity from the final model. Please note that the partial effects are on a log scale ($\ln \mu\text{g L}^{-1}$).

(Pörtner, 2010), and a generally reduced capability to produce antioxidants at higher temperatures (Lannig et al., 2006). Both heat stress and contaminants can also induce costs associated with the production of heat shock proteins (Rhee et al., 2009), further increasing the animals' energy demands. Our initial data exploration suggested no apparent differences in whether animals were fed and non-fed (Fig. A.2 D). Therefore, the energetic state of the individuals might not play a critical role during short-term experiments (96 h), which we considered here.

Warming aquatic habitats might thus worsen the effects of already present pollutants in the sea and freshwater habitats. Interactions with multiple stressors will exacerbate the impact of climate change-induced warming in small lakes and temperate coastal areas, where summer temperatures can exceed 25 °C. The climate crisis will strongly affect the coasts (Nicholls et al., 2007), and with that, unfavorable temperature conditions are likely to occur more often in the future.

Knowledge about the higher toxicity of copper at elevated temperatures could also inform the timing of copper application to aquaculture net pens or crops to minimize negative interactions with non-target organisms. While antifouling paints continuously release small amounts of copper ions, the initial deployment and pressure rinsing of the nets release highly concentrated pulses of copper into the water column (Valkirs et al., 2003). Avoiding, for example, the warmest months in the year could potentially minimize collateral damage. It has to be noted that slow uptake kinetics might cause the lack of an apparent response at lower temperatures, mainly since we restricted our analysis to data from experiments up to 96 h. Adverse copper effects at low temperatures still exist, but due to the slow uptake kinetics, the consequences will only become apparent after a more extended exposure period (see also A.0.2 G).

Contrary to our expectation, salinity had little influence on copper toxicity in copepods. This link is well established within species (Kwok and Leung, 2005) and is often related to favorable copper speciation at higher salinities, i.e., less free copper ions. Our analysis, however, also revealed a lack of studies between salinities of 20 and 25 PSU, which might hide a humpback-shaped response like the one found in juvenile killifish *Fundulus heteroclitus* (Grosell et al., 2007). We thus encourage more studies on the role of salinity on toxicants in brackish water conditions.

Future climate scenarios include increased ocean acidification (Pörtner et al., 2019). In this study, we did not model the effect of pH (see methods). The predicted associated increase in free copper ions with pH (Richards et al., 2011) will likely increase the toxicity of copper in the ocean (Fitzer et al., 2013). However, the net effect depends also on

the availability of other substances. Dissolved organic matter (DOM) is one of the substances that complexes with metals and reduces their bioavailability (Plette et al., 1999). Higher future precipitation, reduced sulfur deposition, and increased forest productivity will likely increase the influx of DOM from terrestrial sources, at least in Northern latitudes (Larsen et al., 2011; de Wit et al., 2016) and thereby potentially reduce copper toxicity.

4.2. Biotic factors

Copepod species have a wide range of copper sensitivities, and in our model, the factor species accounts for a lot of the variation in the data. A negative size dependency of toxicity is expected and observed in zooplankton (Reeve et al., 1976), mainly due to a change in surface-to-volume ratio. In the model with the factor body size instead of species, we indeed see a positive relationship between size and LC50. However, this is driven mainly by one large species (Fig. 4). However, size differences explain much less of the variation in LC50 than species. Interestingly, according to the variance partitioning analysis, there is no overlap in which part of the variation the two factors explain. Therefore, other inherent traits must explain the differences between species.

Size can also not explain the difference between calanoids and harpacticoids since the latter are on average 0.2 mm smaller (see Table A.2) but generally less susceptible to copper toxicity. In a model with body size, the difference between these taxonomic groups became more pronounced. Hence inherent species-specific differences that are stronger than the size influence might hide the differences between higher taxonomic levels in the best model since species is included as a random factor. Such size-independent relationships between phylogenetic relatedness and species sensitivity also exist in terrestrial invertebrates such as nematodes (Heaton et al., 2020).

