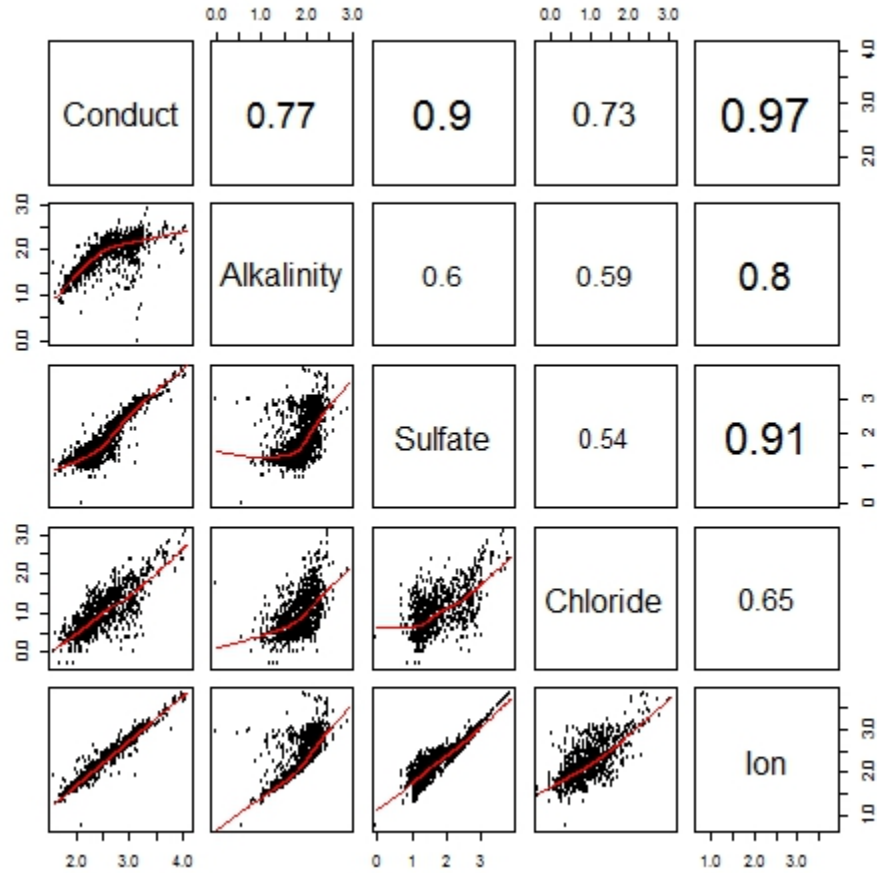
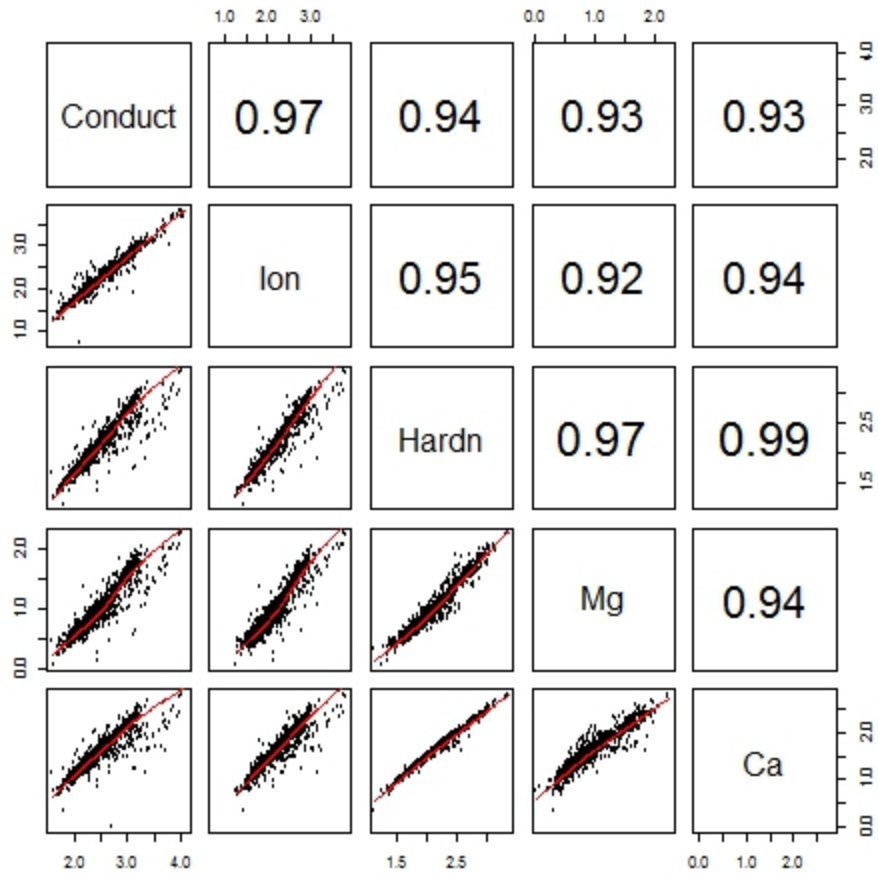


APPENDIX B. CASE STUDY II: SUPPORTING MATERIALS

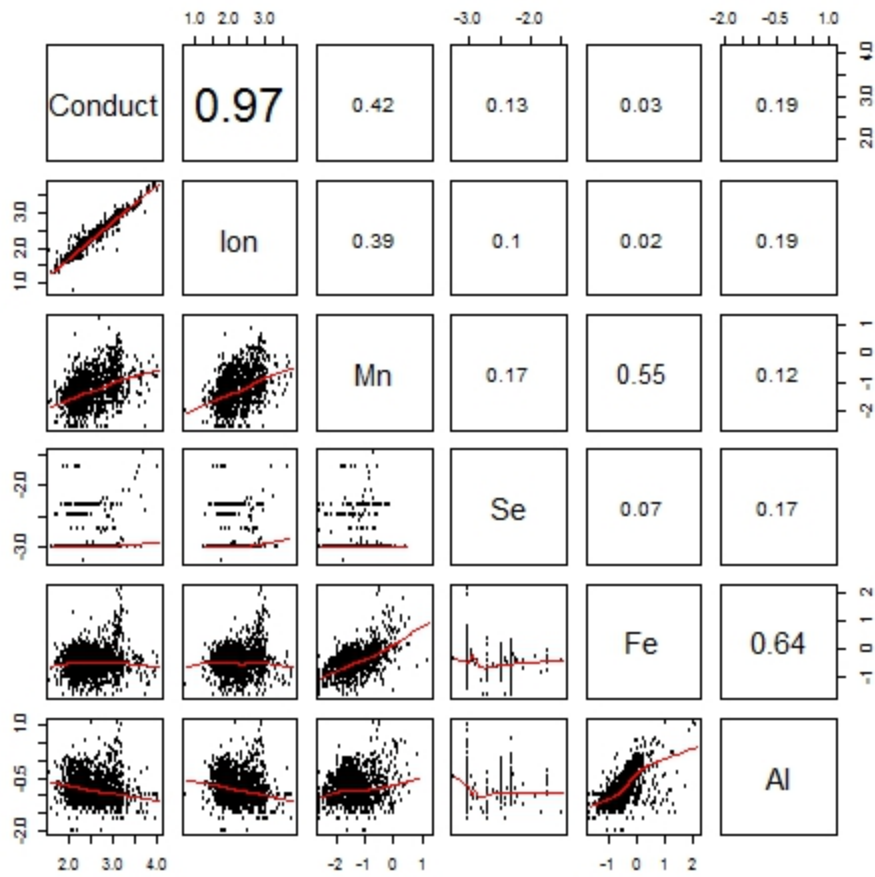
B.1. CASE STUDY II: MATRICES OF SCATTER PLOTS AND ABSOLUTE SPEARMAN CORRELATION COEFFICIENTS



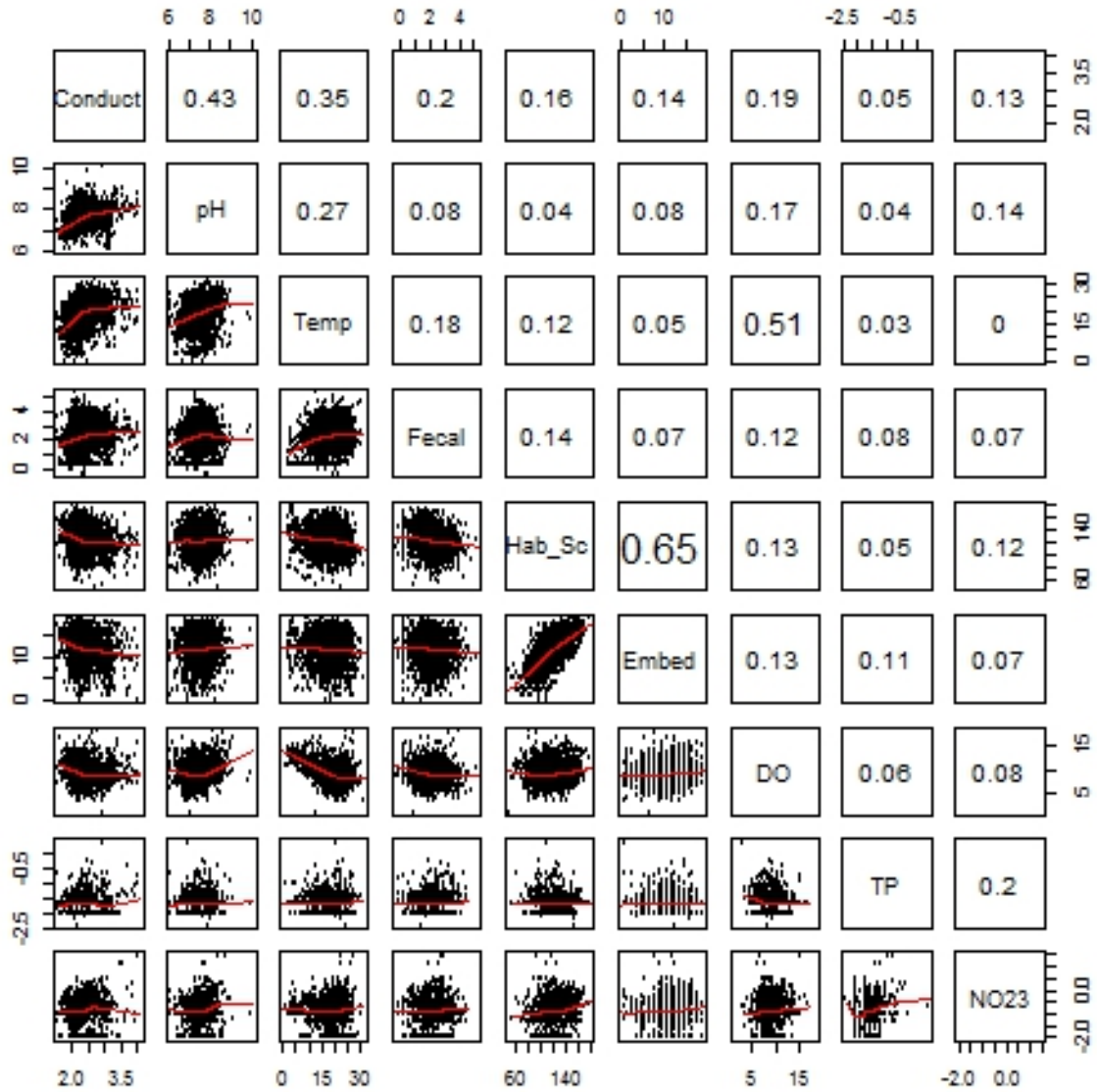
**Figure B-1. Anions.** Matrix of scatter plots and absolute Spearman correlation coefficients between specific conductivity ( $\mu\text{S}/\text{cm}$ ), alkalinity ( $\text{mg}/\text{L}$ ), sulfate ( $\text{mg}/\text{L}$ ), chloride ( $\text{mg}/\text{L}$ ), and ion ( $[\text{HCO}_3^- + \text{SO}_4^{2-}] \text{mg}/\text{L}$ ) concentrations in Case Study II. All variables are logarithm transformed. The smooth lines are the locally weighted scatterplot smoothing (LOWESS) lines (span = 2/3).



