The use of WHAM-F_{TOX}, parameterized with laboratory data, to simulate zooplankton species richness in acid- and metal- contaminated lakes

5   E Tipping[a]*, S Lofts[a], W Keller[c]

6   *[a] UK Centre for Ecology and Hydrology, Lancaster Environment Centre, Lancaster, LA1 4AP,*

7   *United Kingdom; [b] Cooperative Freshwater Ecology Unit, Laurentian University, Sudbury,*

8   *Ontario, P3E 2C6, Canada*

9

10   *Corresponding author: Dr Edward Tipping

11   Centre for Ecology & Hydrology

12   Lancaster Environment Centre

13   Lancaster

14   LA1 4AP

15   United Kingdom

16   et@ceh.ac.uk

17

18   Declarations of interest: none

19 **Abstract**

20 The WHAM-F_{TOX} model quantifies cation toxicity towards freshwater organisms, assuming

21 an additive toxic response to the amounts of protons and metals accumulated by an organism.

22 We combined a parameterization of the model, using data from multi-species laboratory

23 toxicity tests, with a fitted field species sensitivity distribution, to simulate the species richness

24 (n_{sp}) of crustacean zooplankton in acid- and metal-contaminated lakes near Sudbury, Ontario

25 over several decades, and also in reference (uncontaminated) lakes. A good description of

26 variation in toxic response among the zooplankton species was achieved with a log-normal

27 distribution of a new parameter, β, which characterizes an organism's intrinsic sensitivity

28 towards toxic cations; the greater is β, the more sensitive is the species. The use of β assumes

29 that while species vary in their sensitivity, the relative toxicities of different metals are the same

30 for each species (common relative sensitivity). Unbiased agreements between simulated and

31 observed n_{sp} were obtained with a high correlation ($r^2 = 0.81$, $p < 0.0001$, $n = 217$). Variations

32 in zooplankton species richness in the Sudbury lakes are calculated to be dominated by toxic

33 responses to H, Al, Cu and Ni, with a small contribution from Zn, and negligible effects of Cd,

34 Hg and Pb. According to the model, some of the Sudbury lakes were affected predominantly

35 by acidification (H and Al), while others were most influenced by toxic heavy metals (Ni, Cu,

36 Zn); for lakes in the latter category, the relative importance of heavy metals, compared to H

37 and Al, has increased over time. The results suggest that, if common relative sensitivity

38 operates, n_{sp} can be modelled on the basis of a single set of parameters characterizing the

39 average toxic effects of different cations, together with a species sensitivity distribution.

1. Introduction

The quantitative prediction of the responses of freshwater ecosystems to the effects of potentially toxic metals and acidification is a continuing challenge in ecotoxicology. Most effort has been devoted to risk assessment for environmental protection. For metals, environmental quality standards have been based upon the results of laboratory toxicity testing for individual metals (Mance, 1987; Cairns and Mount, 1990), with recent major progress in taking bioavailability into account (Erickson et al., 1996) notably using Biotic Ligand Models (BLMs) (Di Toro et al., 2001, Paquin et al., 2002, Ardestani et al., 2015). In the case of acidification (in the implied absence of toxic metals other than aluminium), the most widely applied criterion is simply alkalinity (Sverdrup and De Vries, 1994; Henricksen and Posch, 2001).

Risk assessment aims to set acceptable limits for metal and acidity levels, but a more difficult task is to account for actual ecosystem responses to contamination. In the first place, it requires toxicity models that can deal with mixture effects. Examples of these are modified BLMs (Playle, 2004; Santore and Ryan, 2015), WHAM-F_{TOX} (Tipping and Lofts, 2013, 2015), and related models (Balistrieri et al., 2015). In this work we used WHAM-F_{TOX}, which is based upon the relationship between metal body burdens and toxicity (Meador, 2006; Borgmann et al., 2008). It estimates the "metabolically active" proton and metal body burden by assuming that the cation-binding sites of humic acid (HA) are a proxy for those of the toxicity-responsive biomolecules of the organism in question. The toxic effect of each cation is the product of its fractional occupancy of the binding sites and its toxicity coefficient (α), and the sum of the products is equal to F_{TOX}, which quantifies the total toxicity. The WHAM speciation code (UK CEH, 2020) is used to calculate cation binding, assuming equilibrium with the bathing solution. The model has provided reasonable fits of laboratory toxicity data (Tipping and Lofts, 2013, 2015), and the approach is supported by field evidence; measured metal (Al, Ni, Cu, Zn, Cd, Pb) contents of stream bryophytes (Tipping et al., 2008), and macroinvertebrates (Stockdale et al., 2010; Tipping and Lofts, 2013; De Jonge et al., 2014) are correlated with WHAM-calculated loadings of HA.

Secondly, an appropriate field variable has to be simulated. An obvious one is species richness (n_{sp}), the number of species of a chosen taxon in a given water. As well as being a fundamental ecological univariate measure, richness has been widely used to demonstrate the toxic effects of contaminants in the field (Rainbow, 2018). There have been several studies in which the variable n_{sp} has been related to water chemistry, in relation to metals and acidity.

73   Macroinvertebrate species richness in mountain streams affected by metal mining was

74   measured by Clements et al. (2000), and shown to be interpretable using the cumulative

75   criterion unit, defined as the ratio of the instream metal concentration to the U.S. Environmental

76   Protection Agency criterion concentration for freshwater aquatic organisms (based on

77   laboratory tests), summed for all metals measured. Khan et al. (2012) demonstrated correlations

78   between zooplankton species richness in acid- and metal-damaged lakes near Sudbury, Ontario

79   and toxicity endpoints from laboratory studies with *Daphnia magna*, using toxic units to

80   quantify metal mixture effects, and including BLM applications. Stockdale et al. (2010,

81   2014a,b) fitted WHAM-F_{TOX} to field n_{sp} data for stream invertebrates and lake zooplankton,

82   using quantile regression to allow for additional variables, other than toxic cations, that might

83   affect richness. Balistrieri et al. (2015) applied models based on the WHAM-F_{TOX} approach to

84   stream invertebrate and lake zooplankton species richness.

85   In all of these studies, n_{sp} was used to summarize ecosystem response to contamination,

86   covering all species together. But to be more precise, the observed n_{sp} arises because individual

87   species respond differently to the toxic cations; the greater is the combined toxic stress in a

88   given water, the fewer species can survive, and the smaller is n_{sp}. Therefore, in the present

89   work, we explored whether field data could be simulated by considering variations in

90   sensitivity among species.