Our model LC50 estimate was 360 $\mu\text{g Cu L}^{-1}$ lower for calanoids than harpacticoids. Cyclopoids, another mostly pelagic group, are understudied for copper tolerance. Here we could only find LC50s for two species, and their copper sensitivity seems to exceed that of calanoids (Fig. A.1). Copepods, especially pelagic ones, are the main link between primary producers and higher trophic levels, such as fish. Therefore, the adverse effects of copper on copepods will likely potentiate through the marine food web. In addition, many phytoplankton species are susceptible to copper pollution (Brand et al., 1986), which, given the current trends in copper use, will increase the vulnerability of aquatic ecosystems, both in marine and limnic waters.

Many harpacticoids are adapted to benthic and intertidal zones

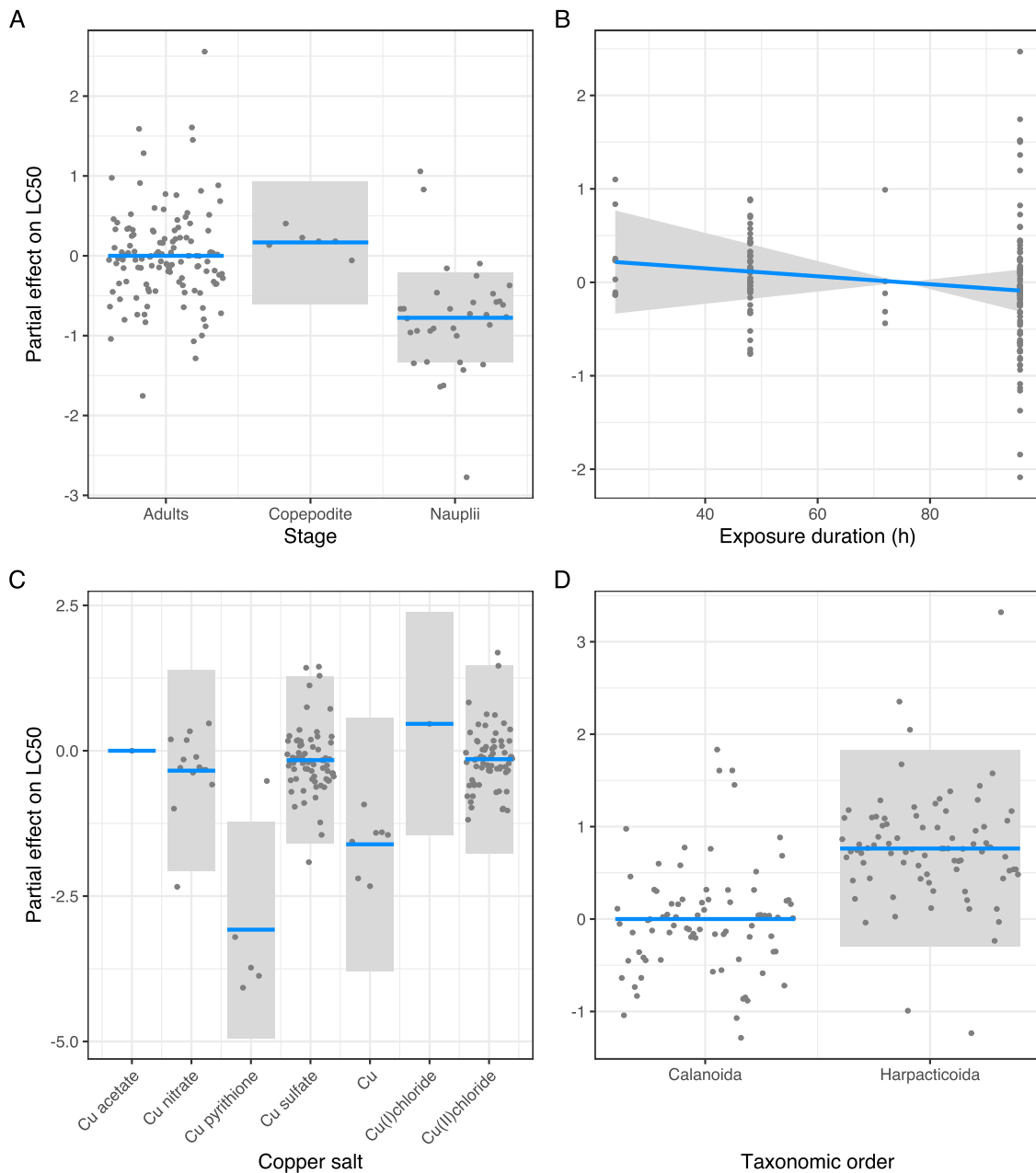


Fig. 3. Partial effects (i.e., effects on the expected value by moving away from the average value) \pm 95% confidence interval of the parametric model components of the final model. Please note that the partial effects are on a log scale ($\ln \mu\text{g L}^{-1}$).