**Figure B-2. Cations.** Matrix of scatter plots and absolute Spearman correlation coefficients between specific conductivity ( $\mu\text{S}/\text{cm}$ ), ion ( $[\text{HCO}_3^- + \text{SO}_4^{2-}]$  mg/L), hardness (mg/L), Mg (mg/L), and Ca (mg/L), in Case Study II. All variables are logarithm transformed. The smooth lines are the locally weighted scatter plot smoothing (LOWESS) lines (span = 2/3).



**Figure B-3. Dissolved metals.** Matrix of scatter plots and absolute Spearman correlation coefficients among specific conductivity ( $\mu\text{S}/\text{cm}$ ), ion ( $[\text{HCO}_3^- + \text{SO}_4^{2-}]$  mg/L), and dissolved metal concentrations (mg/L) in Case Study II. All variables are logarithm transformed. The smooth lines represent the locally weighted scatterplot smoothing (LOWESS) lines (span = 2/3).



**Figure B-4. Other water-quality parameters.** Matrix of scatter plots and absolute Spearman correlation coefficients between environmental variables in Case Study II. The smooth lines are locally weighted scatter plot smoothing (LOWESS) lines (span = 2/3). Specific conductivity is logarithm transformed specific conductivity ( $\mu\text{S}/\text{cm}$ ); temp is water temperature ( $^{\circ}\text{C}$ ); Hab\_Sc is habitat score from Rapid Bioassessment (Habitat) Protocol (Barbour et al., 1999) score (possible range from 0 to 200); fecal is logarithm transformed fecal coliform bacteria count (per 100 mL water); embeddedness is a parameter score from the Rapid Bioassessment Protocol (possible range from 0 to 20); DO is dissolved oxygen (mg/L); TP is logarithm transformed total phosphorus (mg/L); NO23 is logarithm-transformed nitrite  $[\text{NO}_2^-]$  plus nitrate  $[\text{NO}_3^-]$  in mg/L.

## **B.2. CASE STUDY II: ASSESSMENT OF POTENTIAL CONFOUNDERS**

Previous assessments of the factors potentially influencing the model of the causal relationship between ionic concentration and extirpation of benthic invertebrates (Suter and Cormier, 2013; U.S. EPA, 2011, Appendix B) indicated that the following factors did not substantially confound the causal relationship between specific conductivity (SC) and benthic macroinvertebrate assemblages: rapid bioassessment protocol (RBP) habitat scores (Barbour et al., 1999), sampling date, organic enrichment, nutrients, deposited sediments, high pH, selenium, heat (temperature), lack of headwaters, size of catchment area, settling ponds, dissolved oxygen (DO), and metals. However, low pH could possibly affect the model (Suter and Cormier, 2013; U.S. EPA, 2011, Appendix B) because its mode of action is associated with increased solubility of metals which are toxic (e.g., Wren and Stephenson, 1991; Ormerod et al., 1987). As a result, sampling sites with acidic waters (pH <6) were excluded from the analysis in order to minimize any effects, but no other modification of the data set was required to address confounding.

New analyses described below are consistent with the analyses reported by the (U.S. EPA, 2011).

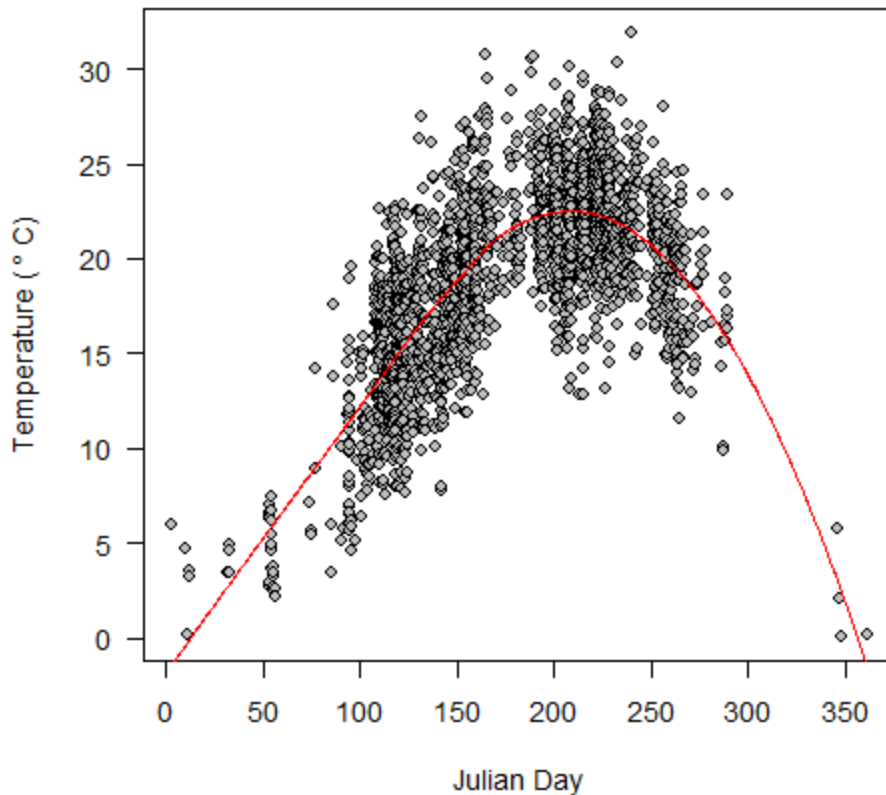
### **B.2.1. Multivariate Analysis**

Potential confounding of the model for Case Study II was reassessed for habitat (total RBP score), embeddedness (RBP subscore), temperature, and organic enrichment (fecal coliform) using a two-step process.

Habitat quality and fecal coliform together had little effect on the slope in multiple regression analyses with the dependent variable of occurrence of the genera with the 36 lowest extirpation concentration ( $XC_{95}$ ) values (see Table B-1). However, to ensure that they were not influential, their combined effect on the hazardous concentration ( $HC_{05}$ ) was determined (see Section B.2.2). The most influential parameter other than SC was temperature (slope =  $-0.202$ , Spearman standard error [SE] 0.21). Because there is a relationship with the life history of salt-intolerant taxa and because there is a nonlinear relationship between temperature and sampling date (see Figure B-5), further analyses were performed to evaluate temperature/sampling date related to the  $HC_{05}$  (see Section B.2.3).

**Table B-1. An output table for two generalized linear models.** The first is the simple model predicting the number of mayfly genera from specific conductivity. The second is a multivariate model with the additional covariates rapid bioassessment protocol (RBP) score, temperature, and fecal coliform count. These variables were chosen based on previous analyses as likely confounders that could co-occur and have combined effects. Fecal coliform count and specific conductivity were first log10 transformed to normalize the data, then all four variables were centered and scaled (subtracting the means and then dividing the centered values by their standard deviation) so that all four variables are at the same scales. The response variable is assumed to follow a Poisson distribution which appropriate for counts of occurrences.

<b>Parameter</b>	<b>Estimate</b>	<b>Standard error</b>
<b>Univariate model</b>		
Intercept	0.848	0.017
Specific conductivity slope	-0.852	0.018
<b>Multivariate model</b>		
Intercept	0.842	0.017
Specific conductivity slope	-0.703	0.019
RBP slope	0.037	0.013
Temperature slope	-0.202	0.013
Fecal coliform slope	-0.077	0.013

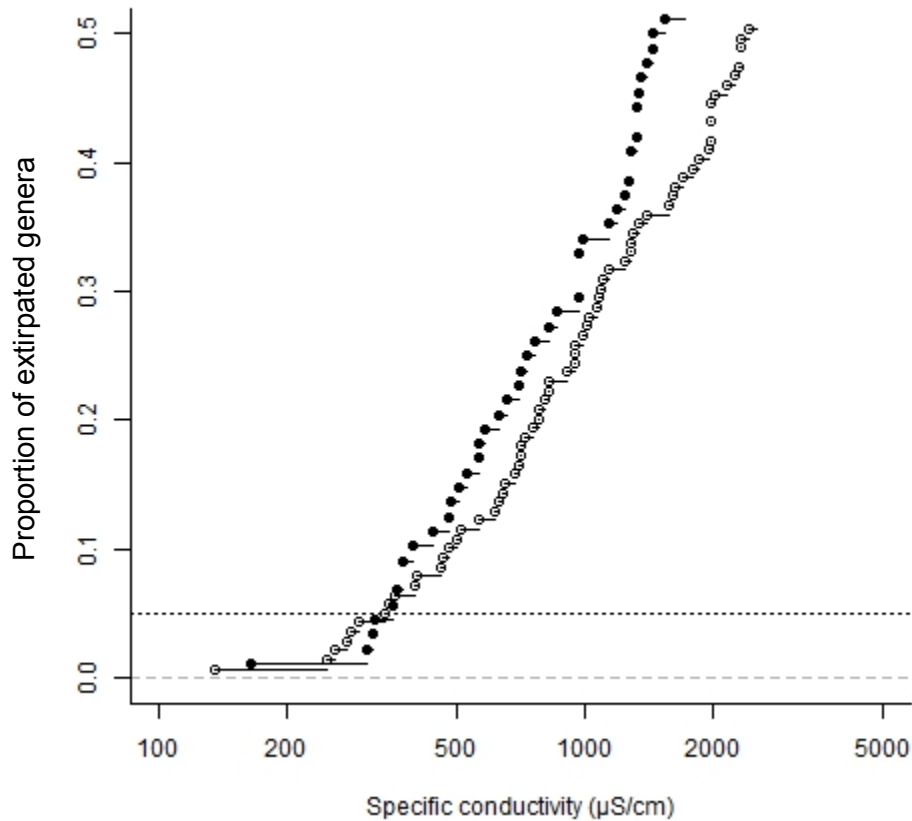


**Figure B-5. Scatter plot showing inter-relatedness of stream temperature and sampling date.** The fitted line is a locally weighted scatterplot smoothing spline (LOWESS, quadratic polynomial, span = 0.75).