91   Our modelling approach assumes that all species in a given water accumulate protons and

92   metals to the same extent, and that the relative toxicities of the cations are the same for each

93   species. However, the species are assumed to differ in their intrinsic toxic sensitivity; some

94   respond strongly to all cations, others weakly to all cations. This gives rise to a distribution of

95   species sensitivities, and thereby to variations in n_{sp} with water chemistry. Here, the relative

96   responses of species to different cations were taken from the results of a meta-analysis (Tipping

97   et al., 2019) in which WHAM-F_{TOX} was used to analyse data from c. 2000 toxicity tests,

98   thereby providing averaged, and presumably representative, values of the toxicity coefficient

99   α for different metals. We introduced a new model parameter, β, that characterizes the

100   sensitivity of each species (see Section 2.3). The aims of the study were first to determine

101   whether the distribution of β values could be optimized so that the model could match field n_{sp}

102   data, and second to use the results to interpret changes in n_{sp} in response to changing water

103   chemistry.

104   We conducted our analysis using crustacean zooplankton species richness data, and water

105   chemistry data, for nine lakes near Sudbury Ontario, which have been affected for more than

100 years by atmospherically-deposited acidity and metals, and for a number of uncontaminated reference lakes. Changes in lake water chemistry and species richness, as the Sudbury lakes recovered from initially high contaminating inputs, have been comprehensively monitored over several decades (Keller and Yan, 1991; Keller et al. 2019). The large data set arising from this sustained fieldwork provides an excellent test of the modelling approach. It cannot be assumed that zooplankton species richness in the lakes is determined only by the effects of toxic cations (protons and metals); other likely factors include changes in climate and calcium concentrations (Keller et al., 2019) and fish predation (Yan et al., 2016). Nonetheless, the high degree of contamination in these lakes means that such toxicity is a dominating factor, so the events at Sudbury come close to a "field ecotoxicity experiment". An advantage of using lake data is that they vary relatively slowly over time, owing to the large buffering volume of lakewater in comparison to inlet and outlet volumes, so that annual average water chemistry provides a good estimate of toxicant exposure.

119   **2. Methods**

120   *2.1 Field data*

121   Emissions from metal smelters at Sudbury, northeastern Ontario, caused contamination of

122   surrounding lakes by acid deposition, while lakes closest to the smelters were also

123   contaminated with metals, mainly Ni and Cu, and to lesser extents Zn, Cd and Pb. Reductions

124   in emissions, which were started in the early 1970s, together with experimental neutralization

125   in some cases, led to chemical and biological improvements (Keller et al., 2007, 2019). We

126   used annually averaged chemistry and zooplankton richness data from 9 Sudbury lakes that

127   had been monitored, approximately monthly during the open-water period, between 1973 and

128   2006, to obtain a total of 171 matched individual lake/year data points. We also employed data

129   from 23 reference lakes in northeastern Ontario, each sampled once in the midsummers of 2003

130   and 2005, making a total of 217 data points for the analysis.

131   The water chemistry data are provided in Table S1. Temporal changes varied among the lakes,

132   but generally the concentrations of Ca, SO_4 and trace metals (Ni-Cu-Zn) declined over the

133   period of our data, while pH and DOC concentration increased. In four of the lakes (Clearwater,

134   Hannah, Lohi and Middle) there were appreciable increases in Na and Cl concentrations, due

135   to road salting, while in the other five, concentrations of these solutes remained steady.

136   Values of n_{sp} for zooplankton in the 9 Sudbury lakes ranged from 1.2 to 12.7, and in the

137   reference lakes from 8 to 16 (Table S1). Further information about the biological and chemical

138   variations in the Sudbury lakes is available in Keller et al. (2019), and references therein.

139   *2.2. Speciation calculations*

140   We used WHAM7 (Tipping et al., 2011; UK CEH, 2020) to calculate lakewater chemical

141   speciation, taking into account the competitive complexation of major and trace metals with

142   inorganic ligands and dissolved organic matter. The measured data refer to unfiltered samples

143   and therefore contain some suspended particulate matter (SPM, including zooplankton) with

144   associated metals. However, the concentrations of SPM in these lakes are low, < 1 mg L^{-1} on

145   average, and therefore we could assume that the data referred to dissolved chemical species;

146   this assumption is justified in more detail in the Supplementary Information. As in previous

147   work (Tipping et al., 2008; Stockdale et al., 2010) we attributed DOC to fulvic acid (FA), with

148   the standard conversion [FA] (g L^{-1}) = 1.3 [DOC] (g L^{-1}), where square brackets indicate

149   concentrations. We assumed that measured Al concentrations represented truly dissolved metal

150 (in inorganic forms and complexed with dissolved organic matter), and that Fe(III)

151 concentrations were controlled by equilibrium with $Fe(OH)_3$ (Lofts et al., 2008). We assumed

152 a temperature of $10^{\circ}C$ for all calculations.

153 The key variables characterizing the exposure of organisms to cations are $v_{HA,H}$ and $v_{HA,M}$ (mol

154 gHA^{-1}), the amounts of protons and metals bound to humic acid (HA) in equilibrium with the

155 lakewater solutions; the values of $v_{HA,H}$ and $v_{HA,M}$ are employed in the WHAM-F_{TOX} model

156 calculations, described in Section 2.3. On the assumption that the measured water chemistries

157 represent dissolved concentrations (see above), the proton and metal contents of the organisms

158 themselves were considered negligible (see Supplementary Information), and therefore in order

159 to compute $v_{HA,H}$ and $v_{HA,M}$ we included HA in the calculation inputs at a concentration (10^{-9} g

160 L^{-1}), sufficiently low that the solution speciation calculation would be unaffected.

2.3. WHAM-F_{TOX} theory

162 The WHAM-F_{TOX} model, recently slightly modified (Tipping et al., 2019), is based on the

163 assumptions (a) that the toxic effects of protons and metal cations are additively related to their

164 occupancies of binding sites possessed by biological organisms, and (b) that those binding sites

165 can be represented by the binding sites of humic acid (HA). This enables the WHAM chemical

166 speciation code to be used to calculate proton and metal binding by living organisms, taking

167 into account competition effects, including the influence of pH.

168 The fractional site occupancies (θ_H and θ_M) of the biological binding sites are assumed to be

169 the same as those of HA, obtained by dividing the $v_{HA,H}$ and $v_{HA,M}$ values (Section 2.2) by the

170 HA content of proton-dissociating groups (5.1×10^{-3} mol g^{-1}). Values of θ_H and θ_M are

171 dimensionless, and can vary from zero to unity.

