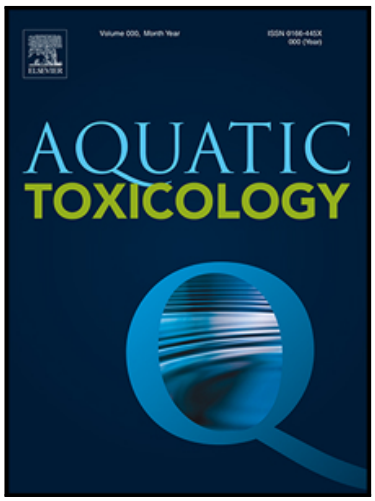

Derivation of toxicity parameters from field data: analysis of lake zooplankton species responses to metals and acidity

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Highlights

- Long-term response to toxic metals and acidity varies among lake zooplankton species
- Data analysis with WHAM-F_{TOX} allowed derivation of species-specific parameter β
- β values for lake zooplankton species fall within the range of β from laboratory data

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Derivation of toxicity parameters from field data: analysis of lake zooplankton species responses to metals and acidity

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Abstract

The WHAM-$F_{TOX}\beta$ model describes the toxic effects of mixtures of protons and metal cations towards biological species, using a set of intrinsic parameters for the cations (α_H, α_M*) and a sensitivity parameter (β) for each species. We applied the model to extensive water chemistry and zooplankton species occurrence data for four lakes contaminated with acidity and metals (Al, Ni, Cu, Zn) at Sudbury, Ontario, over the period 1973-2018, during which cation contamination declined, and zooplankton species numbers increased. Assuming that the appearance of a species resulted solely from decreases in water toxicity, and that α_H and α_M* values previously derived from laboratory toxicity test data could be applied in the field, we used the field data to estimate values of β for individual lake zooplankton species. Results for lake-species pairs with 20 or more species occurrences (from six samplings per year) were analysed. In most cases, the number of occurrences increased over time from zero to five or six per year, then remained at the high level. For a minority of pairs, occurrences per year increased initially, but subsequently declined, and so data only from the initial period were used to estimate β. The β values derived for the lake zooplankton are reasonably consistent with values derived from laboratory data for a range of other species. The findings support the application of WHAM-$F_{TOX}\beta$ to describe toxic effects of mixtures of cations in the field, and the toxicity model might be combined with ecological theory to interpret natural population responses.

Keywords: chemical speciation, lakes, metals, toxicity, WHAM-$F_{TOX}\beta$, zooplankton

1. Introduction

The toxic effects of metals and acidity in the field are widely assessed using the response of the catch-all variable n_{sp} (total number of species present) to variations in water chemistry. Examples include invertebrates in streams (Griffith et al., 2004; Stockdale et al., 2010, 2014a) and crustacean zooplankton in lakes (Khan et al., 2012; Stockdale et al., 2014b; Tipping et al., 2021). This may be a robust and useful approach, but it is only correlative; the actual interactions responsible for the effects must operate at the individual species level. Therefore, by analysing how individual species respond to varying toxic conditions, greater insight into field toxicity might be achieved. In this study we performed such an analysis using long-term zooplankton and chemistry data collected for four acid- and metal-contaminated lakes at Sudbury, Ontario.

We used the WHAM-$F_{TOX}\beta$ toxicity model (Tipping et al., 2023) an extension of WHAM-F_{TOX} (Tipping et al., 2021); see Fig. 1 and Table 1. These models assume that the cation-binding sites of a biological organism can be approximated by those of isolated humic acid (HA); the accumulation of metabolically-active toxic cations by the organism is estimated by assuming chemical equilibrium with the surrounding solution and applying the WHAM chemical speciation model (Tipping et al., 2011; UKCEH 2020). This approach permits the combined toxicities of protons and cationic metal mixtures to be calculated and combined, in the variable F_{TOX}, while accounting for the solution chemical speciation.

In WHAM-$F_{TOX}\beta$, each metal cation has an intrinsic toxicity, α_M*, which is the same for all biological species, each of which has an intrinsic sensitivity, β, which is the same for all metals. Thus, biological attributes of the species are separated from the chemical system. Tipping et al. (2023) parameterised WHAM-$F_{TOX}\beta$ with data from laboratory toxicity test experiments covering results for 76 test species and 15 different metals, and provided evidence that the intrinsic sensitivity concept, i.e. the use of the single parameter β for each species, approximately holds.

The effects of atmospherically deposited acidity and toxic metals (chiefly Al, Ni, Cu and Zn) on the chemistry and biology of lakes in the vicinity of metal smelters at Sudbury, Canada, have been documented and analysed for half a century (Keller et al., 2019). A major part of the research has been devoted to crustacean zooplankton species richness (Keller & Yan, 1991; Palmer et al., 2013). The results show severe depletions in annual average species richness in the early 1970s, followed by a long-term increase in richness over the next several decades, as

metal concentrations declined, while pH and concentrations of dissolved organic carbon (DOC) increased. These results offer an excellent, perhaps unique, opportunity to test toxicity modelling with field data.

In previous work on zooplankton from the Sudbury lakes (Tipping et al., 2021), we used a preliminary version of WHAM-$F_{TOX}\beta$ to fit, with reasonable success, monthly-averaged n_{sp} for 9 lakes covering the period 1973 to 2006. In that work, we assumed a set of model zooplankton species, and summed their calculated presences to obtain n_{sp}, which was compared with the observed values. Here, the approach is different, in that the analysis focuses on fitting data for the field occurrences of individual zooplankton species. This has been facilitated by the availability, for four of the lakes, of longer data runs (1973 to 2018), which cover wider ranges of water chemistry and zooplankton responses.

Our null hypothesis is that the number of occurrences of a regionally-common individual zooplankton species in a given year depends only upon the combined effect of toxic cations, expressed through the model variable F_{TOX}. For values of F_{TOX} less than a lower threshold ($F_{TOX,LT}$), the species is expected to occur on every sampling visit. When F_{TOX} exceeds an upper threshold ($F_{TOX,UT}$), the water toxicity is so high that the species cannot occur. In between the two thresholds, the number of occurrences is in the range zero to 100%. This behaviour is taken to parallel the laboratory responses of test species to toxic cations, which vary from zero to 100% effect (e.g. complete mortality) as F_{TOX} increases from the lower to the upper threshold.

The data from the Sudbury lakes are suitable for analysis because they are numerous and cover a wide range of water chemistries, so that substantial variations in F_{TOX} have taken place. The variable has generally decreased over time in the Sudbury lakes owing to improvements in water quality. Moreover, within the field data set there are many "replicates" where the behaviour of a given species can be observed in the different lakes. We aimed to answer the following questions.

(1) Is the null hypothesis followed, such that field species appear over the decreasing F_{TOX} range and then persist in the lake, without the involvement of other ("ecological") factors?

(2) Are β values estimated from field data similar to laboratory-based values?

(3) Are differences between the upper and lower thresholds ($F_{TOX,UT}$ - $F_{TOX,LT}$, or Δ_T) estimated from field data similar to laboratory-based values?

2. Methods

2.1. The study lakes

The study lakes, ranging in surface area from 27 to 77 ha, are located near the large Copper Cliff metal smelting complex, Sudbury, Ontario, Canada. They comprise two lake pairs; Middle and Hannah lakes are within 5 km of the smelters, while Clearwater and Lohi lakes are 10 to12 km away. When monitoring of these lakes began, in 1973, they were among the most acid and metal contaminated lakes in the region due to atmospheric contaminant deposition. Between 1973 and 1975 (Scheider et al., 1975; Dillon et al., 1979) Lohi, Middle and Hannah lakes received additions of $CaCO_3$ and $Ca(OH)_2$ to test the efficacy of lake liming as a remedial measure. To attempt to promote biological recovery, these lakes further received phosphorus additions between 1975 and 1978 (Yan et al., 1996). Lohi Lake quickly reacidified and was not treated further; however, between 1983 and 1984 the watersheds of Middle and Hannah lakes also received applications of lime and fertilizer as part of regional land reclamation efforts (Yan et al., 1996). Clearwater Lake, upstream of Lohi Lake, did not receive any lake or watershed treatments to allow tracking of its natural recovery as smelter emissions were reduced.