### **B.2.2. Influence of Poor Habitat and Organic Enrichment on the Hazardous Concentration (HC<sub>05</sub>)**

To assure that the genus extirpation concentration distribution (XCD) model was detecting effects from SC and not a response to poor habitat, the HC<sub>05</sub> was recalculated using the example criterion-data set in which samples were removed with an RBP score <130 total, the HC<sub>05</sub> was 337 µS/cm. The threshold of RBP <130 was selected as an upper bound on acceptable habitat by Gerritsen et al. (2010) that also provided an adequate and to maximize sample size (relevant  $n = 581$ ). This threshold of RBP <130 represents, on average, habitat that is not pristine, but which is adequate for maintenance of biological assemblages. Removal of poor habitat and high fecal coliform samples from the data set had almost no effect on the XCD model

or HC<sub>05</sub> (see Figure B-6). With this constrained data set (RBP score >130) the HC<sub>05</sub> was 337  $\mu\text{S}/\text{cm}$  (95% confidence interval [CI] 265–360  $\mu\text{S}/\text{cm}$ ). The confidence interval overlaps with the HC<sub>05</sub> for the example criterion continuous concentration (CCC; 338  $\mu\text{S}/\text{cm}$ ; 95% CI 272–365  $\mu\text{S}/\text{cm}$ ). Therefore, no correction was made for habitat quality or organic enrichment.



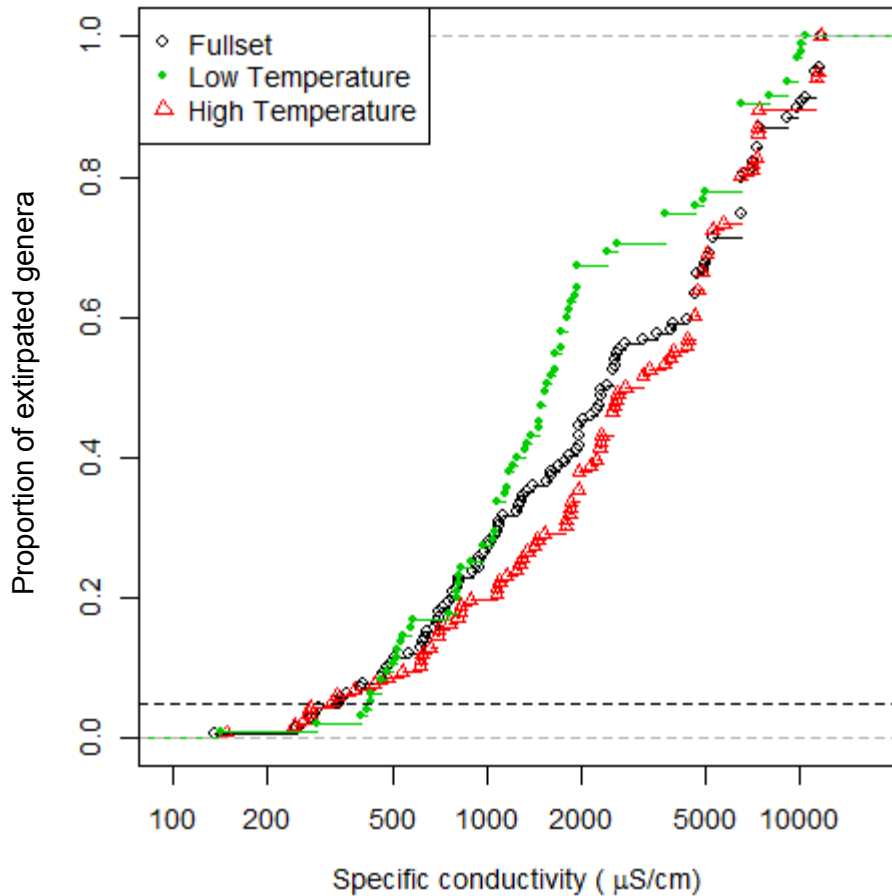
**Figure B-6. Lower portion of genus extirpation concentration distribution with and without removal of sites with poor habitat.** For both data sets the pH is >6, Rapid Bioassessment Protocol score is >130 and fecal coliform is  $\leq 400$  colonies/100 mL. The full (unconstrained, open circles) data set has 139 genera and the constrained data set has 88 genera. Habitat disturbance and organic enrichment have little influence; the hazardous concentration (HC<sub>05</sub>) for the constrained data set is 337  $\mu\text{S}/\text{cm}$  (95% confidence interval [CI], 265–360  $\mu\text{S}/\text{cm}$ ), based on 88 genera (closed circles).



### **B.2.3. Potential Influence of Temperature on the Hazardous Concentration (HC<sub>05</sub>)**

To assure that the genus XCD model was detecting effects from SC and not a response to warmer temperatures, the example criterion data set was constrained to samples with pH <6 and fecal coliform <400 colonies/100 mL and either a temperature  $\geq 17^{\circ}\text{C}$  or  $< 17^{\circ}\text{C}$ . The threshold of  $17^{\circ}\text{C}$  was selected based on reported upper temperature tolerance values for aquatic insects (Nebeker and Lemke, 1968; Vieira et al., 2006) and provided adequate sample sizes. If low temperature is a confounder, the XCD  $< 17^{\circ}\text{C}$  is expected to move to the right because lower temperatures are less stressful and organisms may be able to tolerate higher SC levels. If high temperature is a confounder, conditions are more stressful and the XCD  $\geq 17^{\circ}\text{C}$  is expected move to the left; that is, lower XC<sub>95</sub> values and a lower HC<sub>05</sub>.

Removal of cooler samples from the data set decreased the HC<sub>05</sub> (see Figure B-7). With the data set constrained to temperatures  $\geq 17^{\circ}\text{C}$ , the HC<sub>05</sub> was 315  $\mu\text{S}/\text{cm}$  (95% CI = 256–365, 116 genera, relevant  $n = 1,416$  samples). This is consistent with confounding by higher temperatures. Removal of warmer samples from the data set increased the HC<sub>05</sub> (see Figure B-7). With the data set constrained to temperatures  $< 17^{\circ}\text{C}$ , the HC<sub>05</sub> was 425  $\mu\text{S}/\text{cm}$  (95% CI = 292–455, 95 genera, relevant  $n = 658$  samples). This is also consistent with the direction that is expected to occur if temperature is a confounder. Hence, the results are logically inconsistent with temperature acting as a cause of extirpation along with SC. The confidence interval overlaps with the unconstrained example HC<sub>05</sub> (338  $\mu\text{S}/\text{cm}$ ; 95% CI 272–365  $\mu\text{S}/\text{cm}$ , 139 genera). Furthermore, the reduced sample sizes reduced the overall number of genera in the model by 17–32%. Also, the XCD overlaps the full data set through most of the lower range of the models. In such cases, correction for confounding may increase error in the estimated HC<sub>05</sub>, therefore, no correction was made for temperature.



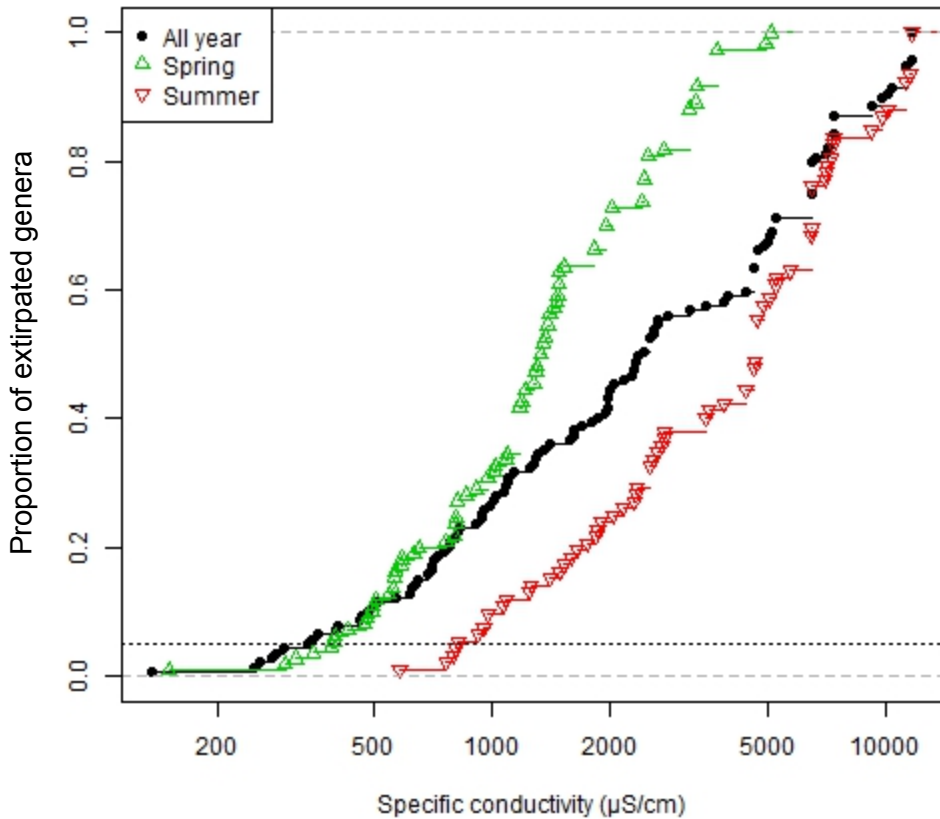
**Figure B-7. Genus extirpation concentration (XC<sub>95</sub>) distributions for example criterion data set and temperature constrained data sets.** Samples with pH ≤6 and (SO<sub>4</sub> + HCO<sub>3</sub>)/Cl ≤1 were removed from all data sets. The example criterion (unconstrained, 0–32°C) data set has XC<sub>95</sub> values for 139 genera (open black diamonds). The ≤17°C constrained data set has 95 genera (closed green diamonds [*N* = 658]). The ≥17°C constrained data set has 116 genera (open red triangles [*n* = 1,416]). For comparison the 5<sup>th</sup> centile is 338 µS/cm in the unconstrained data set, 425 µS/cm in the ≤17°C constrained data, and 315 µS/cm in the ≥17°C constrained data.