172 The key variable in the model is F_{TOX}, defined by the equation

173 $$F_{TOX} = \alpha_H\theta_H + \Sigma\ \alpha_M\theta_M \qquad (1)$$

174 where α_H and α_M are toxicity coefficients (dimensionless) for protons and metals, and the

175 summation is over all toxic metals present in the bathing solution. Values of α_M depend upon

176 the exposure time employed in a toxicity experiment. Here, we use values that apply to infinite

177 exposure time, $\alpha_{M,max}$, estimated by extrapolation (Tipping et al., 2019) (Table 1).

178 The toxic response (TR) depends upon lower and upper thresholds (LT and UT) of F_{TOX},

179 between which TR increases linearly from zero to unity. Thus

180 $$F_{TOX} \leq F_{TOX,LT} \qquad TR = 0 \qquad \qquad (2)$$

181 $$F_{TOX,LT} < F_{TOX} < F_{TOX,UT} \qquad TR = (F_{TOX} - F_{TOX,LT}) / (F_{TOX,UT} - F_{TOX,LT}) \qquad (3)$$

182 $$F_{TOX} \geq F_{TOX,UT} \qquad TR = 1 \qquad \qquad (4)$$

183 From fitting laboratory toxicity data (Tipping and Lofts, 2013, 2015; Tipping et al., 2019),

184 average values of $F_{TOX,LT}$ and $F_{TOX,UT}$ of 0.446 and 1.170 respectively have been derived, and

185 these are used in the present work.

186 Here, we extend the model for application to a system with a number of different biological

187 species, as in the lakes of the present study. This requires values of $\alpha_{M,max}$ for each species i,

188 expressed as

189 $$\alpha_{M,max,i} = \beta_i \, \alpha_{M,max,mean} \qquad \qquad (5)$$

190 where $\alpha_{M,max,mean}$ is the average value of $\alpha_{M,max}$ over all species, and β_i (dimensionless) is a

191 metal-independent constant characterizing the sensitivity of each species towards toxic cations.

192 In the absence of values of $\alpha_{M,max}$ for the zooplankton species that occur in the study lakes, we

193 made the approximation that the $\alpha_{M,max,mean}$ values for the zooplankton are equal to those

194 derived by Tipping et al. (2019) in a meta-analysis of data for laboratory test species (Table 1);

195 these test data covered all major taxa (invertebrates, plants, vertebrates).

196 Equation (5) means that the relative values of $\alpha_{M,max}$ for different metals are the same for each

197 species; the species differ simply according to their β values. For example, from the values of

198 $\alpha_{Al,max,mean}$, $\alpha_{Ni,max,mean}$, $\alpha_{Cu,max,mean}$, and $\alpha_{Zn,max,mean}$ of 2.6, 31.1, 34.6, and 17.0 respectively

199 (Table 1), a species with $\beta = 0.5$ has $\alpha_{Al,max} = 1.3$, $\alpha_{Ni,max} = 15.6$, $\alpha_{Cu,max} = 17.3$, $\alpha_{Zn,max} = 8.5$,

200 while another species with $\beta = 2.0$ has $\alpha_{Al,max} = 5.2$, $\alpha_{Ni,max} = 62.2$, $\alpha_{Cu,max} = 69.2$, $\alpha_{Zn,max} = 34.0$.

201 The proportions of the $\alpha_{M,max}$ values are 1 : 12.0 : 13.3 : 6.5 for both species. We refer to this

202 behaviour as common relative sensitivity.

203 From equations (1) and (5), the value of F_{TOX} for species i is given by

204 $$F_{TOX,i} = \alpha_H \theta_H + \beta_i \, \Sigma \, \alpha_{M,max,mean} \, \theta_M \qquad \qquad (6)$$

205 In a given water, all biological species possess the same values of θ_H and θ_M, but their

206 sensitivities to the toxic cations vary through β_i. The greater is β_i, the more sensitive is the

207 species to metals, since this gives greater $\alpha_{M,max,i}$ values from equation (5). It should be noted

208 that the leading term in equation (6), $\alpha_H \theta_H$, is not affected by the value of β_i. This is because,

209 thus far in the model development, α_H has been fixed at unity for all organisms, owing to the

210 lack of toxicity testing data for H^+ alone.

211 The model assumes that, in the absence of toxic effects, the number of species present in a lake

212 during the summer period is $n_{sp,max}$, which is an integer. The model species are not equated

213 with real, identified, species; they simply represent the range of sensitivities to toxic cations

214 possessed by real species. The model species have values of β equal to β_1, β_2, β_3, etc, up to

215 $\beta_{nsp,max}$.

216 Taking a probabilistic approach to the presence or absence of an individual species, values of

217 $F_{TOX,i}$ are computed from equation (6), and compared with $F_{TOX,LT}$ and $F_{TOX,UT}$ to obtain the

218 probability of the model species being present (Pr_i) according to the following relationships

219 $\qquad F_{TOX,i} < F_{TOX,LT} \qquad\qquad Pr_i = 1 \qquad\qquad\qquad\qquad\qquad\qquad (7)$

220 $\qquad F_{TOX,LT} < F_{TOX,i} < F_{TOX,UT} \qquad Pr_i = (F_{TOX,UT} - F_{TOX,i}) / (F_{TOX,UT} - F_{TOX,LT}) \qquad (8)$

221 $\qquad F_{TOX,i} > F_{TOX,UT} \qquad\qquad Pr_i = 0 \qquad\qquad\qquad\qquad\qquad\qquad (9)$

222 The value of n_{sp} is obtained by the summing the Pr_i values over all the possible model species.

223 The results are non-integral because, between $F_{TOX,LT}$ and $F_{TOX,UT}$, the model produces

224 probabilities of presence, rather than binary presence/absence.

225 Alternatively, a simplified calculation can be performed in which the average of $F_{TOX,LT}$ and

226 $F_{TOX,UT}$ is taken as a single cut-off, below which the species is present and above which it is

227 absent. This produces integral values of n_{sp}.

228 *2.4. Data fitting and statistics*

229 We used Microsoft Excel to perform statistical computations, calculate percentiles of normal

230 distributions, and carry out optimizations using the Solver function.

231 We set $n_{sp,max}$ to 13 for all the lakes, based on the 95%ile of the data set, and the choice of the

232 same value (rounded) by Khan et al. (2012) in their study of these lakes.