(Holan et al., 2016). These habitats have a harsher and more fluctuating environment (O'Brien et al., 1988) and are prone to higher contaminant levels (Medina et al., 2008). A comparison of Antarctic species of the intertidal zone and those from more constant subtidal zones (Holan et al., 2016) shows a similar habitat-specific tolerance. Further, given the differences in LC50 between calanoids and harpacticoids, one should be cautious about transferring safe concentrations and environmental assessments between them. Most environmental monitoring efforts for copper and other heavy metals focus on sediments and the benthic community and less on short- and long-term copper exposure in the water column.

Predictions for adult LC50s were 70% higher than for nauplii, which could be caused by a higher metabolic activity due to development or size differences. Such a difference between nauplii and adults in LC50, although only 10%, has also been detected in the freshwater copepod *Notodiaptomus conifer* (Gutierrez et al., 2010). However, when looking at

the raw data of the present study, intraspecific stage differences are not very pronounced (Fig. A.4).

Although adults are less prone to die from copper, copper ions or their free radicals might still interrupt or hamper sub-lethal developmental and physiological processes such as gametogenesis and reproduction. These effects are not captured by measuring mortality. Most LC50 values in our data originate from experiments with adult copepods, which calls for more studies focusing on the developmental differences in susceptibility to copper. Therefore, future testing should focus on the most vulnerable life stages and life-history processes, such as metamorphosis events (Heuschele et al., 2019; Lode et al., 2018) and reproduction (Fitzer et al., 2013).

Differences in the susceptibility to copper between and especially within copepod species could also stem from different historical exposure and a subsequent plastic or potentially evolutionary adaptation of local populations (Kwok et al., 2009; Moraítou-Apostolopoulou et al.,

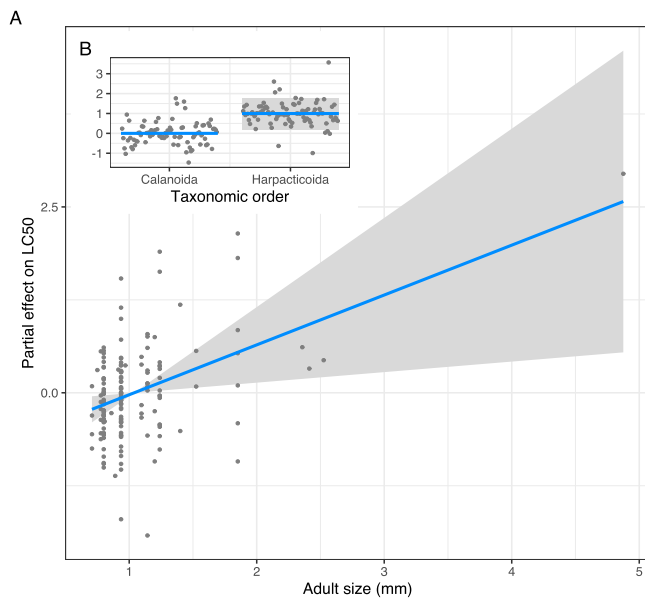


Fig. 4. Partial effects \pm 95% confidence interval for adult size and taxonomic order (smaller window) for the model, including size instead of copepod species. Please note that the partial effects are on a log scale ($\ln \mu\text{g L}^{-1}$).

1983). One might expect naturally higher background concentrations of trace elements in sediments and following episodes of anoxic conditions. These factors, especially anoxic episodes, could potentially lead to a rise in antioxidant levels, ROS-handling capacity, and prepare the animals for future oxidative stressors (Hermes-Lima et al., 2015). Copper values are often higher close to harbors, industries, and urban areas (Dassenakis et al., 1996); hence copepod populations from these areas might be more resistant to adverse copper effects. For example, this could explain the highly resistant *T. japonicus* in Hongkong harbor, where background copper concentrations in field sediments can reach 140 mg Cu/kg dry mass (Bao et al., 2018).