At the beginning of monitoring, the study lakes were completely devoid of fish. As chemical conditions improved, planktivorous fish, particularly the acid-tolerant yellow perch (*Perca flavescens*) were able to colonize the lakes. Perch recolonization occurred first in Middle and Hannah lakes, during the mid-1980s, and perch later became established in Clearwater and Lohi lakes by the late-1990s (Luek et al., 2010). Piscivorous fish later appeared in all the lakes at various points during the mid- to late-2000s and showed varying success at establishment (Luek et al., 2010).

2.2. Zooplankton data and derived variables

The available data for the four lakes comprise species identities of crustacean zooplankton collected at different times during nearly all the growing seasons (May to October or November) between 1973 and 2018. Zooplankton sampling and species identification followed the methods described previously (Palmer et al., 2013). To ensure fair comparisons between years and lakes, we only used data for years in which at least 6 samples were taken, and for those years with more than 6 samples, we chose 6 samples at random. This procedure meant

that we had data for 35 years in Clearwater Lake, 37 years in Lohi Lake, 35 years in Hannah Lake, and 36 years in Middle Lake. For each zooplankton species, the occurrences per year were expressed as a fraction of the maximum (f_{occ}) by dividing by 6.

2.3. Lake chemistry

We used annually-averaged chemistry data for each lake over the period 1973 to 2018; data for the most important variables are plotted in Fig. S1. The chemical changes and the reasons for them (decline in atmospheric deposition, lake liming and fertilization, and land reclamation by catchment liming, tree planting and nutrient additions) have been described and discussed in detail elsewhere (Keller et al., 2019, and references cited therein). The main features of relevance to the present study in the paired lakes (Clearwater and Lohi, Hannah and Middle) are as follows.

Since Clearwater Lake was not subjected to any remediation measures, and Lohi Lake was limed only for a short time in the 1970s, these two lakes recovered gradually over the study period (1973-2018). Recovery from acidification occurred markedly during the 1990s, and at the same time concentrations of DOC (dissolved organic carbon) increased. Metal concentrations declined over the entire study period, but Al and Cu showed marked falls during the 1990s. There were modest increases in the concentrations of Na and Cl, and modest declines in the concentrations of Mg and Ca.

Hannah and Middle lakes showed similar chemistries. There were sharp increases in pH and DOC concentrations in the late 1970s and early 1980s, owing to lake and catchment liming. Metal concentrations declined approximately exponentially over the study period. Concentrations of Na and Cl rose markedly, due to road salting.

2.4. Chemical speciation calculations

We used WHAM7 (Tipping et al., 2011; UKCEH, 2020) to calculate lakewater chemical speciation, taking into account the competitive complexation of major and trace metals with inorganic ligands and dissolved organic matter. As in previous work (Stockdale et al., 2010) we attributed DOC to fulvic acid (FA), with the standard conversion [FA] (g L^{-1}) = 1.3 [DOC] (g L^{-1}), where square brackets indicate concentrations. We used the quadratic equation for Al^{3+} activity, derived for surface waters by Tipping (2005), to constrain total concentrations of

dissolved Al (in inorganic forms and complexed with dissolved organic matter); if the activity of Al^{3+} calculated using the measured Al concentration exceeded the value given by the equation, then the equation value was assumed. We assumed that total dissolved Fe(III) concentrations were controlled by equilibrium with $Fe(OH)_3$ (Lofts et al., 2008). Our calculation inputs included humic acid (HA) at a very low assumed concentration of 10^{-9} g L^{-1}, in order to compute values of $v_{HA,H}$ and $v_{HA,M}$ (mol gHA^{-1}), which are the amounts of protons and metals bound to HA in equilibrium with the lakewater solutions; the assumed very low value of [HA] ensures that the bulk solution speciation calculation is unaffected. The values of $v_{HA,H}$ and $v_{HA,M}$ are used in the WHAM-$F_{TOX}\beta$ model (Section 2.5).

2.5. Modelling cation toxicity

A schematic is given in Fig. 1. The WHAM-$F_{TOX}\beta$ model (Tipping et al., 2023) is based on the assumptions (a) that the toxic effects of protons and metal cations are additively related to their occupancies of binding sites possessed by biological organisms, and (b) that those binding sites can be represented by the binding sites of isolated humic acid (HA). This enables the WHAM chemical speciation code to be used to calculate proton and metal binding by living organisms, taking into account competition effects, including the influence of pH. In this way we can account for metal mixture toxicity and the effect of solution composition etc, and so apply the model to field situations.

The fractional site occupancies (θ_H and θ_M) of the biological binding sites are assumed to be the same as those of HA, obtained by dividing the $v_{HA,H}$ and $v_{HA,M}$ values (Section 2.4) by the HA content of proton-dissociating groups (5.1×10^{-3} mol g^{-1}). Values of θ_H and θ_M are dimensionless and can vary from zero to unity. They are used in the following equation to obtain, for a given species, the value of the toxicity variable F_{TOX}

$$F_{TOX} = \alpha_H\theta_H + \beta \Sigma\, \alpha_M\, \theta_M \qquad (1)$$

where α_H and α_M are toxicity coefficients (dimensionless) for protons and metals, β is a constant that quantifies the intrinsic susceptibility of the species to toxic metals (the greater is β the more sensitive is the species, and this applies to all metals), and the summation is over all toxic metals present in the bathing solution. The value of α_H is time-independent, and the same for all species. The value of α_M is the same for a given metal irrespective of the biological species. It increases with time of exposure, up to a maximum value of α_M*.

The toxic response (*TR*) depends upon lower and upper thresholds (LT and UT) of F_{TOX}, between which *TR* increases linearly from zero to unity. Thus

$$F_{TOX} \leq F_{TOX,LT} \qquad TR = 0 \tag{2}$$

$$F_{TOX,LT} < F_{TOX} < F_{TOX,UT} \qquad TR = (F_{TOX} - F_{TOX,LT}) / \Delta_T \tag{3}$$

$$F_{TOX} \geq F_{TOX,UT} \qquad TR = 1 \tag{4}$$

Here, Δ_T (= $F_{TOX,UT} - F_{TOX,LT}$) is the range of F_{TOX} over which the toxic response is incomplete; for example, in a mortality-based laboratory test, conditions usually vary from those in which all the test specimens survive (*TR* = 0, eq. 2) to those in which none do so (*TR* = 1, eq. 4), with fractional responses (1 > *TR* > 0) in between (eq. 3).

Since F_{TOX} is only a relative measure of toxic effect, the WHAM-$F_{TOX}\beta$ model cannot have absolute parameter values. In deriving values of α_M* and β from laboratory toxicity testing data, we therefore specify *a priori* two parameter values. These are α_H, fixed at 1.00, and the median value of β, also fixed at 1.00 (Tipping et al., 2023). With these fixed values, the value of $F_{TOX,50}$ (F_{TOX} when *TR* is 0.5) is equal to 0.82.