233 For data fitting, we chose to minimize deviations in $\log_{10} n_{sp}$, so as to give due weight to results

234 at low n_{sp}. Ideally, the fitted distribution of β should give a small root-mean-square deviation

235 (RMSD) in $\log_{10} n_{sp}$, and the slopes of the linear and logarithmic plots of observed vs calculated

236 n_{sp} should be unity. We fitted the field data to the probabilistic version of the model (equations

237 7 – 9), using the generalized reduced gradient nonlinear algorithm in Solver, and to the single

238 cut-off version using the evolutionary algorithm. We tried several two-parameter distributions

239 to fit the field data, assuming the β values to be evenly-distributed in all cases. We also fitted

240 all 13 β values individually.

3. Results

Using the probabilistic version of the model, the best choice of distribution for β was log-normal, with an unconstrained average value (Table 2). The fitted mean and standard deviation were -0.117 and 0.483 respectively. The derived distribution is shown in Figure 1. Panels (a) and (b) of Figure 2 show that unbiased fits were achieved with high statistical significance; for the logarithmic regression $r^2 = 0.81$, $p < 0.0001$; for the linear regression $r^2 = 0.84$, $p < 0.0001$. The probabilistic model was also fitted by optimizing all 13 β values; this gave similar goodness-of-fit results (Table 2), and a distribution similar to the two-parameter log-normal distribution (Figure 1).

Application of the single cut-off version of the model led to a similar log-normal distribution, with fitted mean and standard deviation values of -0.098 and 0.519 respectively (Figure 1). Again, unbiased fits were obtained (Figure 2, panels (c) and (d)), with high correlations (logarithmic $r^2 = 0.81$, $p < 0.0001$; linear $r^2 = 0.84$, $p < 0.0001$). The fitting statistics were marginally poorer than those for the probabilistic log-normal fit (Table 2).

The importance of the different metals to the modelled values of log10 n_{sp} can be gauged from Table 3, which shows RMSD in $\log_{10} n_{sp}$ obtained after the omission of individual metals from the analysis. The results are most sensitive to Cu, then Al and Ni, with a small dependence on Zn. There are negligible contributions from Cd, Hg and Pb to the estimation of $\log_{10} n_{sp}$; although these metals have high values of $\alpha_{M,max,mean}$ (Table 1), their low lakewater concentrations mean that their loadings of the zooplankton binding sites (θ_{Cd}, θ_{Hg}, θ_{Pb}) are too small to affect F_{TOX} (equation 1).

Temporal variations of n_{sp} are matched fairly well for most of the Sudbury lakes, whether the probabilistic or single cut-off versions of the model are applied (Figure 3). However, there is a tendency for the model to underestimate the rates of increase in n_{sp}, most noticeably for Middle Lake. Observed patterns of inter-annual variability in n_{sp} are reproduced in some instances, but they are often missed. In particular, the observed n_{sp} values for Clearwater Lake are much more variable than the calculated ones.

Contributions of the different cations to F_{TOX} are shown for the 9 Sudbury lakes in Figure 4, based on results from the probabilistic version of the model. Results are shown for the central model species in the distribution of 13 species, that is, model species number 7, for which $\beta = 0.764$ (Figure 1). Results for model species 3 and 11 are shown in Figures S1A and S1B. The relative contributions of the metal cations are the same for each model species, but their

273 absolute contributions vary. The contribution of H^+ to F_{TOX} is the same for each model species

274 (see Methods).

275 As expected from the results in Table 3, the main effects were due to H, Al, Ni and Cu, with

276 small contributions from Zn. The lakes can be distinguished by the contributions of different

277 toxic cations to F_{TOX}, and the consequent effects on n_{sp}. In Laundrie and Whitepine Lakes,

278 acidification has been dominant, the main toxic effects coming from H and Al. At the other

279 extreme, Hannah and Middle Lakes have been most affected by heavy metals (Ni, Cu, Zn)

280 especially in the later years. The other five lakes have been affected to similar extents by

281 acidification and heavy metals. Overall, the relative influence of the heavy metals has increased

282 with time, even though their lakewater concentrations have been falling. According to the

283 model, this was because metal accumulation by the zooplankton increased due to declining

284 competition by H and Al for complexation by the organisms; the effect applies especially to Ni

285 in Clearwater, Hannah, Lohi and Middle Lakes.

286 **4. Discussion**

287 In this work we combined the average values of metal-related parameters (αM,max,mean,

288 Table 1) estimated from laboratory toxicity test results (Tipping et al., 2019) with fitted model

289 species sensitivity distributions for lake zooplankton. Optimization of only two parameters (the

290 average and standard deviation of $\log_{10} \beta$) produced remarkably good fits (Figures 2 and 3) to

291 field observations of zooplankton species richness in 217 lakewaters covering substantial

292 ranges of pH, and concentrations of major ions and toxic metals (Table S1). Extending the

293 optimization to all $\log_{10} \beta$ values of the assumed total of 13 model species made almost no

294 difference to the goodness of fit (Table 2), and so the assumption of an evenly-spaced log-

295 normal distribution is justified.

296 We applied the model in two ways. Firstly, in line with laboratory toxicity testing, we assumed

297 that the toxic effect leading to the presence or absence of a species could vary between lower

298 and upper threshold values of F_{TOX} (equations 2 – 4), related to conventional dose-response

299 curves. We then interpreted the intermediate values in terms of the probability of the species

300 being present (equations 7 – 9). Alternatively, a simpler approach could be taken, whereby a

301 single cut-off value of F_{TOX} (the average of the lower and upper thresholds) was taken as the

302 condition where presence changes to absence, or vice versa. The single cut-off version of the

303 model is perhaps more closely related to field measurements, which also deal with simple

304 presence or absence. On the other hand, the probabilistic version might provide a better

305 representation of the average n_{sp} over a sampling season, providing non-integer values to

306 compare with the non-integer observations that arise from averaging results for different dates

307 within the yearly period of sampling a given lake. However, the results obtained with the two

308 approaches are very similar, and do not affect any conclusions that might be drawn.