#### **B.2.4. Potential Influence of Sampling Date on the Hazardous Concentration (HC<sub>05</sub>)**

To assess effects of date of sampling on the XCD model, three lines of evidence were analyzed to address potential confounding by lack of seasonal observation of apparently salt-intolerant genera. First, the HC<sub>05</sub> using the spring (March to June) only data set was compared with the full-year data set. (Seasons are defined by the phenology of the aquatic insects and the changes in SC, not the conventional intervals.) The confidence bounds of the

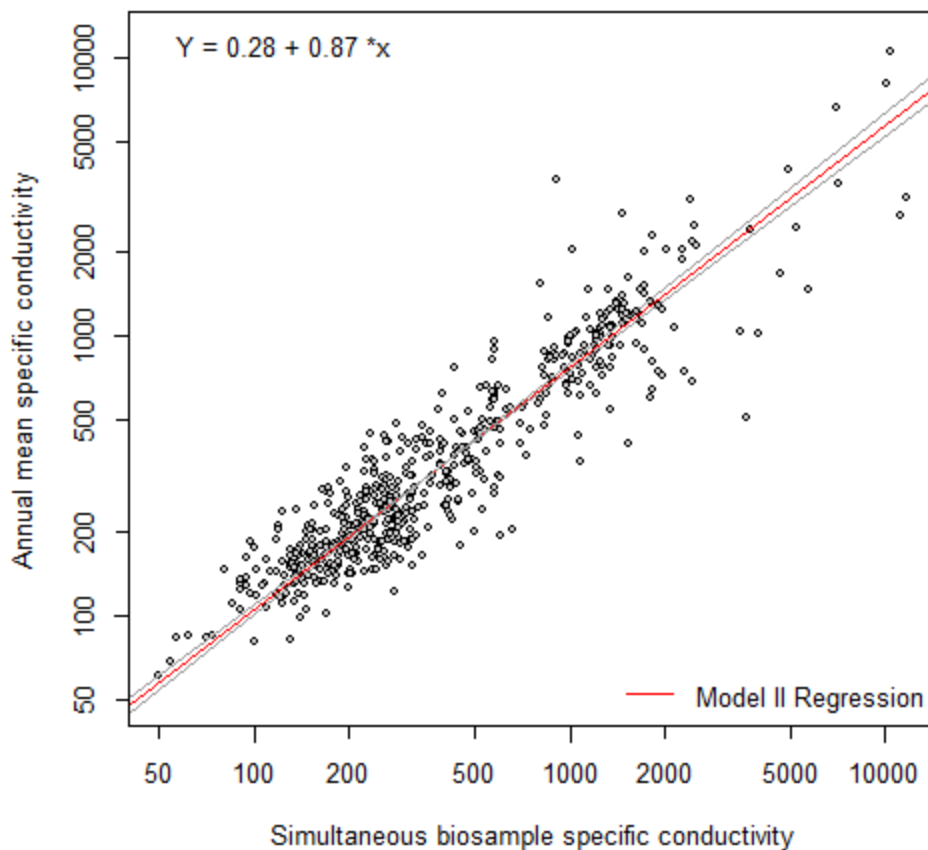
spring HC<sub>05</sub> overlap with the confidence bounds of the full-year data set (see Figure B-8). The summer (July to October) only XCD lacks taxa known to be intolerant to ionic stress which can be seen by the overall shift of the XCD to the right. The shift in the upper portion of the XCD in spring is mostly due to the narrower SC sample range during the spring compared to the all year data set.

Next, a scatter plot and regression model was developed for the relationship between measurements of SC at the time of the biological sample and annual mean SC (see Figure B-9). The annual geometric mean SC values were calculated from at least six water samples collected before biological samples were taken. At least one spring and one summer sample were required in order to be included in the data set. There were 325 sites with paired SC and biological data (see Figure B-9) meeting these additional data requirements. On the *x*-axis is the SC value when biological samples were collected and on the *y*-axis is the annual geometric mean value during that rotating year for a site. A Model II Regression was fitted for this data set which takes into account for error variance in both variables. The mean relationship between measurements of SC at the time of the biological sample and annual mean SC is nearly 1:1. For example, when SC is 340  $\mu\text{S}/\text{cm}$  on the biological sampling date, the regression prediction for an annual mean SC for the same site is 304  $\mu\text{S}/\text{cm}$ .



**Figure B-8. Comparison of genus extirpation concentration distributions (XCD) full data set and subsets in different months.** Example criterion data set (black circles) and subsets of March to June (spring, inverted green triangles) and July to October (summer, filled red triangles) collected samples from the Case Study II Criterion-data set. The all year XCD has 139 genera, the spring XCD has 110 genera ( $N = 1,044$ ), summer XCD has 92 genera ( $N = 989$ ). The horizontal dotted line is the 5<sup>th</sup> centile. The spring and summer hazardous concentrations' ( $HC_{05}$ ) 95% confidence bounds overlap with the all-year data set. The summer XCD model lacks salt-intolerant genera in the data set.

### Model II Regression and 95% confidence Intervals



**Figure B-9. Relationship between specific conductivity sample at the time of biological sampling and annual mean specific conductivity (using 6–12 intra-annual samples) in the Case Study II data set (1999–2011).**

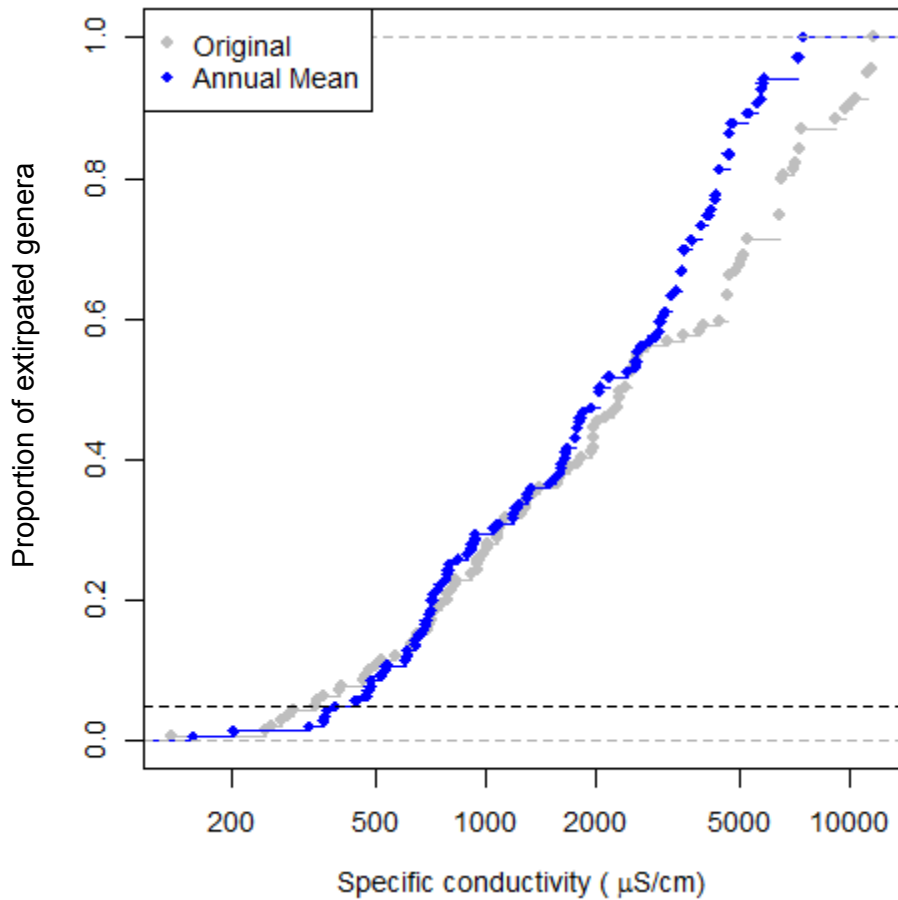
Though the relationship is nearly 1:1, some variability may be attributable to different seasonal specific conductivity regimes. Model II Regression with 95% confidence intervals. Specific conductivity is expressed as  $\mu\text{S}/\text{cm}$  on log10 scales; therefore,  $x$  and  $y$  are log10 expressions.

Lastly, to account for the seasonal variability, SC values collected at the time of biological sampling were adjusted to estimate annual mean SC values as described in Section 3.1.4. The weighting factors vary slightly for different months 0.94 to 1.05 (see Table B-2). June through November SC values are slightly higher than the annual average, so the weighting factors are generally lower, while the earlier spring weighting factors are generally higher. The  $\text{HC}_{05}$  calculated with weighted SC measurements is  $385 \mu\text{S}/\text{cm}$  (CI 327–468  $\mu\text{S}/\text{cm}$ ;

see Figure B-10). These three analyses suggest that sampling date is at most a minor confounder. Correction for confounding may increase error in the estimated HC<sub>05</sub>, therefore, no correction was made for sampling date.

**Table B-2. Weighting factors used to normalize specific conductivity on date of biological sample to annual average**

Month	1	2	3	4	5	6	7	8	9	10	11	12
Weighting factor	1.03	1.05	1.04	1.05	1.05	0.99	0.96	0.95	0.94	0.94	0.97	1.00



**Figure B-10. Case Study II comparison of annual weighted and original extirpation concentrations (XC<sub>95</sub>).** Genus extirpation concentration distribution (XCD) of unweighted XC<sub>95</sub> values (gray) and XCD of XC<sub>95</sub> derived from specific conductivity normalized to an annual geometric mean (blue). Hazardous concentration (HC<sub>05</sub>) values are 338, and 385 µS/cm, respectively.

### **B.2.5. Conclusion**

Previous assessments of the factors potentially influencing the model of the causal relationship between ionic concentration and extirpation of benthic invertebrates indicated that 13 factors had little or no effect on the causal relationship between SC and benthic macroinvertebrate assemblages (Suter and Cormier, 2013; U.S. EPA, 2011, Appendix B). The 13 factors that were considered included RBP habitat scores, sampling date, organic enrichment, nutrients, deposited sediments, high pH, selenium, heat (temperature), lack of headwaters, size of catchment area, settling ponds, dissolved oxygen, and metals.

The additional analyses described in this Appendix Section B.2 using data from Ecoregion 70 indicate that SC remains the strongest influence in the multivariate model of genera with low  $XC_{95}$  values (see Table B-1). Organic enrichment (estimated based on fecal coliform counts) did not significantly contribute to the multivariate model and no further analyses were warranted. Habitat score showed a minor effect in the multivariate model, but recalculating the  $HC_{05}$  in a data set with sites with RBP score >130 resulted in an  $HC_{05}$  with confidence intervals that overlapped with the  $HC_{05}$  from the example criterion data set. Temperature and sample date are nonlinearly associated; therefore, three different analyses were performed to assess potential effects on the XCD model. They indicated that neither temperature nor sample date confounds the  $HC_{05}$  of the XCD model for the example criteria. Therefore, the example criterion data set was not altered and no corrections were made for habitat, temperature, or sample date.

### **B.3. CASE STUDY II: COMPARISON OF WATER CHEMISTRY BASED CRITERION MAXIMUM EXPOSURE CONCENTRATION (CMEC) AND BIOLOGICAL SURVIVAL**

The criterion maximum exposure concentration (CMEC) is the maximum SC level that may occur for a short duration and be protective of 95% of macroinvertebrate genera. The CMEC for Ecoregion 70 was calculated using the water chemistry approach in Section 3.2. In this method, the CMEC is estimated at the 90<sup>th</sup> centile of observations at sites with water chemistry regimes meeting the CCC. It estimates the protective maximum using only water chemistry data without biological data.