To quantify the effect of toxicity on a species in the field, we used values of f_{occ} (Section 2.2). If the species occurred in all samples, $F_{TOX} \leq F_{TOX,LT}$, and f_{OCC} = 1; if it occurred in none of the samples, $F_{TOX} \geq F_{TOX,UT}$, and f_{OCC} = 0; if it occurred in three samples, $F_{TOX} = F_{TOX,LT} + 0.5 (F_{TOX,UT} - F_{TOX,LT})$, and f_{OCC} = 0.5. This approach means that we are equating f_{occ} with (1 – TR), in terms of cation toxicity. Thus, for the field situation, with f_{occ} as the response variable instead of *TR*, equations (2) to (4) are rewritten as

$$F_{TOX} \leq F_{TOX,LT} \qquad f_{occ} = 1 \tag{5}$$

$$F_{TOX,LT} < F_{TOX} < F_{TOX,UT} \qquad f_{occ} = (F_{TOX,UT} - F_{TOX}) / \Delta_T \tag{6}$$

$$F_{TOX} \geq F_{TOX,UT} \qquad f_{occ} = 0 \tag{7}$$

2.6. Data fitting

From the time-dependence of α_M reported in previous work (Tipping et al., 2019) we calculate that one month's exposure of an organism to dissolved cations is sufficient for α_M to reach 95% of the α_M* value, and therefore we consider it justified to use α_M* for α_M in equation (1), to model toxicity in the Sudbury lakes, over the ice-free season. Values of α_M* for the relevant

metals in the lakes (Al, Ni, Cu, Zn, Cd, Hg, Pb) were taken from the analysis of laboratory toxicity data by Tipping et al. (2023).

We analysed field data by calculating values of $\alpha_H\theta_H$ and $\Sigma\ \alpha_M\theta_M$ (equation 1) and then fitting values of f_{occ} obtained in different years to equations (5 –7) by optimising the values of β and Δ_T. Calculations were done with Microsoft Excel, using Solver, to minimise the sum of the squared differences between observed and calculated f_{occ}. We evaluated the results by regressing observed f_{occ} against calculated f_{occ} to obtain the slope (m) and significance (p value) for each species-lake pair; the expected value of m is 1.00. We restricted our analysis to those species occurring in at least 20 samples from a given lake. This potentially allowed analysis of results for 20 different zooplankton species. We used the jackknife "leave-one-out" method (e.g. Hinkley, 1977) to estimate confidence limits on the two parameters extracted from the data.

2.7. Species Types

During the analysis, we found that some species behaved as expected from the null hypothesis, in that f_{occ} increased from near-zero to a maximum value over a definable period, and then remained at the higher value. We refer to these species as Type A. Other species (Type B) showed the same behaviour in their early years, but f_{occ} subsequently declined. We objectively distinguished the two types as follows. First, the five-point running mean of f_{occ} as it changed through time (year of observation) was calculated; it was obtained from the value of f_{occ} for the year in question, the values for the two preceding years, and the values for the two following years. Running mean values ranged from zero to 1.00. Second, the difference was calculated between the maximum and final values of the running mean. If this was less than 0.2, the species was assigned to Type A, and if it was greater than 0.2 to Type B. Examples of the derivations are shown in SI, Fig. S2. If Type B behaviour was followed, data fitting was restricted to the period during which f_{occ} increased to a maximum and then levelled off.

2.8. Lag time effects

If there had been time lags between chemistry reaching a certain state and the species responding, then the observed responses (values of f_{occ}) would have been the consequences of earlier water chemistries. The studies of Watson et al. (1999) and Audet et al. (2013) have demonstrated that within five years many species can colonize newly created aquatic habitats

in the Sudbury area from external sources. Therefore, to explore and quantify the possible effects of time lags, we moved the f_{occ} values earlier by two or five years, and then refitted the data.

3. Results

3.1. Background to the analysis

Fig. 2 shows values of F_{TOX} over time, calculated for each lake for three hypothetical species with β values of 0.25, 1.00 and 1.75 (approximately the 12th, 50th and 80th percentiles of the values from laboratory studies; Tipping et al., 2023). The downward trends in F_{TOX} reflect the chemical recoveries of the lakes, as concentrations of protons and metal cations diminished and DOC concentrations rose (Fig. S1). The species with $\beta = 0.25$ has $F_{TOX} < F_{TOX,50}$ over the entire period of observation, and so is expected to occur in all years, although f_{occ} may fall below 1.00 in the early years of the record. The species with $\beta = 1.75$ has $F_{TOX} > F_{TOX,50}$ over nearly all the period, and is expected to occur only in the later years. The species with $\beta = 1.00$ is expected to appear during the intermediate years. The behaviours of the three hypothetical species differ among the lakes, especially in the early years of the record, mainly because Hannah and Middle lakes have greater ranges of F_{TOX} than Clearwater and Lohi lakes. It is also evident from Fig. 2 that the declines in F_{TOX} during the monitoring period show scatter, and therefore the model predicts overall temporal increases in f_{occ}, but with short-term variability.

Additional background information is provided in Fig. S3 to show the relative contributions of the different cations to F_{TOX}. The most important were H, Al, Ni and Cu, with a minor contribution from Zn, but negligible contributions from Cd, Hg and Pb. Considering only the toxic metals, Al and Cu contributed most to F_{TOX} in the early years, but the contribution of Al declined and that of Ni rose as the lakes became less acid.

3.2. Derivation of toxicity parameters

Results for 47 lake-species pairs with 20 or more occurrences of the species were subjected to analysis (Table 2). Twenty-six pairs showed Type A behaviour (see Section 2.7), and data that could be fitted to obtain values of β and Δ_T with a significant p-value for a plot of observed vs predicted f_{occ}. Examples are shown in Fig. 3, and the remaining results in Fig. S4. Seven pairs showed Type B behaviour with data giving significant fits (Fig. 4). A further three pairs, each showing Type B behaviour, gave fitting results that were not significant (Table 2). Although mostly consistent across species, the Type A and B assignments are not fully definitive, which is demonstrated by the finding that a few species displayed different behaviours in different lakes (Table 2). Thus, *Daphnia mendotae* was Type A in Clearwater and Middle lakes, but Type B in Hannah Lake, *Diaphanosoma birgei* was Type A in Lohi, Hannah and Middle lakes but

Type B in Clearwater Lake, and *Orthocyclops modestus* was Type A in Lohi Lake but Type B in Middle Lake.

In the 33 cases with significant fits, the values of β were well-defined, having an average 95% CL value of 0.044 (4.4 % of the average β). The CL values for $F_{TOX,UT} - F_{TOX,LT}$ were relatively wide, with a mean of 0.109 which is 37% of the average.

In all, values of β were estimated for 16 different species, and maximum values were estimated for two (Table 3). For nine species, the availability of data for more than one lake meant that replicate β estimates could be made; of these, six show good or fair agreement (values of range/mean < 0.4) while three have more widely-dispersed values (range/mean > 0.6).

There were no apparent similarities in values of Δ_T obtained for the same species in different lakes (Table 4). The values ranged from zero, the change from low to high f_{occ} being essentially instantaneous, to 0.4 or more, for which the transition took 10 years or more (Table 2, Figs. 3, 4, S4). The values of Δ_T averaged 0.28 (SD 0.26, n = 33), and this is significantly smaller ($p < 0.001$) than the average for laboratory species calculated from the results of Tipping et al. (2023), which was 0.63 (SD 0.44, n = 61).

Eleven of the 47 lake-species pairs did not yield parameter estimates, because the species were sufficiently insensitive to toxic effects to be fully established at the start of the record. Therefore the transitional periods required to obtain data for fitting were lacking. However, maximum values of β could be estimated (Table 2); note that the maximum β values vary among lakes because of differences among lake chemistries at the outset of the observation period. Four of these pairs showed Type A behaviour (Table 2, Fig. 5) and 7 showed Type B behaviour (Table 2, Fig. 6).

3.3. Lag-time effects

Simulations to explore the possible effects of lag-times (see Section 2.8), using nine representative examples with well-behaved responses (Table S1), showed that two-year and five-year lag-times would result in lower β values. For the two-year lag, the average decrease was 0.07 (9 %); for the five-year lag, it was 0.14 (16 %). No systematic differences in the Δ_T values were found.