309 With the approximation (Section 2.3) that average values of αM,max,mean for the zooplankton are

310 the same as those derived for laboratory test species (Table 1), the optimized average values of

311 $\log_{10} \beta$ are -0.117 (probabilistic) and -0.098 (single cut-off), equivalent to β values of 0.76 and

312 0.80. From the fit of all 13 $\log_{10} \beta$ values, we obtain a mean β of 0.72 These values imply that

313 the average lake model species is somewhat less sensitive to toxic metals than the average

314 species used in toxicity testing, for which an average β near to 1.00 is expected. But the

315 difference in average sensitivity is not great, suggesting that the toxicity parameters

316 (αM,max,mean) derived from laboratory results (Tipping et al., 2019) are strongly relevant to field

317 behaviour.

318   The fitting parameters can be used to gauge the range of sensitivities of zooplankton species to

319   toxic cations, by considering ratios of high and low β values. Taking the values for model

320   species 1 (least sensitive) and 13 (most sensitive), we obtain ratios of 22, 51 and 68 respectively

321   from the 13-parameter fit, the probabilistic log-normal fit, and the single cut-off log-normal fit.

322   Alternatively, the corresponding ratios from species 2 and 12 are 12, 14 and 18, which are

323   perhaps more realistic because the field data may not define the extremes of the distributions

324   very well. These ratios indicate a considerable range in the abilities of different model species

325   to resist cation toxicity.

326   The assumption of common relative sensitivity, in which the sensitivity of a model species

327   towards toxic metals is characterized by the parameter, β, allows the model to attribute toxic

328   effects separately, to variations among metals on the one hand, and among species on the other.

329   If such a differentiation cannot be made, then it is difficult to see how modelling based on the

330   responses of individual species could be done, unless comprehensive toxicity data were

331   available for every species. In the present case, common relative sensitivity only has to apply

332   to three metals, Al, Ni and Cu, since the toxic effects of the others are calculated to be small or

333   negligible (Table 3). Moreover, the results in Figure 4, predicting that some lakes have

334   responded principally to Ni and Cu, while others have been mainly affected by acidification,

335   including the effect of Al, means that the field data do not provide a strong test of the need for

336   common relative sensitivity. For example, it is quite possible for there to be no relationship

337   between the sensitivities of zooplankton towards Ni and Cd. As far as we have been able to

338   ascertain, there was no published evidence for or against common relative sensitivity until

339   Fettweis et al. (2020) reported correlations among toxic endpoints for the effects of Ni, Cu and

340   Zn on the growth rates of 8 phytoplankton species. More research on this topic is warranted.

341   An implied assumption in our approach is that the accumulation of metals via dietary intake

342   does not represent a quantitatively different exposure route. We have previously argued

343   (Stockdale et al., 2014a) that because the dietary organisms on which zooplankton feed are

344   exposed to the same lakewater as the zooplankton themselves, they are loaded with metals in

345   the same proportions experienced by the zooplankton in water-borne exposure. Therefore,

346   relative metal loadings of zooplankton are the same by both routes. It can also be noted that the

347   WHAM-F_{TOX} model does not entail a specific mode of cation uptake; it merely asserts that the

348   loading of cations by organisms is related, via equilibrium complexation reactions, to the

349   bathing water composition.

350   A limitation to this study is the approximate nature of the WHAM-F_{TOX} model, including the

351   assumption that HA is an adequate surrogate for toxicity-sensitive biomolecules. The model

352   only provided a broad-brush encapsulation of laboratory toxicity testing data (Tipping et al.,

353   2019), and those data did not evenly cover a wide range of species and metals, being biased

354   towards one metal, Cu (76% of all endpoints used) and with 72% of the endpoints applying to

355   just five test species. Correction for water chemistry was poorer than has been obtained in

356   focused multi-parameter modelling in individual studies. Another aspect that requires

357   improvement is the necessary use of a constant value (1.0) of α_H, owing to a lack of studies of

358   toxicity by H^+ alone. However, it seems unlikely that improving WHAM-F_{TOX} and its

359   parameterization would necessarily improve the field modelling reported here, without more

360   research into common relative sensitivity and species sensitivity distributions.

361   It should be noted that the method of application of WHAM-F_{TOX} in the present study differs

362   from that in earlier work in which the model was applied to zooplankton data from a wide range

363   of lakes, including those in the vicinity of Sudbury (Stockdale et al., 2014a). In the previous

364   work, a larger number of model parameters were fitted to the field data, and account was taken,

365   via quantile regression, of effects other than cation toxicity in determining values of n_{sp}. This

366   means that the parameter values determined in this and the previous study are not comparable.

367   Furthermore, as explained in the Introduction, the important difference between the studies is

368   that in the present analysis, the toxic responses of individual species are summed to obtain n_{sp},

369   whereas in the earlier work n_{sp} was treated as an overall ecosystem response.

370   Whereas the model allows 13 invariant species, characterized only by their sensitivities towards

371   toxic cations (β values), the real zooplankton populations in the lakes are drawn from a larger

372   number of different species; some 65 different species were observed at different times in the

373   9 Sudbury lakes. Although many of these occurred only rarely, the observed average n_{sp} values

374   are certainly made up of more than 13 different species. In the absence of toxic effects, n_{sp}

375   depends on a variety of factors, including water body morphometry (Dodson, 1992), climate

376   (Hessen et al., 2006; Keller et al., 2019), ultraviolet radiation (Marinone et al 2006), chemical

377   and thermal variability (Shurin et al., 2010), non-toxic chemistry (DeSellas et al 2011, Sinclair

378   & Arnott 2017), lake productivity (Dodson, 1992, Hessen et al., 2006), predation (Knapp et al

379   2001, MacLennan et al 2015), niche availability (Walseng et al., 2006), long-range dispersal

380   (Shurin et al., 2000), and competition (Hebert, 1982). The combination of some or all of these

381   factors determines which species succeed in a given lake in a given year, and although some

382   species may occur regularly, others come and go. This compositional variation in the

383   zooplankton populations means that the 13 model species cannot be equated with real

384   zooplankton species, but it does appear, from the reasonably successful fitting of the data, that

385   the fitted distribution of β values covers the range of toxic sensitivities of species that occur in

386   the field.

387   Although the modelling accounts for much of the temporal variation in n_{sp} as the lakes

388   recovered from the most extreme toxic conditions (Figure 3), other factors may have played a

389   role. For example, the changes in water chemistry are likely to have affected biota other than

390   zooplankton, and thereby have had indirect effects on n_{sp}, resulting from changes in food

391   sources and predation pressure. Yan et al. (2016) concluded that in the later years of recovery

392   the introduction of piscivorous fish relieved the pressure on zooplankton by planktoniferous

393   fish, allowing faster recovery from contaminant effects. Furthermore, climate change has likely

394   affected (increased) zooplankton richness at Sudbury (Keller et al., 2019). A full explanation

395   of zooplankton species richness in the Sudbury lakes, and for field systems in general, would

396   require the combined modelling of ecology and toxicity.