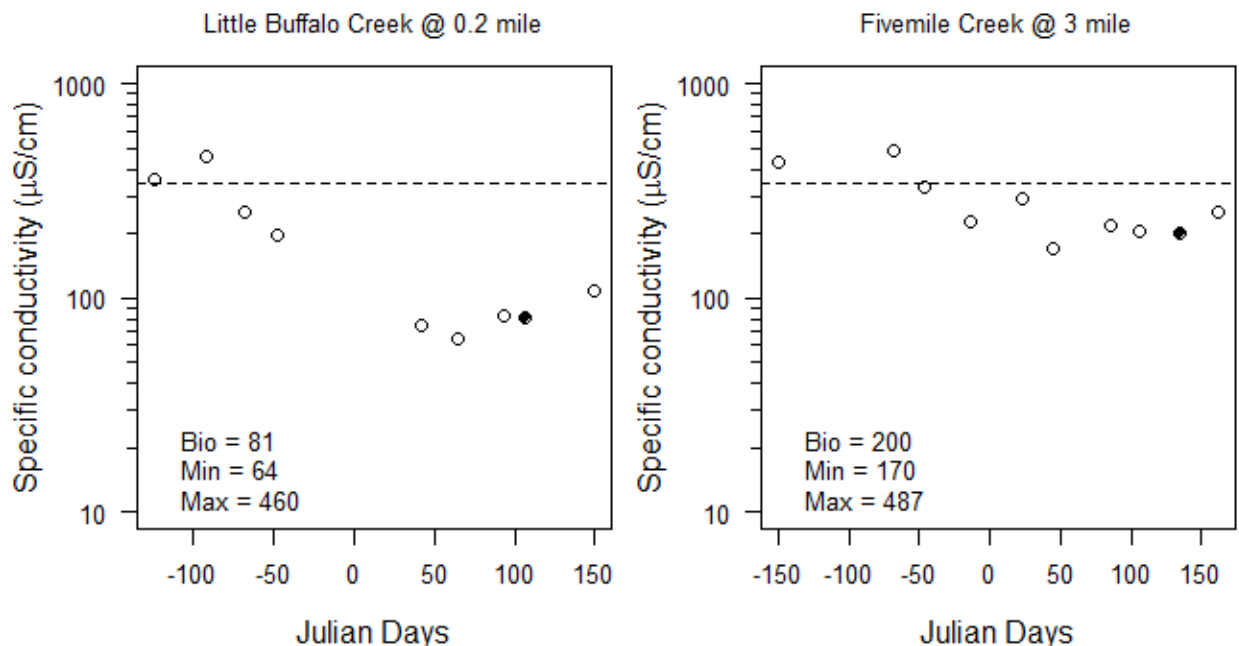
Owing to the moderate number of biological samples with multiple seasonal sampling of water chemistry available for Ecoregion 70, it was possible to estimate a maximum SC that could

occur and salt-intolerant genera had survived until the following year shortly before emergence as winged adults. Salt-intolerant genera are more commonly observed when they are larger and nearing emergence usually in April through June. The maximum SC of streams in Ecoregion 70 usually occurs between August and September.

A data set was constructed from the Ecoregion 70 criterion-data set. For a site to be included, it required a minimum of six water chemistry samples taken samples of water chemistry data taken over the course of the year prior to biological sampling. A minimum of six samples were required for inclusion in the data set which was defined as a rotating year. Of the 819 sites sampled in the data set, 317 met the stringent criteria for inclusion in the data set for this analysis. The data set tended to represent long term reference sites and sites monitored for remediation. Therefore, the data set is not optimal for this analysis. However, it is useful for illustrating the analysis and for evaluating the degree the protectiveness of the CMEC estimated from SC measurements alone.

The relationship between SC and the presence of salt-intolerant taxa were inspected for each of the 317 sites that met the inclusion criteria in the data set. The most salt-intolerant taxa are those taxa with and  $XC_{95} < 340 \mu\text{S}/\text{cm}$ , the CCC for Ecoregion 70. Figure B-11 depicts two plots where the annual average SC is well below 340  $\mu\text{S}/\text{cm}$ . Fivemile Creek is an exceptional site with three of the seven salt-intolerant taxa, moderate temperature with some higher levels in summer months, and low SC year-round with an annual average of 240  $\mu\text{S}/\text{cm}$  and a maximum of 487  $\mu\text{S}/\text{cm}$ . Fivemile Creek meets both the CCC and the CMEC calculated for the case example. Buffalo Creek has four salt-intolerant taxa with an annual average of 158.5  $\mu\text{S}/\text{cm}$  and a maximum grab sample of 460  $\mu\text{S}/\text{cm}$ . Buffalo Creek would meet the recommended SC CCC and for Ecoregion 70.

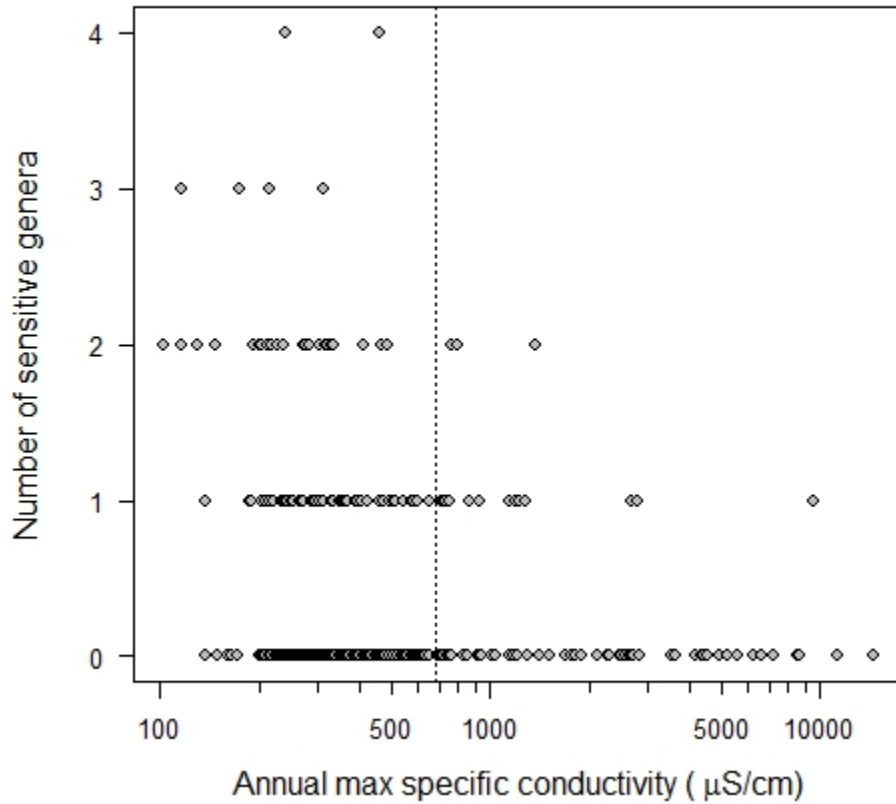




**Figure B-11. Specific conductivity and temperature variations in stations with multiple observations.** Julian day, 0 = January, is on the  $x$ -axis. Specific conductivity is on the left  $y$ -axis with water chemistry samples as open circles; a filled circle is date of biological sampling. Dashed line is at 340  $\mu\text{S}/\text{cm}$  for orientation. One rotating year is defined as the year prior to biological sample, a minimum of six samples were required for inclusion in the data set. Specific conductivity minimum (min), maximum (max), and date of biological sampling (bio) are shown in the lower left corner. The count of the seven most salt-intolerant genera (extirpation concentration  $[\text{XC}_{95}] < 340 \mu\text{S}/\text{cm}$ ) are 4 for Little Buffalo and 3 Fivemile Creeks.

As an evaluation of the CMEC, an analysis was performed to compare the calculated CMEC with an estimate of a tolerated maximum SC using biological survival as the assessment endpoint. A scatter plot was constructed of the count of the seven most salt-intolerant taxa and maximum SC that occurred in the year prior to biological sampling (see Figure B-12). The analysis showed that there is a negative relationship between maximum SC and salt-intolerant genera. There are few observations of salt-intolerant genera were observed at sites with SC  $> 680 \mu\text{S}/\text{cm}$  in this data set, the CMEC calculated from chemistry only data. The chemistry only analysis used a much more representative sample of sites comprised of 819 rotation years from 805 unique stations, with at least one sample from July to October (J–O) and one from March to June (M–J), and at least six samples within a rotation year (see Table 5-3). Because the CMEC

analysis is based on a much larger and more representative sample, the CMEC of 680  $\mu\text{S}/\text{cm}$  was retained. However, as data becomes more available, the method using biological samples may become preferable.



**Figure B-12. Scatter plot of count of salt-intolerant genera (extirpation concentration  $[\text{XC}_{95}] < 340 \mu\text{S}/\text{cm}$ ) and maximum conductivity in preceding year.** Few salt-intolerant genera are observed at sites ( $N = 317$ ) with a specific conductivity greater than the criterion maximum exposure concentration (CMEC) of  $680 \mu\text{S}/\text{cm}$  (vertical dashed line). Specific conductivity expressed as  $\mu\text{S}/\text{cm}$ .