4. Discussion

4.1. Type A and Type B behaviours

The Type A zooplankton-toxicity responses were as expected from the null hypothesis, in that f_{occ} increased from near-zero to near-unity over a well-defined time period, during which water quality improved. This meant that if the data for a Type A lake-species pair covered a transitional period, they could be transformed to fit the WHAM-$F_{TOX}\beta$ model (Figs. 3, S4). The species assigned to Type A correspond to those observed to thrive in the pelagic (open water) zones of regional lakes in terms of reaching detectable abundances, based on their common detection in spatial surveys (Keller and Pitblado, 1989).

Type B behaviour involves two phases. In the first, Type A behaviour is followed, with f_{occ} increasing as toxic conditions lessened, to reach high values in several cases (Fig. 4). Then in the second, f_{occ} declines, most likely owing to ecological factors. The most general and obvious reason for Type B behaviour is that the species in question are either less common regionally, or more typically associated with littoral areas (Keller and Pitblado, 1989). Their early establishment in the pelagic zone may therefore have been possible due to lack of competition, with subsequent displacement by Type A species as water quality improved. Therefore, although Type B behaviour overall does not follow the null hypothesis, it is justifiable to use data from its first phase to derive toxicity parameters.

4.2. Lag times

For the default modelling, we assumed that a species responds instantaneously to changes in lake water chemistry. However, there might be a lag between the time when non-limiting chemistry first arises and the time when a species colonises the lake at sufficient density to be detectable with our field sampling protocols. According to previous work, colonisation is likely to be rapid, given that (a) the landscape contains a high density of source populations which provide a constant supply of propagules arriving at the focal lakes (Yan et al., 2016), and (b) emergence from resting stages in sediments also provides an ongoing internal source of zooplankton colonists (Arnott and Yan, 2002). To explore possible lag effects (Section 3.3), we derived the toxicity parameters on the assumptions of 2-year and 5-year time lags between species response and water chemical change; As would be expected, given the improvements in water chemistry through time, the greater the assumed time lag, the smaller was the estimated

β value. However, the calculated decreases in β were modest, averaging 0.07 (9%) with a 2-year lag and 0.14 (16%) with a 5-year lag (Table S1). Therefore, it is unlikely that the β values we report exceed true sensitivity by more than 10%, if indeed lag effects apply.

4.3. Values of β

The null hypothesis is based on the idea that β for a given species should be the same in each lake, but the expected agreement may be obscured by several factors. These include (a) uncertainty and error associated with zooplankton sampling, water chemistry analysis and the annual averaging of water chemistry, (b) approximations and assumptions made in chemical speciation and toxicity modelling, and (c) ecological effects including the availability of colonists from internal refuges or from other lakes and physical and biological barriers to their dispersal and successful colonization. (Keller and Yan, 1998; Yan et al., 2003), Despite these factors, in the nine cases for which values of β were estimated for the same species in two or more lakes, reasonable overall agreement was obtained (Table 3).

The largest variation was shown for *D. mendotae*, with a much higher β in Clearwater Lake than in Hannah and Middle lakes. A possible explanation is that abundant planktivorous perch suppressed the population during the early- to mid-1990s in Clearwater Lake, despite the absence of toxic effects, but the later appearance of piscivores reduced perch abundance and permitted the species to establish (see Fig. S4). In both Hannah and Middle lakes, the species was established by 1990, having overcome toxic effects (Yan et al., 1996), which suggests that the lower β values derived for these two lakes are more reliable.

When it comes to comparing β values derived from field data with those from the laboratory, two points must be borne in mind. First, from the field, we could not derive the lowest β values (which apply to insensitive species present in all the field samples) because there are no or few data with F_{TOX} and f_{occ} values covering the transition from species absence to presence in the lakes. In such cases, we could only say that the β value is less or equal to some maximum; for example, *Bosmina* sp. and *Chydorus sphaericus* have $\beta \leq 0.28$ in Hannah Lake (Table 2(c)). Second, the highest β values were inaccessible to estimation because even in the most recent years the lakes remain contaminated to some degree, and so the lowest F_{TOX} values are still sufficient for toxicity towards sensitive species (Fig. 2). For these two reasons, we should expect a smaller range in the observable β values, even if the true range of field β coincides with the laboratory values.

The laboratory-based values of β estimated by Tipping et al. (2023) for 76 species had a (forced) median β of 1.00 and were approximately normally distributed with a 5th percentile of 0.17 and a 95th percentile of 2.16. For the 31 invertebrates in the laboratory data set, the median was 0.70, and the 5th and 95th percentiles were 0.17 and 2.80 respectively. Considering all the individual field-based estimates, but not the less-than values, 33 in all (Table 3), we obtain a median of 0.96, with 5th and 95th percentiles of 0.45 and 1.39. If we assume that all the field values were overestimated by 10% due to lag effects (Section 4.1.2), the median, 5th and 95th percentile values would be 0.86, 0.41 and 1.25, respectively. Therefore, with or without the allowance for possible lag-times, the field values fall within the laboratory-based range and have a similar median.

The analysis in the previous paragraph applies broadly to the properties of the β distributions, but for daphnids more direct comparisons are possible. The β values derived by Tipping et al. (2023) from laboratory test data show that daphnid species tend to have high β values, with mean values as follows; *Ceriodaphnia dubia* 1.91 (from 135 estimates), *Daphnia magna* 1.27 (540), *Daphnia obtusa* 1.44 (53), *Daphnia pulex* 1.90 (54), and *Daphnia pulicaria* 1.90 (34). For two species, *Daphnia ambigua* and *D. pulex-pulicaria*, lower values of 0.45 and 0.76 respectively were obtained from the laboratory data, but in both cases the mean was based on only two estimates; moreover, bioassay results (Celis-Salgado et al., 2016) suggest that *D. ambigua* has a sensitivity to metals comparable to several other daphnids. Overall, most of the field results for daphnids agree with the laboratory data, relatively high β values (>1.00) being obtained for *Ceriodaphnia lacustris*, *Ceriodaphnia* sp., *D. ambigua* and *D. pulex* (Table 3). Notwithstanding this agreement, it must be acknowledged that the appearance of these daphnids in the lakes may also be related to the suppression of planktivorous fish by piscivores in the later years of the record (Section 2.1).

Further insight would no doubt be obtained from the results of laboratory toxicity studies with a range of zooplankton species covering those encountered in the four lakes of this study, building on the work of Celis-Salgado et al. (2016).

4.4. Threshold differences (Δ_T)

The second parameter in the model, after β, is Δ_T which occurs in equation (3) for the description of laboratory data, and in equation (6) for field data; see Section 2.5 and Fig. 1. This part of the model covers the “intermediate zone of partial response”, within which the

variable (1 – TR) in laboratory studies, or f_{occ} in the field, increases from zero to 1.00. Before trying to compare Δ_T values for laboratory and field, we must first consider why this intermediate zone exists.

For laboratory tests, the two possibilities identified by Ashauer et al. (2015) apply. First, the intermediate zone may arise because the individuals in the test population have a distribution of tolerance thresholds, the exceedance of which leads to the measured effect (mortality or other response). Second, all individuals may share the same tolerance threshold, but the effect occurs stochastically. In both cases the incomplete response arises from differences among individuals in the population under test. However, the intermediate zone in our field observations is to do with the population of a species as a whole; in some of the six sampling visits in a given year the population is present, in others it is absent. This might reflect the fact that the population is under toxic stress but is able to survive if other factors (physical conditions, predation, competition, food supply etc) are favourable.

Therefore, the parameter Δ_T derived from field data is qualitatively different from the laboratory-based version. Moreover, in neither case is it an intrinsic property of a given species; rather it is an *ad hoc* correction for extrinsic factors. In this respect Δ_T differs from β, which, being an intrinsic property of the species, should have the same value in both laboratory and field (see Section 4.2. above). For these reasons, comparison of the field and laboratory values of Δ_T is not meaningful.