397   This study has demonstrated the possibility that species richness can be modelled on the basis

398   of laboratory-derived parameters for metals (α values) together with a fitted species sensitivity

399   distribution (β values). Further progress with respect to toxic effects in the field requires the

400   issue of common relative sensitivity to be resolved, and testing of the approach on other

401   ecosystem types. Potentially, this could lead to a generally applicable method of quantifying

402   and predicting ecosystem damage by, and recovery from, toxic cations, including attribution

403   of the relative toxic effects of different cations.

5. Conclusions

(a) Species richness data for zooplankton in contaminated lakes near Sudbury, and in reference lakes, were successfully fitted with WHAM-F_{TOX}, parameterized with laboratory toxicity data for metals, and with an optimized log-normal species sensitivity distribution.

(b) The most important toxic cations were H, Al, Ni and Cu, with a small contribution from Zn, and negligible toxic effects attributable to Cd, Hg and Pb.

(c) The results highlight the issue of common relative sensitivity, which means that a species is intrinsically sensitive, or insensitive, to toxic cations. Further research is need to establish the validity or otherwise of this concept.

(d) Some of the lakes at Sudbury were affected predominantly by acidification (H and Al), while others were most influenced by toxic heavy metals (Ni, Cu, Zn). For lakes in the latter category, the relative importance of heavy metals to toxicity has increased over time, despite their decreased concentrations.

Acknowledgments

References

426

427 Ardestani, M.M., Van Straalen, N.M., Van Gestel, C.A.M., 2015. Biotic ligand modeling
428   approach: synthesis of the effect of major cations on the toxicity of metals to soil and
429   aquatic organisms. Environ. Toxicol. Chem. 34, 2194–2204.

430 Balistrieri, L.S., Mebane, C.A., Schmidt, T.S., Keller, W., 2015. Expanding metal mixture
431   acute toxicity models to natural stream and lake invertebrate communities. Environ.
432   Tox. Chem. 34, 761–776.

433 Borgmann, U., Norwood, W.P., Dixon, D.G., 2008. Modelling bioaccumulation and toxicity
434   of metal mixtures. Hum. Ecol. Risk Assess. 14, 266–289.

435 Cairns, J., Mount, D.I., 1990. Aquatic toxicology. Environ. Sci. Technol. 24, 154-161.

436 Clements, W. H., Carlisle, D. M., Lazorchak, J. M., Johnson, P. C., 2000. Heavy metals
437   structure benthic communities in Colorado mountain streams. Ecol. Appl. 10, 626−638.

438 De Jonge, M., Lofts, S., Bervoets, L., Blust, R., 2014. Relating metal exposure and chemical
439   speciation to trace metal accumulation in aquatic insects under natural field conditions.
440   Sci. Tot. Environ. 496, 11-21.

441 DeSellas, A. M., Paterson, A. M., Sweetman, J. N., Smol, J. P., 2011. Assessing the effects of
442   multiple environmental stressors on zooplankton assemblages in Boreal Shield lakes
443   since pre-industrial times. J. Limnol. 70, 41–56.

444 Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. A
445   biotic ligand model of the acute toxicity of metals I. Technical basis. Environ. Toxicol.
446   Chem. 20, 2383–2396.

447 Dodson, S.I., 1992. Predicting crustacean zooplankton species richness. Limnol. Oceanogr. 37,
448   848-856.

449 Erickson, R.J., Benoit, D.A., Mattson, V.R., Nelson, H.P., Leonard, E.N., 1996. The effects of
450   water chemistry on the toxicity of copper to fathead minnows. Environ. Toxicol. Chem.
451   15, 181–193.

452 Fettweis, A., Bergen, B., Hansul, S., De Schamphelaere, K., Smolders, E., 2020. Mixture
453   toxicity of Ni, Cu and Zn on eight freshwater algal species: what determines a
454   something-from-nothing effect? Extended Abstract SETAC Dublin 2020.

455 Hebert, P.D.N., 1982. Competition in zooplankton communities. Ann. Zool. Fennici 19, 349-
456   356.

457 Henriksen, A., Posch, M., 2001. Steady-state models for calculating critical loads of acidity for
458   surface waters. Water Air Soil Pollut. Focus 1, 375–398.

459   Hessen, D. O., Faafeng, B. A., Smith, V. H., Bakkestuen, V., Walseng, B., 2006. Extrinsic and
460   intrinsic controls of zooplankton diversity in lakes. Ecology 87, 433-443.

461   Keller W., Yan, N.D., Gunn, J.M., Heneberry, J., 2007. Recovery of acidified lakes: lessons
462   from Sudbury, Ontario, Canada. Water Air Soil Pollut. Focus 7, 317-322.

463   Keller, W., Heneberry, J., Edwards, B.A., 2019. Recovery of acidified Sudbury, Ontario,
464   Canada, lakes: a multi-decade synthesis and update. Environ. Rev. 27, 1-16.

465   Keller, W., Yan, N.D., 1991. Recovery of crustacean zooplankton species richness in Sudbury
466   area lakes following water quality improvements. Can. J. Fish. Aquat. Sci. 48, 1635–
467   1644.

468   Khan, F.R., Keller, W., Yan, N.D., Welsh, P.G., Wood, C.M., McGeer, J., 2012. Application
469   of biotic ligand and toxic unit modelling approaches to predict improvements in
470   zooplankton species richness in smelter-damaged lakes near Sudbury, Ontario.
471   Environ. Sci. Technol. 46, 1641-1649.

472   Knapp, R. A., Matthews, K. R., Sarnelle, O., 2001. Resistance and resilience of alpine lake
473   fauna to fish introductions. Ecol. Monogr. 71, 401-421.

474   Lofts, S., Tipping, E., Hamilton-Taylor, J., 2008. The chemical speciation of Fe(III) in
475   freshwaters. Aquat. Geochem. 14, 337–358.

476   MacLennan, M. M., Dings-Avery, C., Vinebrooke, R. D., 2015. Invasive trout increase the
477   climatic sensitivity of zooplankton communities in naturally fishless lakes. Freshwat.
478   Biol. 60, 1502-1513.

479   Mance, G., 1987. Pollution Threat of Heavy Metals in Aquatic Environments. Pollution
480   Monitoring Series. Springer, Dordrecht.