#### B.4. CASE STUDY II: EXTIRPATION CONCENTRATION (XC<sub>95</sub>) VALUES

Order	Family	Genus	Symbol	XC <sub>95</sub>	95% CI	N
Ephemeroptera	Ephemerellidae	<i>Drunella</i>		136	127–169	78
Plecoptera	Chloroperlidae	<i>Utaperla</i>		248	193–323	31
Ephemeroptera	Heptageniidae	<i>Cinygmula</i>		258	207–329	120
Plecoptera	Chloroperlidae	<i>Alloperla</i>		275	229–307	71
Ephemeroptera	Ephemerellidae	<i>Ephemerella</i>		283	237–364	316
Ephemeroptera	Heptageniidae	<i>Heptagenia</i>		294	229–563	41
Plecoptera	Perlodidae	<i>Diploperla</i>		338	257–404	152
Ephemeroptera	Ephemerellidae	<i>Eurylophella</i>		346	270–450	146
Ephemeroptera	Heptageniidae	<i>Nixe</i>		359	314–560	200
Diptera	Dixidae	<i>Dixa</i>		398	325–1,247	25
Plecoptera	Chloroperlidae	<i>Haploperla</i>		403	341–566	212
Plecoptera	Perlodidae	<i>Isoperla</i>		460	377–567	541
Trichoptera	Glossosomatidae	<i>Agapetus</i>		466	287–502	25
Ephemeroptera	Heptageniidae	<i>Epeorus</i>		481	359–2,020	303
Trichoptera	Uenoidae	<i>Neophylax</i>		499	317–577	144
Diptera	Ceratopogonidae	<i>Bezzia</i>		514	281–563	37
Ephemeroptera	Ameletidae	<i>Ameletus</i>		567	272–4,884	244
Diptera	Chironomidae	<i>Demicryptochironomus</i>		618	297–857	66
Diptera	Chironomidae	<i>Zavrelia</i>		627	275–1,383	60
Diptera	Chironomidae	<i>Conchapelopia</i>		640	393–1,175	121
Plecoptera	Perlidae	<i>Eccoptura</i>		648	440–1,028	51
Ephemeroptera	Baetidae	<i>Plauditus</i>		688	567–756	365
Ephemeroptera	Baetidae	<i>Dipheter</i>		701	565–951	133
Ephemeroptera	Leptophlebiidae	<i>Paraleptophlebia</i>		706	508–812	400
Ephemeroptera	Baetidae	<i>Procloeon</i>		708	646–1,252	87
Ephemeroptera	Heptageniidae	<i>Leucrocuta</i>		727	358–1,082	217
Ephemeroptera	Baetiscidae	<i>Baetisca</i>		757	264–762	34
Ephemeroptera	Heptageniidae	<i>Maccaffertium</i>		783	672–1,017	440
Trichoptera	Limnephilidae	<i>Pycnopsyche</i>	~	784	456–1,228	26
Ephemeroptera	Leptophlebiidae	<i>Leptophlebia</i>		805	277–912	59
Coleoptera	Dytiscidae	<i>Hydroporus</i>		822	347–822	37
Plecoptera	Peltoperlidae	<i>Peltoperla</i>	~	824	379–1,175	32
Plecoptera	Nemouridae	<i>Amphinemura</i>		911	531–3,725	618
Diptera	Chironomidae	<i>Pothastia</i>		944	480–1,059	32
Ephemeroptera	Heptageniidae	<i>Stenonema</i>		945	653–1,075	614

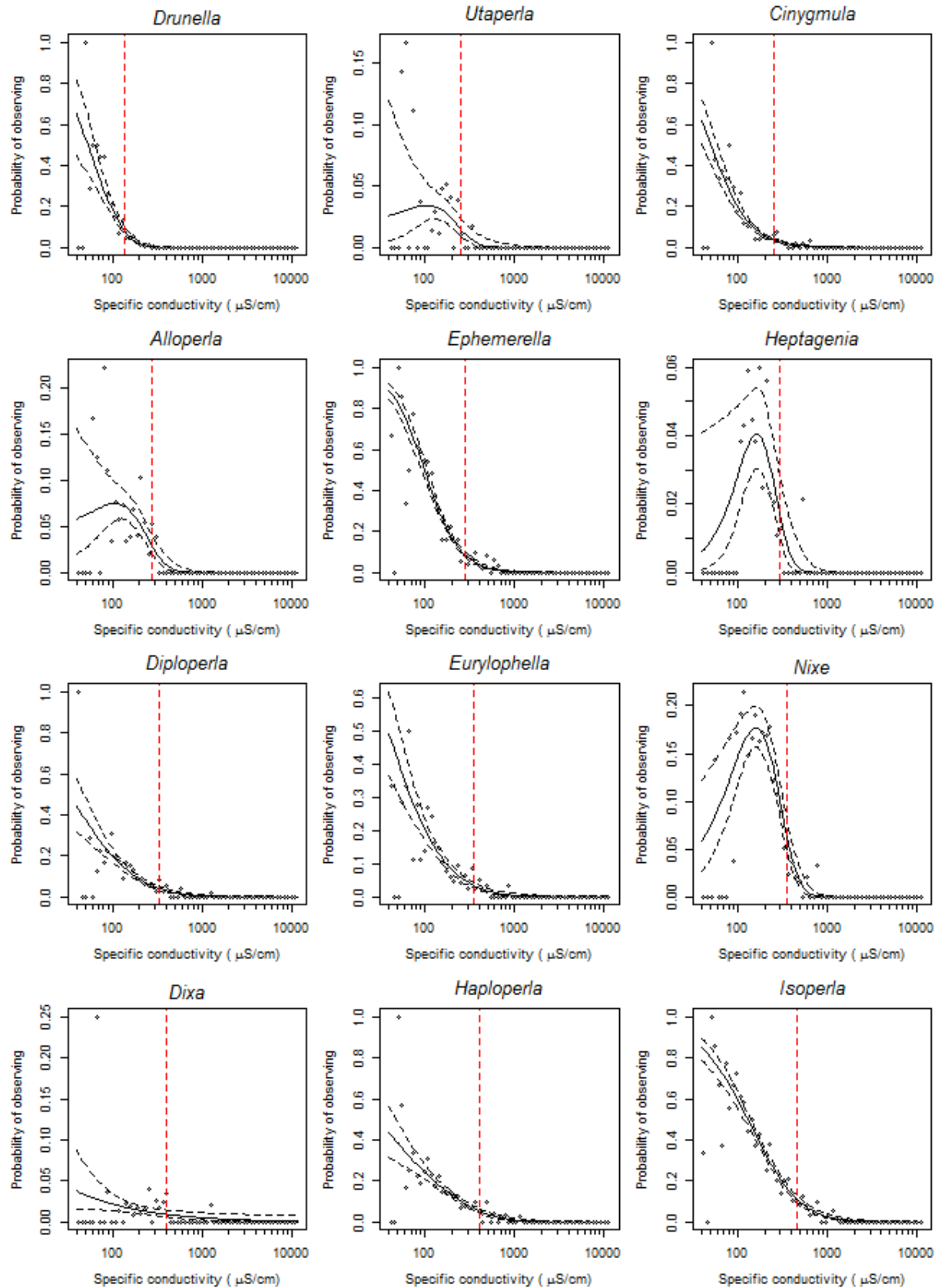
Order	Family	Genus	Symbol	XC <sub>95</sub>	95% CI	N
Trichoptera	Philopotamidae	<i>Wormaldia</i>	~	947	459–1,261	35
Ephemeroptera	Baetidae	<i>Acentrella</i>		986	505–3,162	567
Isopoda	Asellidae	<i>Asellus</i>		1,014	365–1,014	26
Ephemeroptera	Isonychiidae	<i>Isonychia</i>		1,017	805–1,129	654
Plecoptera	Perlodidae	<i>Cultus</i>	~	1,073	307–1,398	27
Diptera	Chironomidae	<i>Stempellinella</i>		1,077	951–1,338	262
Odonata	Gomphidae	<i>Lanthus</i>	~	1,091	566–1,175	29
Ephemeroptera	Heptageniidae	<i>Stenacron</i>	~	1,100	973–1,195	245
Ephemeroptera	Baetidae	<i>Centroptilum</i>	~	1,137	508–1,195	72
Isopoda	Asellidae	<i>Lirceus</i>		1,247	566–1,534	131
Decapoda	Cambaridae	<i>Cambarus</i>	>	1,278	1,046–1,540	307
Diptera	Chironomidae	<i>Parachaetocladius</i>	>	1,285	1,166–1,665	40
Diptera	Chironomidae	<i>Brillia</i>	>	1,301	582–1,526	45
Coleoptera	Psephenidae	<i>Ectopria</i>	>	1,346	978–2,148	214
Veneroida	Pisidiidae	<i>Pisidium</i>	>	1,402	1,287–1,470	49
Diptera	Chironomidae	<i>Parakiefferiella</i>	>	1,569	1,419–1,700	52
Coleoptera	Elmidae	<i>Macronychus</i>	>	1,605	1,195–1,678	63
Ephemeroptera	Baetidae	<i>Baetis</i>		1,620	1,197–2,580	1,222
Diptera	Ceratopogonidae	<i>Dasyhelea</i>	>	1,696	1,136–1,864	48
Diptera	Chironomidae	<i>Natarsia</i>	>	1,786	1,613–1,842	48
Diptera	Empididae	<i>Chelifera</i>	>	1,845	1,589–1,870	39
Diptera	Chironomidae	<i>Cardiocladius</i>	>	1,951	1,270–1,951	120
Diptera	Chironomidae	<i>Pagastia</i>	>	1,970	1,480–1,970	38
Diptera	Chironomidae	<i>Eukiefferiella</i>	>	1,977	1,598–2,523	305
Trichoptera	Rhyacophilidae	<i>Rhyacophila</i>	~	1,977	631–5,057	191
Amphipoda	Crangonyctidae	<i>Crangonyx</i>	>	1,978	734–1,978	111
Ephemeroptera	Ephemeridae	<i>Ephemerella</i>		1,978	475–1,978	90
Hemiptera	Veliidae	<i>Rhagovelia</i>	>	2,030	1,171–2,030	27
Diptera	Simuliidae	<i>Prosimulium</i>	>	2,148	550–2,439	141
Plecoptera	Leuctridae	<i>Leuctra</i>	>	2,257	1,523–2,791	1,010
Diptera	Chironomidae	<i>Sublettea</i>	>	2,294	1,367–2,294	124
Diptera	Chironomidae	<i>Chaetocladius</i>	>	2,320	1,700–5,057	170
Diptera	Chironomidae	<i>Krenopelopia</i>	>	2,320	1,700–2,320	44
Diptera	Chironomidae	<i>Phaenopsectra</i>	>	2,332	1,348–2,332	61
Diptera	Tipulidae	<i>Tipula</i>	>	2,420	1,902–6,492	532
Hemiptera	Veliidae	<i>Microvelia</i>	>	2,523	978–2,523	31