Notwithstanding the argument in the previous paragraph, the narrowness of the values of Δ_T in the field data (Tables 2 and 3), which have an average less 50% of the average for laboratory-derived values (Section 3.2), is noteworthy. It means that, in the field, zooplankton species respond relatively sharply to changes in water chemistry. Indeed, in several cases a value of zero was found for Δ_T (Table 2), meaning that the decrease in F_{TOX} over a single year was sufficient for f_{occ} to increase from zero to 1.00; in other words, a species that was completely absent from the water column in one year was present in all samples collected in the next.

4.5. Model application

Several published models have been developed to take into account bioavailability effects on the toxicities of metals in aqueous systems (see e.g. Mebane et al., 2020, and references therein), and the effects of metal mixtures have also been considered (Farley et al., 2015; Gong et al., 2020). An original aim of WHAM-F_{TOX} was to contribute to this effort (Tipping & Lofts

2013, 2015), and thereby attempt to improve the risk assessment and regulation of metals. However, our aim in developing and testing the newer model, WHAM-$F_{TOX}\beta$, was to describe the effects of acidification and metal contamination at the ecosystem scale. The central aspect is the separation within the model of the intrinsic toxicities of the proton and metals, through the parameter α, and the intrinsic sensitivities of different species to the toxic cations, through the parameter β. The results of the present work add credence to the model, by showing that in the Sudbury lakes a good number of individual species behave approximately as expected, after suitable optimisation of β and Δ_T.

The modelling approach of WHAM-$F_{TOX}\beta$ is admittedly approximate, as already demonstrated in its application to laboratory data (Tipping et al., 2023), but it has distinct advantages for the description of ecosystem response to toxic cation contamination. Thus, in its simplest application, the model can combine α values, derived from laboratory data, with an assumed or estimated distribution of β values, to predict species richness; this has been demonstrated in an exploratory study on the Sudbury lakes zooplankton (Tipping et al., 2021). But use of the model is by no means restricted to zooplankton in lakes; for example, earlier work (Stockdale et al., 2010; 2014a) suggests that toxicity towards river invertebrates might also be modelled by this approach. These applications could aid both the understanding the effects of the toxic cations in the field, and the prediction of how those effects might respond to changes in levels of contamination, for example assisting lake and river management. Further developments might involve the combination of WHAM-$F_{TOX}\beta$ with ecological modelling, to assess the combined influences of contamination and other factors on species richness.

5. Summary and conclusions

We can now answer the three questions posed in the Introduction.

(1) Is the null hypothesis followed? For most lake-species pairs, the WHAM-$F_{TOX}\beta$ model can fit field data via the variable F_{TOX}. This is consistent with the null hypothesis that a species is unable to survive under conditions that are too toxic, then it establishes and persists as toxicity declines (Type A behaviour). But a significant minority of pairs follow Type B behaviour which can be considered as Type A behaviour initially, followed by the replacement of the species in question by more ecologically fit competitors. Another minority comprises insensitive species with low β values, that do not respond under the observed conditions, because they are already able to survive at the start of the monitoring period; but such behaviour does not contradict the hypothesis.

(2) Are β values estimated from field data similar to laboratory-based values? Our results suggest that in terms of both magnitude and range, the β values derived from field data are reasonably consistent with values from laboratory data, either for invertebrates only, or for all taxa (invertebrates, vertebrates, plants). More direct comparisons for daphnid species are largely in agreement; the β values tend to be relatively high, since most daphnids are sensitive to toxic cations.

(3) Are differences between the upper and lower thresholds ($F_{TOX,UT}$ - $F_{TOX,LT}$, or Δ_T) estimated from field data similar to laboratory-based values? We conclude that this question is not meaningful, since the threshold differences occur for different reasons in the two situations (Section 4.4). Moreover, although a value of Δ_T is needed to fit data, it is not intrinsic to a species in the way that β is. The results show that many lake zooplankton species responded over small ranges of F_{TOX}, and in several cases a change in water chemistry from one year to the next was sufficient (Table 4).

We conclude that the findings support the application of WHAM-$F_{TOX}\beta$ to the simulation and prediction of the toxic effects of mixtures of cations in the field, and this might be useful in lake and river management. There is also potential to combine the toxicity modelling reported here with ecological theory to better explain natural population responses.

Data availability

Data will be made available on request.

Acknowledgements

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi: ( ).

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Table 1. Glossary of abbreviations

Abbreviation	**Variable**
α_H α_M	Toxicity coefficients of H^+ and metals
α_M*	Metal toxicity coefficient at infinite time
β	Constant that defines the sensitivity of a species to toxic effects
Δ_T	$F_{TOX,UT} - F_{TOX,LT}$
DOC	Dissolved organic carbon
FA	Fulvic acid
f_{occ}	Fractional occurrence of species in a given year
F_{TOX}	Toxicity variable (equation 1)
$F_{TOX,LT}$ $F_{TOX,UT}$	Lower and upper thresholds of F_{TOX} (equations 2 - 4, 5 - 7)
HA	Humic acid
m	Slope of a plot of observed vs predicted f_{occ}

$v_{HA,H}$, $v_{HA,M}$	Moles of H^+ and metal bound per gHA
p	Probability of significance (* p<0.05, ** p < 0.01, *** p < 0.001)
θ_H, θ_M	Fractional site occupancies of binding sites
TR	Toxic response (varies from 0 to 1); equations 2 - 4

Table 2. Results of data-fitting. NA fitting not applicable; NS fitting not significant; n_{occ} total number of occurrences.

(a) Clearwater

Species	Type	n_{occ}	β	Δ_T	m	p
Acanthocyclops vernalis	B	49	≤0.74	NA	NA	NA
Bosmina sp.	A	197	≤0.74	NA	NA	NA
Chydorus sphaericus	B	99	≤0.74	NA	NA	NA
Daphnia mendotae	A	27	1.35	0.18	0.86	***
Diaphanosoma birgei	B	84	0.96	0.21	0.89	***
Epischura lacustris	A	52	1.13	0.00	0.94	***
Eubosmina longispina	B	21	1.14	0.08	0.98	***
Holopedium gibberum	A	125	0.91	0.00	0.94	***
Leptodiaptomus minutus	A	157	0.83	0.13	1.07	***
Polyphemus pediculus	B	38	NS	NS	NS	NS
Sida crystallina	B	31	NS	NS	NS	NS
Tropocyclops extensus	A	101	0.95	0.07	0.89	***

(b) Lohi

Species	Type	n_{occ}	β	Δ_T	m	p
Acanthocyclops vernalis	B	31	≤0.36	NA	NA	NA
Bosmina sp.	A	221	≤0.36	NA	NA	NA
Chydorus sphaericus	B	69	≤0.36	NA	NA	NA
Daphnia pulex	A	30	1.44	0.36	1.00	***
Diaphanosoma birgei	A	156	0.82	0.67	1.01	*
Epischura lacustris	A	58	1.23	0.13	1.00	***
Holopedium gibberum	A	126	0.96	0.11	0.90	***
Leptodiaptomus minutus	A	199	≤0.36	NA	NA	NA
Orthocyclops modestus	A	60	1.27	0.57	0.99	***
Tropocyclops extensus	A	107	1.00	0.01	0.81	***

(c) Hannah

Species	Type	n_{occ}	β	Δ_T	m	p
Acanthocyclops vernalis	B	25	NS	NS	NS	NS
Bosmina sp.	A	181	≤0.28	NA	NA	NA
Ceriodaphnia lacustris	B	22	1.19	0.32	1.01	***
Ceriodaphnia sp.	A	68	1.30	0.00	0.96	***
Chydorus sphaericus	B	123	≤0.28	NA	NA	NA
Daphnia ambigua	A	29	1.54	0.25	0.98	***
Daphnia mendotae	B	122	0.82	0.33	0.94	***
Diaphanosoma birgei	A	112	0.89	0.51	0.81	***
Epischura lacustris	A	57	1.35	0.00	0.90	***
Leptodiaptomus minutus	A	167	0.50	0.42	0.95	***
Mesocyclops edax	B	93	1.05	0.33	1.00	***
Skistodiaptomus oregonensis	A	102	1.06	0.34	0.91	***
Tropocyclops extensus	A	118	0.94	0.28	0.90	***

(d) Middle

Species	Type	n_{occ}	β	Δ_T	m	p
Acanthocyclops vernalis	B	29	≤0.19	NA	NA	NA
Bosmina sp.	A	197	0.21	0.94	0.97	***
Chydorus sphaericus	B	114	≤0.35	NA	NA	NA
Daphnia mendotae	A	129	0.67	0.24	0.90	***
Diacyclops bicuspidatus thomasi	A	124	0.75	0.05	0.93	***
Diaphanosoma birgei	A	102	0.85	0.78	1.01	***
Epischura lacustris	A	74	1.16	0.21	1.00	***
Leptodiaptomus minutus	A	182	0.38	0.00	0.94	***
Mesocyclops edax	B	71	1.08	0.31	1.00	***
Orthocyclops modestus	B	25	0.69	0.92	0.95	*
Skistodiaptomus oregonensis	A	108	0.89	0.32	0.94	***
Tropocyclops extensus	A	90	0.94	0.31	0.85	***

Table 3. Values of β by species for the four lakes.