481   Marinone, M. C., Marque, S. M., Suárez, D. A., Diéguez, M. d. C., Pérez, P., De Los Ríos, P.,
482   Soto, D., Zagarese, H. E., 2006. UV Radiation as a Potential Driving Force for
483   Zooplankton Community Structure in Patagonian Lakes. Photochem. Photobiol. 82,
484   962-971.

485   Meador J., 2006. Rationale and procedures for using the tissue-residue approach for toxicity
486   assessment and determination of tissue, water, and sediment quality guidelines for
487   aquatic organisms. Hum. Ecol. Risk. Assess. 12, 1018–1073.

488   Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., et al.,
489   2002. The biotic ligand model: a historical overview. Comp. Biochem. Physiol. C 133,
490   3–35.

491   Playle, R.C., 2004. Using multiple metal–gill binding models and the toxic unit concept to help
492   reconcile multiple-metal toxicity results. Aquat. Toxicol. 67, 359–370.

493 Rainbow, P.S., 2018. Trace Metals in the Environment and Living Organisms. Cambridge
494   University Press, Cambridge, 742pp.

495 Santore, R.C., Ryan, A.C., 2015. Development and application of a multimetal multibiotic
496   ligand model for assessing aquatic toxicity of metal mixtures. Environ. Toxicol. Chem.
497   34, 777-787.

498 Shurin, J. B., 2000. Dispersal limitation, invasion resistance, and the structure of pond
499   zooplankton communities. Ecology 81, 3074-3086.

500 Shurin, J. B., Winder, M., Adrian, R., Keller, W., Matthews, B., Paterson, A. M., Paterson, M.
501   J., Pinel-Alloul, B., Rusak, J. A., Yan, N. D., 2010. Environmental stability and lake
502   zooplankton diversity – contrasting effects of chemical and thermal variability. Ecol.
503   Lett. 13, 453-463.

504 Sinclair, J. S., Arnott, S. E., 2018. Local context and connectivity determine the response of
505   zooplankton communities to salt contamination. Freshwat. Biol. 63, 1273-1286.

506 Stockdale, A., Tipping, E., Lofts, S., Ormerod, S.J., Clements, W.H., Blust, R., 2010. Toxicity
507   of proton–metal mixtures in the field: linking stream macroinvertebrate species
508   diversity to chemical speciation and bioavailability. Aquat. Toxicol. 100, 112–119.

509 Stockdale, A., Tipping, E., Lofts, S., Fott, J., Garmo, Ø.A., Hruska, J., et al., 2014a. Metal and
510   proton toxicity to lake zooplankton: a chemical speciation based modelling approach.
511   Environ. Poll. 186, 115–125.

512 Stockdale, A., Tipping, E., Fjellheim, A., Garmoc, Ø.A., Hildrew, A.G., Stephen Lofts, S., et
513   al., 2014b. Recovery of macroinvertebrate species richness in acidified upland waters
514   assessed with a field toxicity model. Ecol. Indic. 37, 341–350.

515 Sverdrup, H., de Vries, W., 1994. Calculating critical loads for acidity with the simple mass
516   balance method. Water Air Soil Pollut. 72, 143-162.

517 Tipping, E., Vincent, C.D., Lawlor, A.J., Lofts, S., 2008. Metal accumulation by stream
518   bryophytes, related to chemical speciation. Environ. Pollut. 156, 936–943.

519 Tipping, E., Lofts, S., 2013. Metal mixture toxicity to aquatic biota in laboratory experiments:
520   application of the WHAM-F_{TOX} model. Aquat. Toxicol. 142–143, 114–122.

521 Tipping, E., Lofts, S., 2015. Testing WHAM-F_{TOX} with laboratory toxicity data for mixtures
522   of metals (Cu, Zn, Cd, Ag, Pb). Environ. Toxicol. Chem. 34, 788–798.

523 Tipping, E., Lofts, S., Sonke, J.E., 2011. Humic Ion-Binding Model VII: a revised
524   parameterisation of cation-binding by humic substances. Environ. Chem. 8, 225–235.

525 Tipping, E., Stockdale, A., Lofts, S., 2019. Systematic analysis of freshwater metal toxicity
526   with WHAM-F_{TOX}. Aquat. Toxicol. 212, 128–137.

527 UK Centre for Ecology and Hydrology. 2020. Windermere Humic Aqueous Model (WHAM7).
528 Available at https://www.ceh.ac.uk/services/windermere-humic-aqueous-model-wham
529 [verified 15 September 2020].

530 Walseng, B., Hessen, D. O. Halvorsen, G., Schartau, A. K., 2006. Major contribution from
531 littoral crustaceans to zooplankton species richness in lakes. Limnol. Oceanogr. 51,
532 2600-2606.

533 Yan, N.D., Bailey, J., McGeer, J.C., Manca, M.M., Keller, W., Celis-Salgado, M.P., Gunn,
534 J.M., 2016. Arrive, survive and thrive: essential stages in the re-colonization and
535 recovery of zooplankton in urban lakes in Sudbury, Canada. J. Limnol. 75(s2), 4-14.

536   Table 1. Values of $\alpha_{M,max,mean}$, obtained by averaging values from laboratory toxicity tests
537   reported by Tipping et al. (2019).

Cation	n	$\alpha_{M,max,mean}$	SE
H		(1.0)	
Al	7	2.6	0.0
Ni	79	31.1	3.4
Cu	1543	34.6	0.7
Zn	118	17.0	1.3
Cd	152	673	35
Hg	5	621	347
Pb	33	126	24

538   The value for H is set to 1.0; n = number of data; SE = standard error.

539   Table 2. Fitting statistics for different distributions of β.

	RMSD in $\log_{10} n_{sp}$	log-log slope	linear slope
Probabilistic, two-parameter distributions			
linear*	0.105	0.930	0.875
linear with intercept ≥ 0.00	0.105	0.905	0.858
normal*	0.106	0.943	0.889
normal with mean fixed at 1.00*	0.108	0.970	0.927
log-normal	0.098	1.013	1.010
log-normal with mean fixed at 0.00	0.111	1.127	1.209
Probabilistic, fit of all 13 β values	0.097	1.008	0.986
Single cut-off, two-parameter log-normal	0.102	1.035	1.019

540   RMSD = root-mean-square deviation

541   *some modelled β values were negative.