4.3. Experimental factors

More prolonged exposure to copper reduces the LC50. We observe a linear (on a log scale), although non-significant, decline over the 96-hour exposure period, which implies that a safe level of copper is likely lower during constant exposure conditions. The raw data (Fig. A.2G), which includes studies with exposure times longer than 96 h, suggests a further drop in LC50s at longer-lasting exposures. Long-exposure studies were conducted at low temperatures; therefore, we cannot rule out trade-offs of copper protection with thermal adaptation. However, dose-time relationships apply to all species and call for longer studies or toxicokinetic-toxicodynamic modeling to fit the dose-time effects (Jager et al., 2011b), especially since the internal metal concentration might not have reached a steady-state during shorter exposures (Chang and Reinfelder, 2002; Wang and Fisher, 1998). Most studies also do not include any observations during recovery phases and might thus miss detecting delayed effects on longevity and other life-history traits such as reproduction.

The source of copper, i.e., the copper salt used in the experiment, has surprisingly little influence on the toxicity. The exception is copper pyrithione, which was much more toxic than the rest of the tested copper compounds. Copper pyrithione differs from the other salts in that it is relatively insoluble and that the toxicity stems mainly from the molecule itself and not from the Cu^{2+} ion. LC50 values and other endpoints are comparable between experiments using commonly applied copper salts.

Similarly, our initial data exploration suggests that artificial or filtered seawater does not influence copper toxicity (Fig. A.2E). The lack of influence of the exposure matrix seems to contrast terrestrial toxicity

tests where soil type is an important driver (Wilson and Frampton, 1996). The distinction is likely because soil types and their specific chemical characteristics differ much more than those of different water types.

4.4. Conclusion

Our meta-analysis shows that, in addition to phylogeny and developmental stage, the copper sensitivity of copepods depends on several environmental factors. These factors can aggravate contaminant effects compared to the predictions from single effects studies done at otherwise optimal environmental conditions. An increase in direct copper toxicity is only expressed at high temperatures, at least during short-term experiments. However, an increase in water temperature can, besides others, reduce phytoplankton biomass and thus alter food availability (Boyce et al., 2010), lower oxygen concentration (Deutsch et al., 2015), and alter community composition on an ecosystem scale (reviewed in Beaugrand and Kirby, 2018). These factors and processes can either relieve or add additional stressors. Adding stressors, or moving species closer to their environmental limits, is likely to increase the impact of toxicant stress (Heugens et al., 2001).

The measured copper concentrations in the field seem to be generally 1–2 magnitudes below the average reported LC50 values, with the bioavailable fraction likely to be even lower (Jones and Bolam, 2007). Thus, at these concentrations, copper should typically not affect copepod survival. Still, given our analysis, high temperatures and other environmental stressors might reduce copper sensitivity to a level that falls in the range to have consequences. This is especially true for areas with high copper influx, such as marinas or close to polluted sediments.

Despite the inherent shortcomings of LC50s (Kooijman, 1996; Jager, 2011a), our analysis indicates that one might be able to use published LC50s to identify environmental factors that influence toxicity across species. The resulting model can be used to plan more process-based experiments and subsequent toxicokinetic – toxicodynamic analyses that allow for a more direct comparison of the underlying effects between species. Our model could also inform environmental impact assessments in the absence of dedicated process-based experiments and models. One could, for example, predict LC50 values for different species for future environmental conditions (reduced salinities, higher temperatures) of a specific region and habitat.

Using the variation in species and experimental conditions in published studies is a straightforward way to determine the underlying factors that influence the toxicity of other contaminants in nature. Our literature search also indicates understudied taxonomic groups, such as cyclopoids in our study, and missing data for specific environmental ranges in temperature and salinity. Moreover, our analysis revealed a lack of studies on the most vulnerable life stages, the nauplii, especially studies that would enable a direct comparison to adults.

Author contribution statement

JH conceived the study, collected the data with help from HSK, did the analysis with the input of the other co-authors, and wrote the original draft. TL, JT, TA, HSK, and KB provided feedback and input on the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2022.113907](https://doi.org/10.1016/j.ecoenv.2022.113907).

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