Species	Clearwater	Lohi	Hannah	Middle	range / mean
Acanthocyclops vernalis	≤0.74	≤0.36		≤0.19	
Bosmina sp.	≤0.74	≤0.36	≤0.28	0.21	
Ceriodaphnia lacustris			1.19		
Ceriodaphnia sp.			1.30		
Chydorus sphaericus	≤0.74	≤0.36	≤0.28	≤0.19	
Daphnia ambigua			1.54		
Daphnia mendotae	1.35		0.82	0.67	0.72
Daphnia pulex		1.44			
Diacyclops bicuspidatus thomasi				0.75	
Diaphanosoma birgei	0.96	0.82	0.89	0.85	0.16
Epischura lacustris	1.13	1.23	1.33	1.16	0.16
Eubosmina longispina	1.14				
Holopedium gibberum	0.91	0.96			0.05
Leptodiaptomus minutus	0.82	≤0.36	0.50	0.38	0.78
Mesocyclops edax			1.05	1.08	0.03
Orthocyclops modestus		1.27		0.69	0.59
Skistodiaptomus oregonensis			1.06	0.89	0.17
Tropocyclops extensus	0.95	1.00	0.94	0.94	0.06

Table 4. Values of Δ_T by species for the four lakes.

Species	Clearwater	Lohi	Hannah	Middle
Bosmina sp.				0.94
Ceriodaphnia lacustris			0.32	
Ceriodaphnia sp.			0.00	
Daphnia ambigua			0.25	
Daphnia mendotae	0.18		0.33	0.24
Daphnia pulex		0.36		
Diacyclops bicuspidatus thomasi				0.05
Diaphanosoma birgei	0.21	0.67	0.51	0.78
Epischura lacustris	0.00	0.13	0.00	0.21
Eubosmina longispina	0.08			
Holopedium gibberum	0.00	0.11		
Leptodiaptomus minutus	0.13		0.42	0.00
Mesocyclops edax			0.33	0.31
Orthocyclops modestus		0.57		0.92
Skistodiaptomus oregonensis			0.34	0.32
Tropocyclops extensus	0.07	0.01	0.28	0.31

Figure captions

Fig. 1. Schematic of the application of WHAM-$F_{TOX}\beta$.

Fig. 2. Values of F_{TOX} over time for the four lakes, for β = 0.25, 1.00 and 1.75. For reference, the dashed line shows the values of $F_{TOX,50}$ (0.82); see Section 2.5. Note the different scales on the y-axis.

Fig. 3. Variations of f_{occ} with time (upper panels for each species) and F_{TOX} (lower panels), for selected Type A species-lake pairs. The points are observed values and the lines are model fits. In the lower plots (F_{TOX} on the x-axis), the direction of time is generally from right to left. The remaining fitted Type A results are shown in Fig. S4.

Fig. 4. Variations of f_{occ} with time (upper panels for each species) and F_{TOX} (lower panels), for Type B species-lake pairs. The points are observed values and the lines are model fits; note that fitting was done only for the results over the period during which f_{OCC} increased to a maximum, indicated by the extents of the fitted lines. In the lower plots (F_{TOX} on the x-axis), the direction of time is generally from right to left.

Fig. 5. Variations of f_{occ} with time for insensitive Type A species that could not be fitted owing to the lack of data at high F_{TOX} (see Section 3.2).

Fig. 6. Variations of f_{occ} with time for insensitive Type B species that could not be fitted owing to the lack of data at high F_{TOX} (see Section 3.2).