Order	Family	Genus	Symbol	XC <sub>95</sub>	95% CI	N
Diptera	Chironomidae	<i>Orthocladius</i>		2,523	500–2,523	167
Diptera	Chironomidae	<i>Rheopelopia</i>		2,523	410–2,523	72
Coleoptera	Elmidae	<i>Microcylloepus</i>	>	2,558	1,397–2,558	76
Diptera	Chironomidae	<i>Tvetenia</i>	>	2,573	1,729–2,791	516
Diptera	Chironomidae	<i>Nilotanypus</i>	>	2,630	1,422–2,630	119
Trichoptera	Polycentropodidae	<i>Polycentropus</i>	>	2,641	1,410–4,713	192
Trichoptera	Hydroptilidae	<i>Ochrotrichia</i>	>	2,791	1,143–2,791	48
Decapoda	Cambaridae	<i>Orconectes</i>	>	3,162	1,520–3,162	211
Diptera	Chironomidae	<i>Paratanytarsus</i>	>	3,489	3,162–5,258	102
Ephemeroptera	Caenidae	<i>Caenis</i>	>	3,884	2,641–4,052	693
Diptera	Chironomidae	<i>Microtendipes</i>	>	3,972	2,437–7,053	462
Diptera	Chironomidae	<i>Rheotanytarsus</i>	>	4,400	2,605–5,468	875
Diptera	Chironomidae	<i>Corynoneura</i>	>	4,636	980–4,636	125
Diptera	Chironomidae	<i>Diamesa</i>	>	4,636	1,924–4,713	463
Diptera	Chironomidae	<i>Polypedilum</i>	>	4,636	3,314–7,093	1,598
Diptera	Chironomidae	<i>Rheocricotopus</i>	>	4,636	1,902–4,884	533
Megaloptera	Sialidae	<i>Sialis</i>	>	4,636	3,714–11,227	241
Isopoda	Asellidae	<i>Caecidotea</i>	>	4,713	1,977–4,713	178
Diptera	Empididae	<i>Clinocera</i>	>	4,713	577–4,713	36
Amphipoda	Gammaridae	<i>Gammarus</i>	>	4,713	2,320–10,350	232
Diptera	Chironomidae	<i>Parametrioctenemus</i>	>	4,713	2,580–4,884	1,266
Diptera	Chironomidae	<i>Zavreliomyia</i>	>	4,884	1,589–4,884	212
Coleoptera	Elmidae	<i>Oulimnius</i>	>	5,000	824–5,000	30
Diptera	Tipulidae	<i>Limonia</i>	>	5,057	1,687–5,057	35
Diptera	Chironomidae	<i>Limnophyes</i>	>	5,120	1,445–5,120	48
Diptera	Chironomidae	<i>Cryptochironomus</i>	>	5,258	2,580–7,093	356
Diptera	Chironomidae	<i>Larsia</i>	>	5,258	875–5,258	98
Diptera	Chironomidae	<i>Tribelos</i>	>	5,258	1,081–5,258	55
Diptera	Tipulidae	<i>Antocha</i>	>	6,468	3,972–7,093	433
Diptera	Chironomidae	<i>Micropsectra</i>	>	6,468	2,471–6,468	173
Diptera	Chironomidae	<i>Paraphaenocladius</i>	>	6,468	863–6,468	43
Diptera	Simuliidae	<i>Simulium</i>	>	6,468	2,874–7,053	1,001
Odonata	Gomphidae	<i>Stylogomphus</i>	>	6,468	2,320–6,468	70
Trichoptera	Hydropsychidae	<i>Ceratopsyche</i>	>	6,492	4,713–7,010	745
Trichoptera	Philopotamidae	<i>Chimarra</i>	>	6,492	3,489–7,053	587
Trichoptera	Hydropsychidae	<i>Diplectrona</i>	>	6,492	1,870–6,492	277

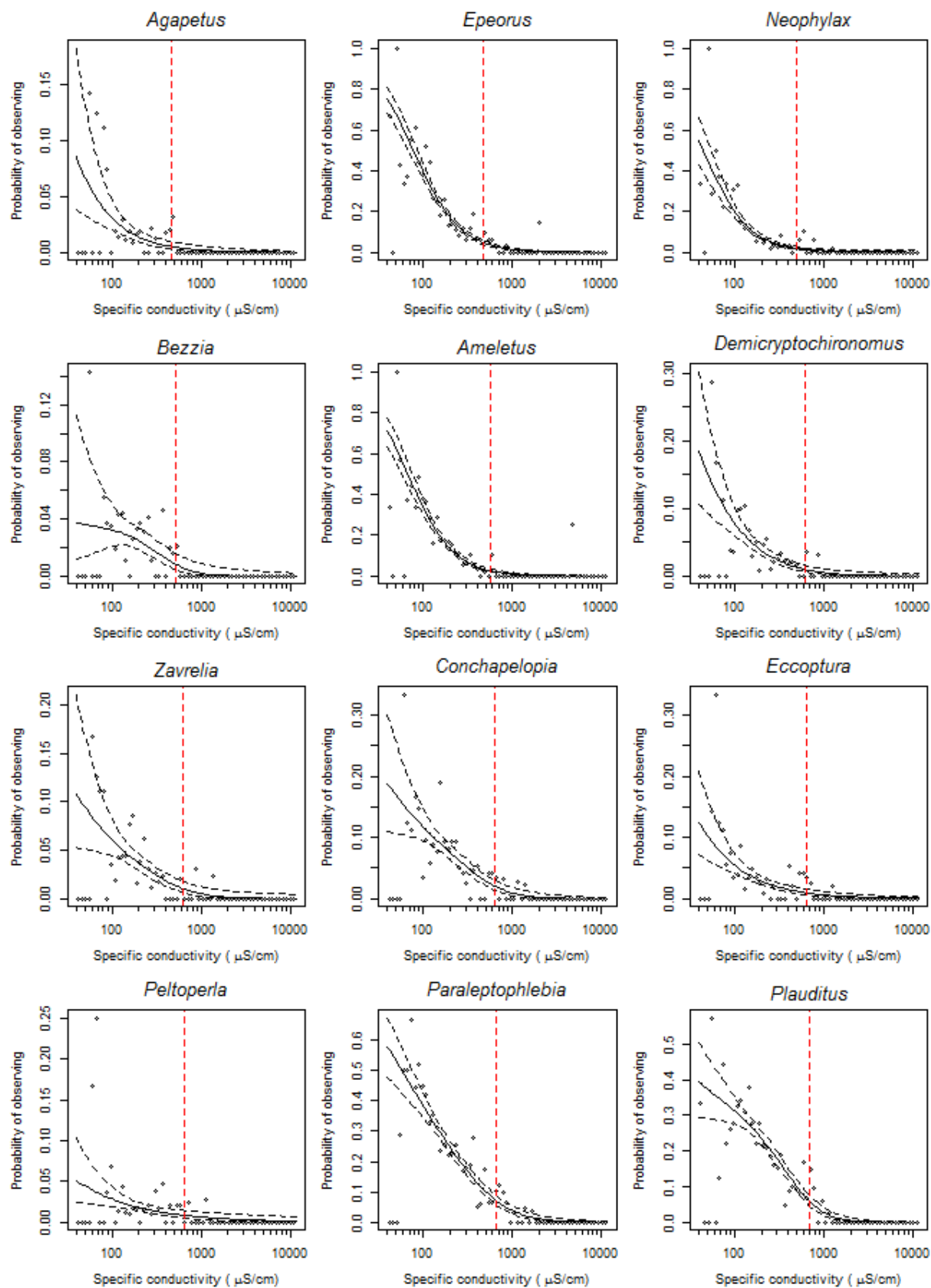
Order	Family	Genus	Symbol	XC <sub>95</sub>	95% CI	N
Plecoptera	Perlidae	<i>Perlesta</i>		6,492	1,139–6,492	453
Diptera	Tipulidae	<i>Pseudolimnophila</i>	>	6,492	1,589–6,492	142
Plecoptera	Chloroperlidae	<i>Sweltsa</i>	~	6,492	916–6,492	256
Diptera	Chironomidae	<i>Thienemannimyia</i>	>	6,492	4,400–7,093	1,361
Coleoptera	Elmidae	<i>Stenelmis</i>	>	6,620	3,972–7,370	1,705
Trichoptera	Philopotamidae	<i>Dolophilodes</i>	>	7,053	588–7,053	85
Coleoptera	Psephenidae	<i>Psephenus</i>	>	7,105	4,884–7,370	1,000
Odonata	Aeshnidae	<i>Boyeria</i>	>	7,340	2,407–7,340	133
Megaloptera	Corydalidae	<i>Nigronia</i>	>	7,340	3,162–9,790	503
Basommatophora	Physidae	<i>Physella</i>	>	7,340	6,468–9,790	183
Diptera	Tipulidae	<i>Dicranota</i>	>	7,370	2,145–7,370	233
Coleoptera	Elmidae	<i>Dubiraphia</i>	>	7,370	3,162–7,370	200
Diptera	Tipulidae	<i>Hexatoma</i>	>	7,370	6,468–9,790	811
Coleoptera	Elmidae	<i>Optioservus</i>	>	7,370	4,713–9,790	1,231
Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i>	>	9,180	5,266–9,180	1,569
Diptera	Chironomidae	<i>Tanytarsus</i>	>	9,180	4,636–9,790	1,183
Diptera	Tabanidae	<i>Tabanus</i>	>	9,790	2,291–9,790	72
Diptera	Chironomidae	<i>Thienemanniella</i>	>	9,790	6,573–11,227	364
Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	>	10,140	4,884–10,140	769
Diptera	Empididae	<i>Hemerodromia</i>	>	10,350	7,010–11,646	427
Megaloptera	Corydalidae	<i>Corydalus</i>	>	11,227	7,340–11,646	257
Diptera	Chironomidae	<i>Cricotopus</i>	>	11,227	6,468–11,646	504
Diptera	Chironomidae	<i>Dicrotendipes</i>	>	11,227	9,790–11,646	313
Trichoptera	Hydroptilidae	<i>Hydroptila</i>	>	11,227	4,884–11,646	386
Diptera	Chironomidae	<i>Procladius</i>	>	11,227	2,630–11,227	35
Diptera	Chironomidae	<i>Ablabesmyia</i>	>	11,582	7,370–11,646	184
Plecoptera	Perlidae	<i>Acroneuria</i>	>	11,646	1,066–11,646	287
Diptera	Athericidae	<i>Atherix</i>	>	11,646	7,340–11,646	80
Diptera	Tabanidae	<i>Chrysops</i>	>	11,646	7,053–11,646	70
Diptera	Chironomidae	<i>Cladotanytarsus</i>	>	11,646	5,253–11,646	121
Coleoptera	Dryopidae	<i>Helichus</i>	>	11,646	1,270–11,646	325
Diptera	Chironomidae	<i>Pseudochironomus</i>	>	11,646	4,400–11,646	50