542   Table 3. Effect of omitting individual metals on the root mean-squared deviation in $\log_{10} n_{sp}$.

543

Metal omitted	RMSD
None	0.098
Al	0.174
Ni	0.116
Cu	0.212
Zn	0.099
Cd	0.098
Hg	0.098
Pb	0.098

544

Figure captions

Figure 1. Distributions of $\log_{10} \beta$ values for lakewater zooplankton species, estimated by fitting the field data. The lines show the two-parameter lognormal fits; the full line is for the probabilistic version of the model, the dashed line refers to the single cut-off version. The points show the 13 values fitted independently.

Figure 2. Comparison of observed species richness ($n_{sp,obs}$) with values calculated using WHAM-F_{TOX} ($n_{sp,calc}$). The top two panels show results with the probabilistic version of the model; the same data are plotted on (a) linear and (b) logarithmic (right) scales. The lower panels show results with the single cut-off version, with (c) linear and (d) logarithmic scales. The 1:1 line is shown in each case. Data for the Sudbury lakes are shown by filled circles, reference lake data by open circles.

Figure 3. Temporal dependence of species richness (n_{sp}) in each Sudbury lake. The points are observations, full lines refer to calculations with the probabilistic version of WHAM-F_{TOX}, dashed lines to calculations with the single cut-off version.

Figure 4. Contributions of different toxic cations to F_{TOX} and change over time for Sudbury lakes. Calculated results from the probabilistic version of WHAM-F_{TOX} are shown for the central species in the distribution of 13 species (species number 7, $\beta = 0.764$ (Figure 1).

562

564   Figure 1.

565

566

567

568

569

570

571

572

573

574

575

576

577

578

579

580

581

582
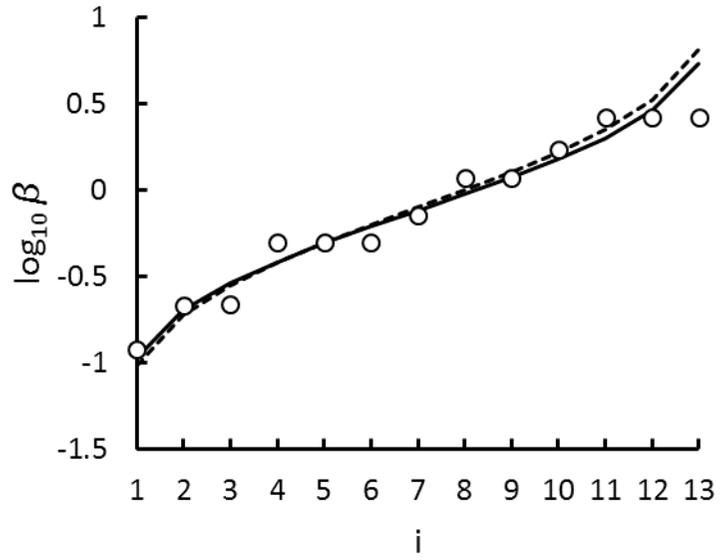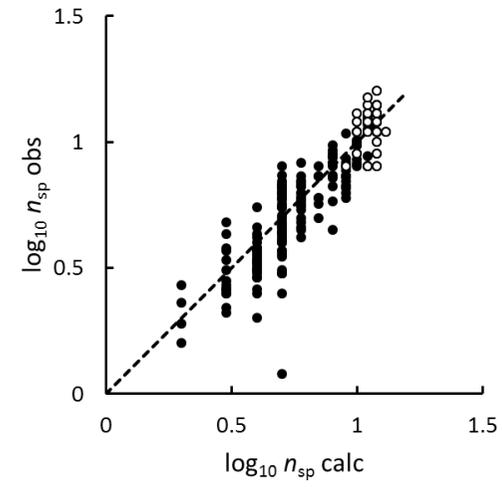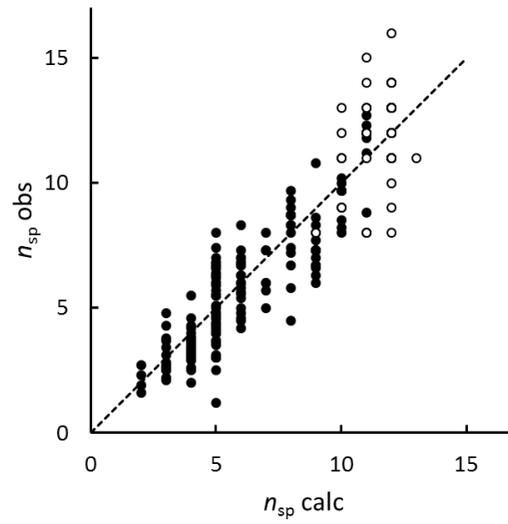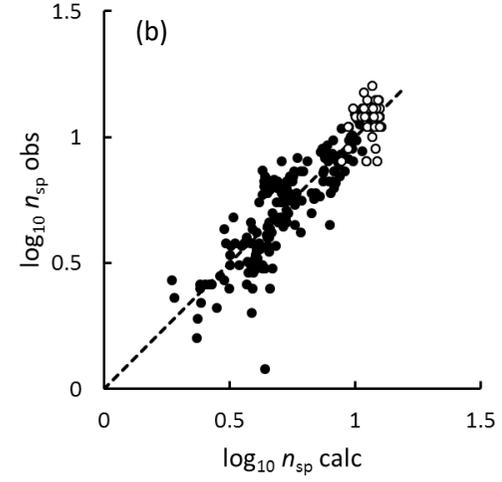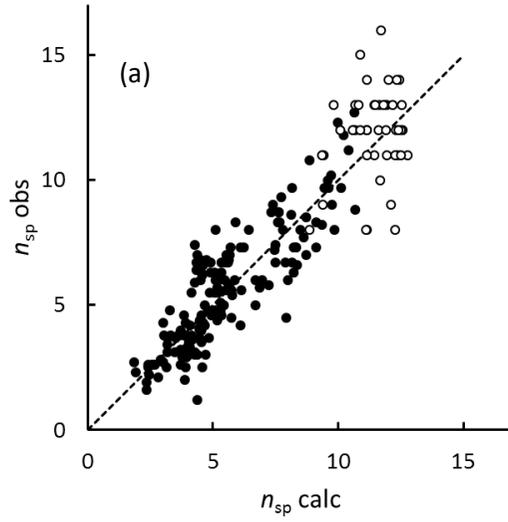
583

584

585   Figure 2.

586

587

588

589

590

591

592

593

594

595

596

597

598

599

600

601

602

603

604   Figure 3.

Figure 4.