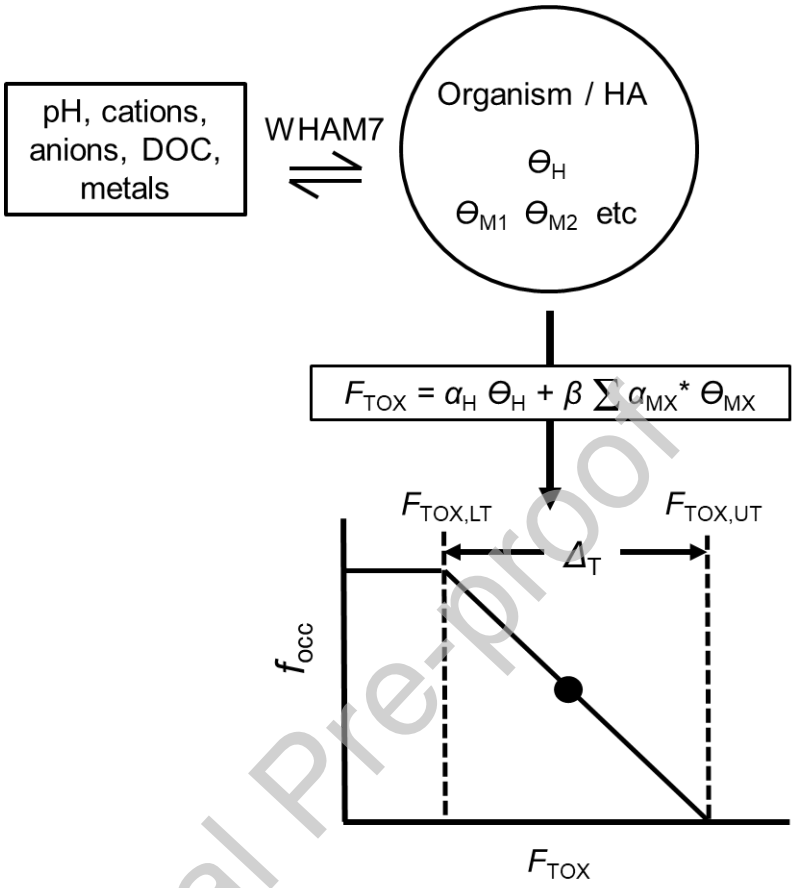


Fig. 1

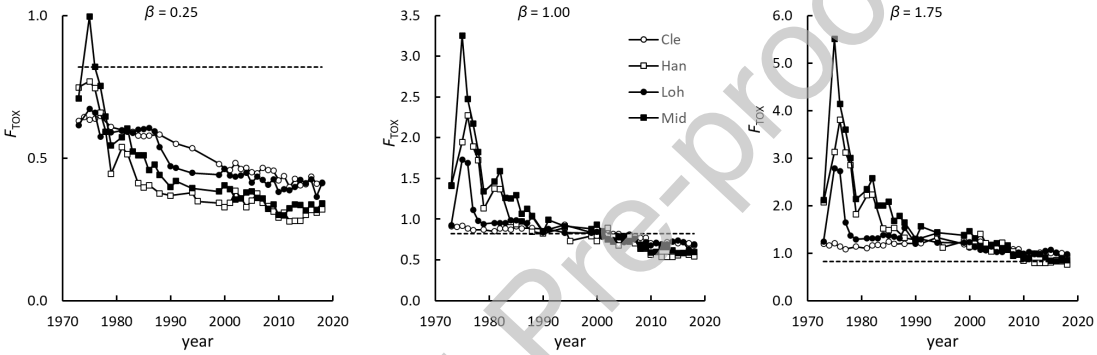
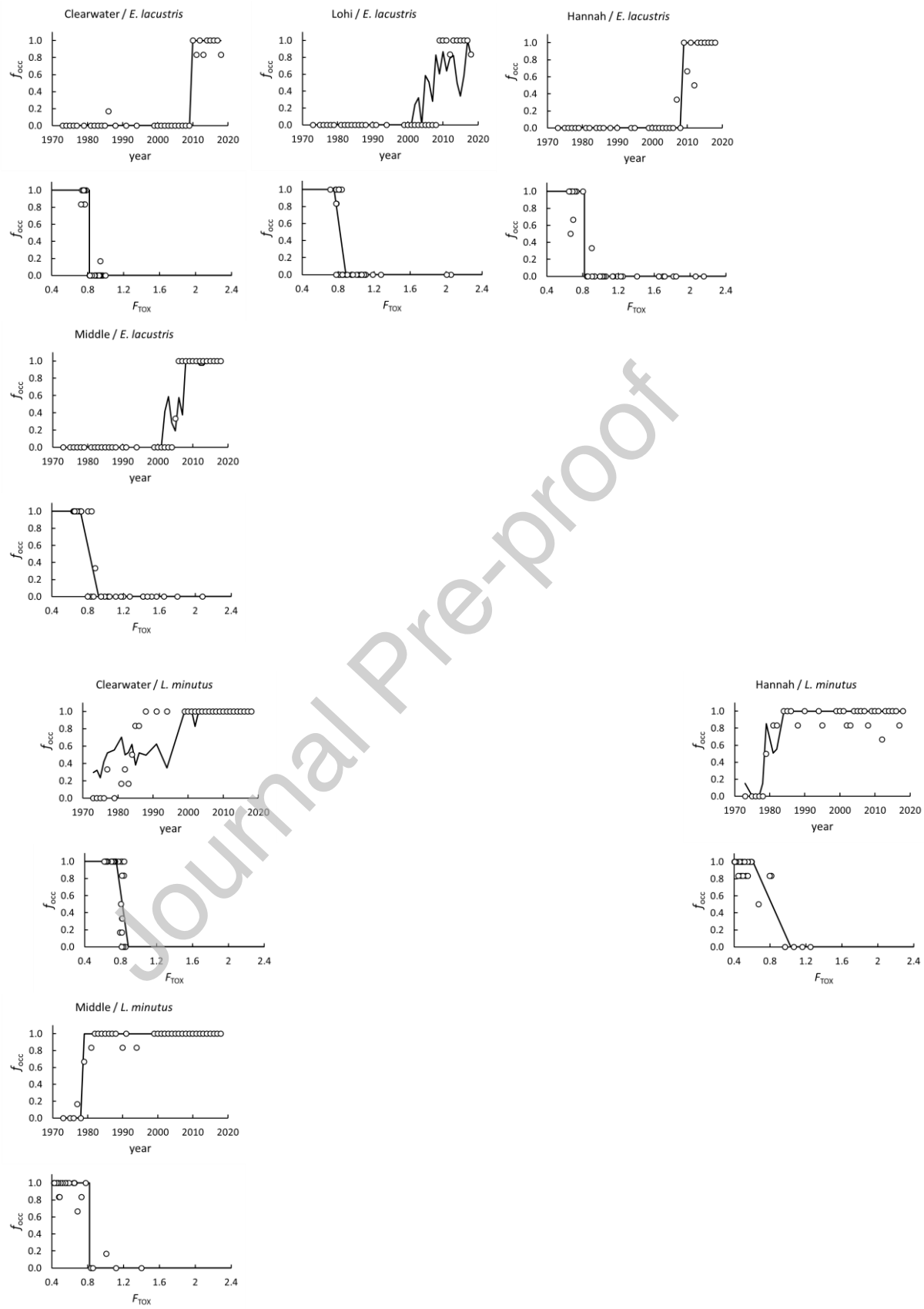


Fig.2



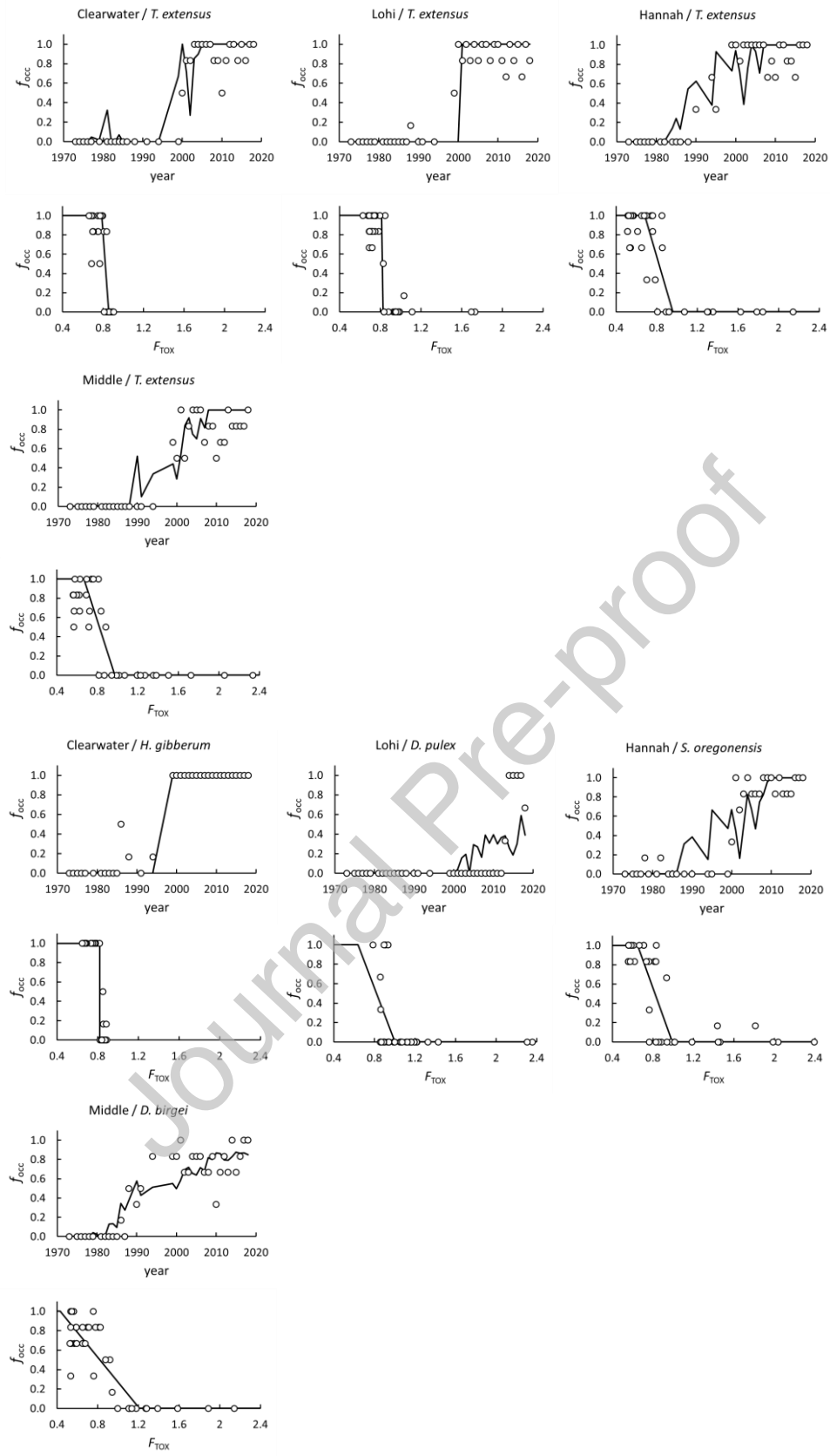


Fig. 3

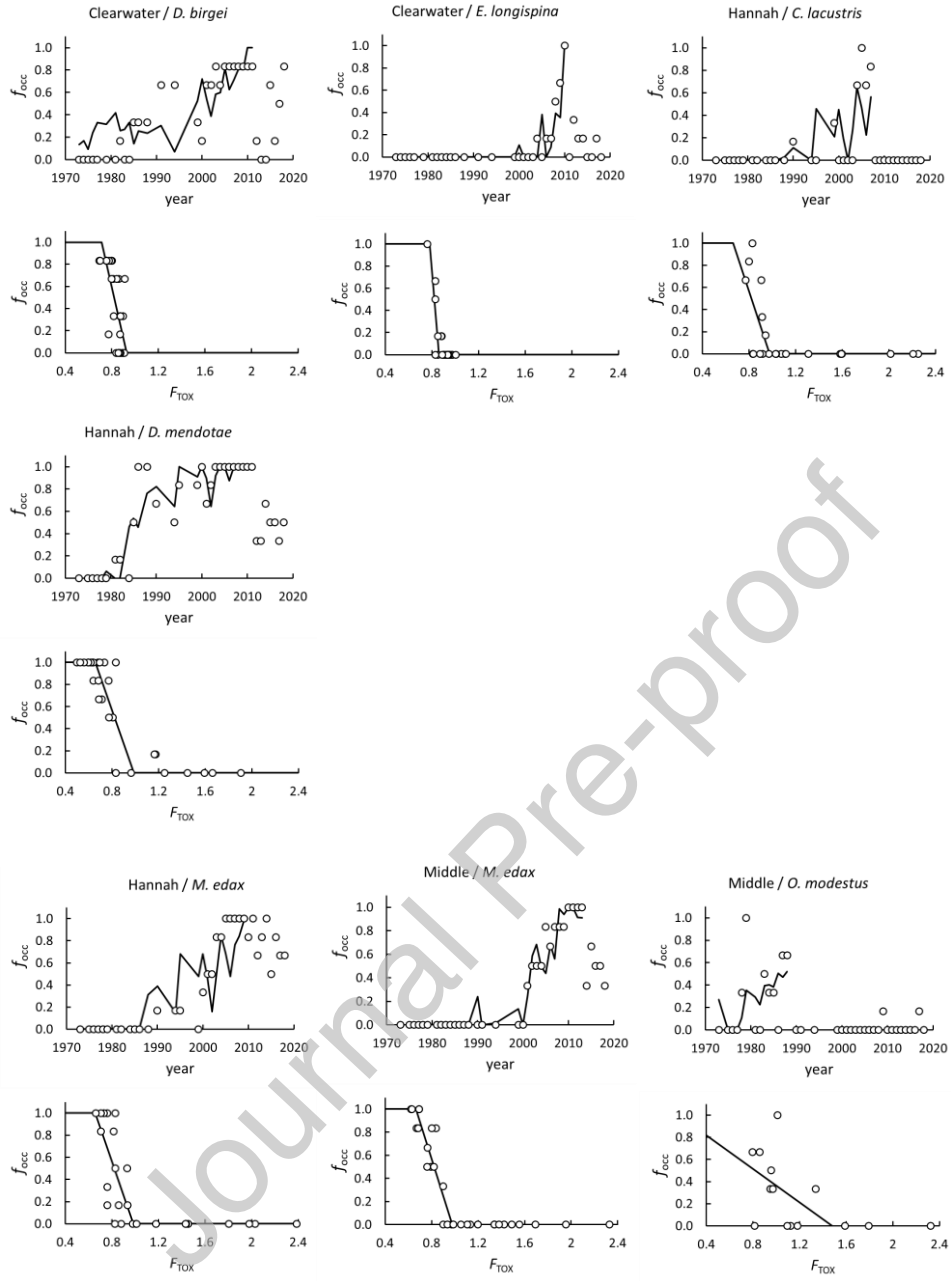


Fig. 4

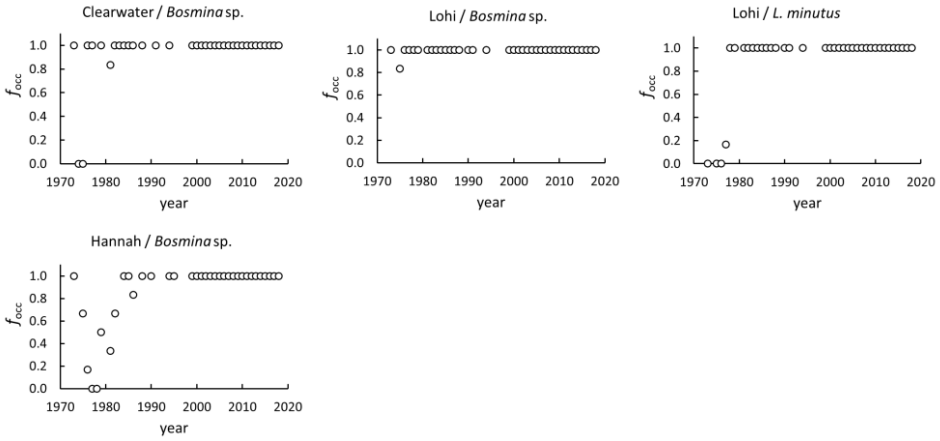


Fig. 5

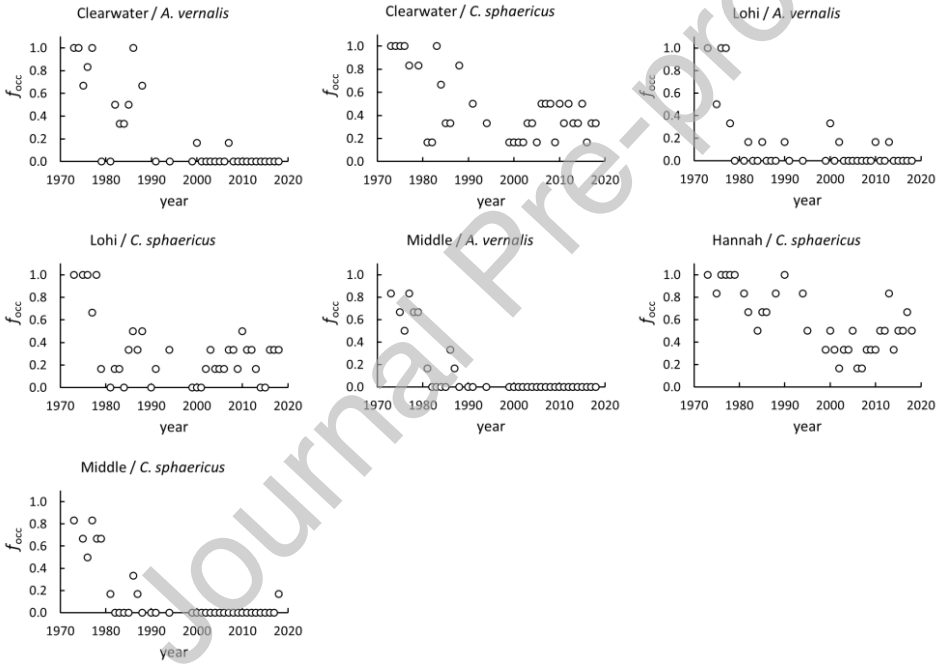


Fig. 6

CRediT authorship contribution statement

ET designed the study, wrote the draft manuscript, performed data analysis, and interpreted the data. SL edited the manuscript, performed data analysis, and interpreted the data. WK wrote parts of the manuscript, edited the manuscript and interpreted the data. BAE collated the data, wrote parts of the manuscript, edited the manuscript and interpreted the data.

Declaration of Competing Interest

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