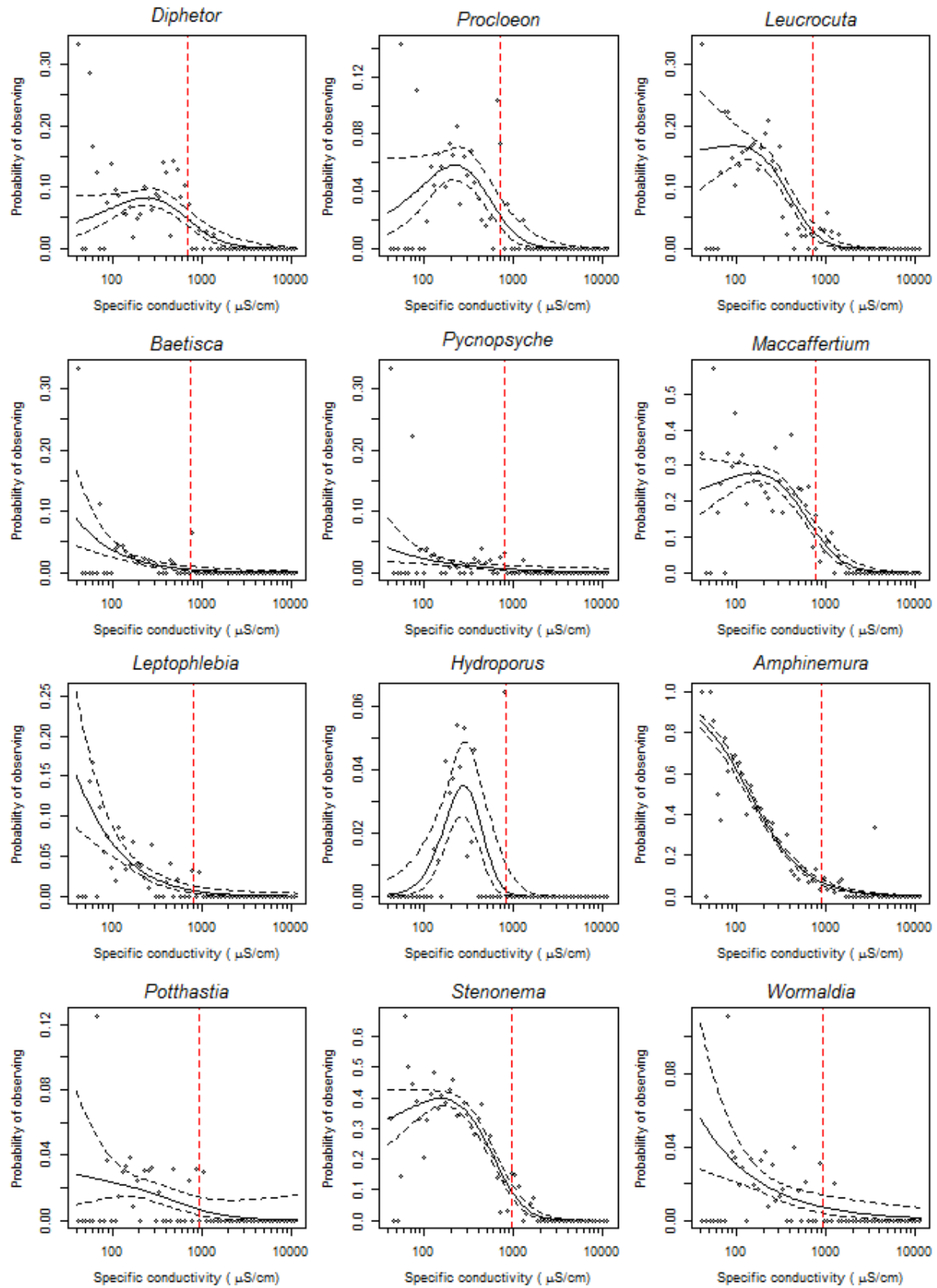
## **B.5. CASE STUDY II: GENERALIZED ADDITIVE MODEL (GAM) PLOTS**

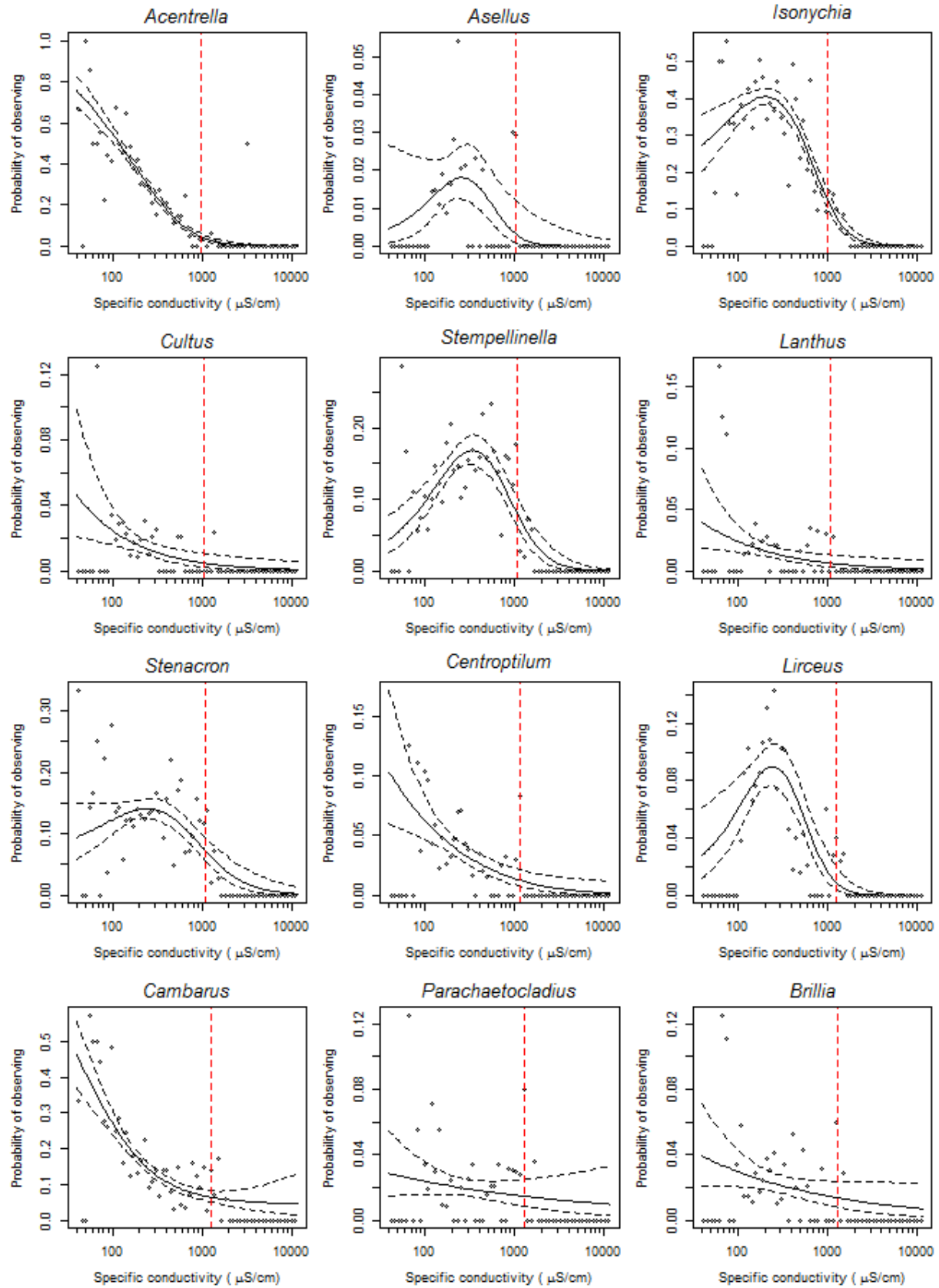
The generalized additive model (GAM) plots in this Appendix Section B.5 were used to designate ~ and > values for those  $XC_{95}$  values listed in Appendix Section B.4. In this example, the probability of observing a genus is the proportion of sampled stations in a conductivity bin with the genus present based on taxonomic identification of 200 individuals per sample. Conductivity is reported as specific conductivity. The red, dashed vertical line is the  $XC_{95}$  value for the genus (see Section B.4) obtained from the plots of the cumulative distribution function's (CDFs) in Appendix Section B.6. Plots are arranged from the lowest to the highest  $XC_{95}$  value.

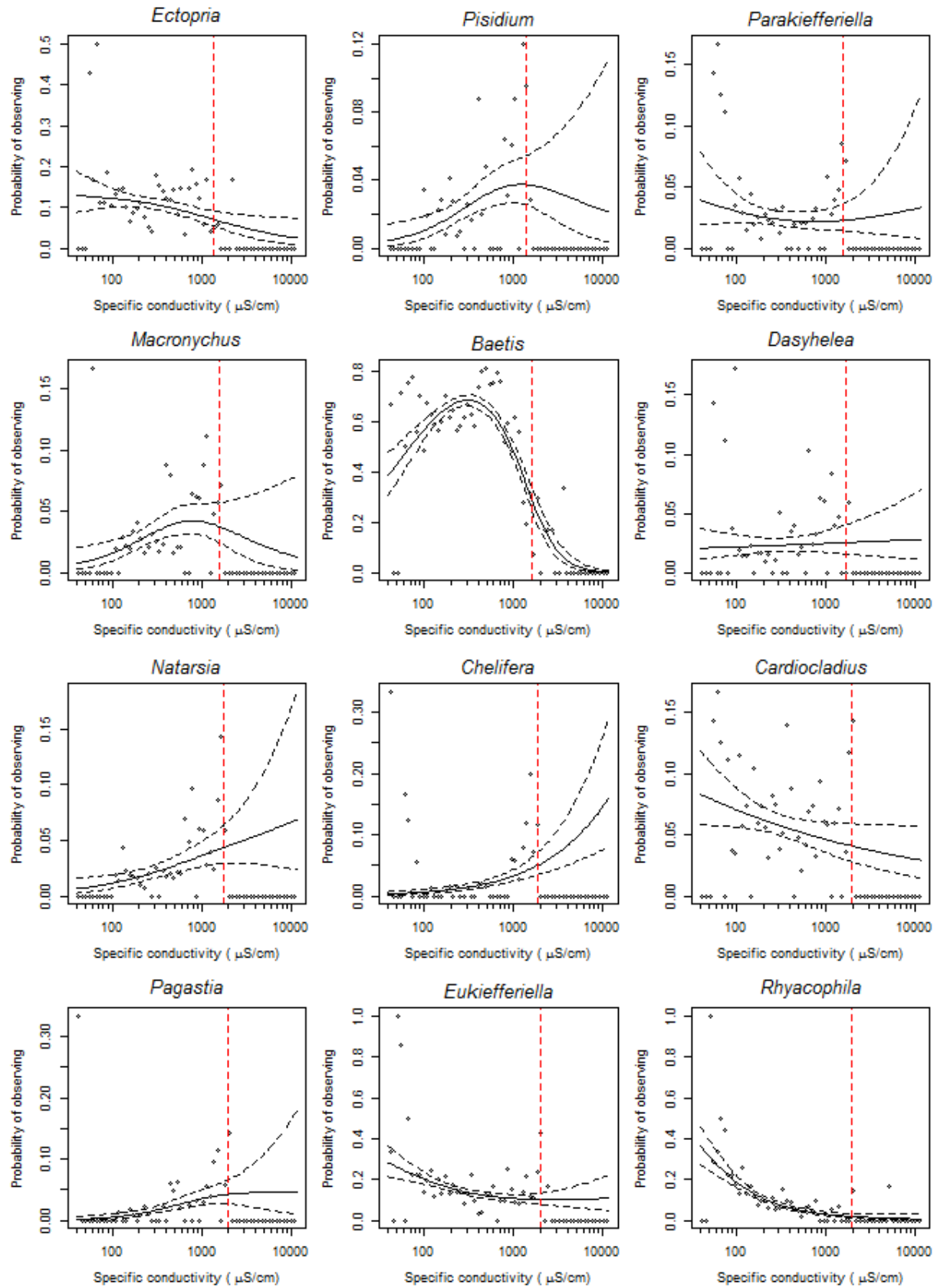


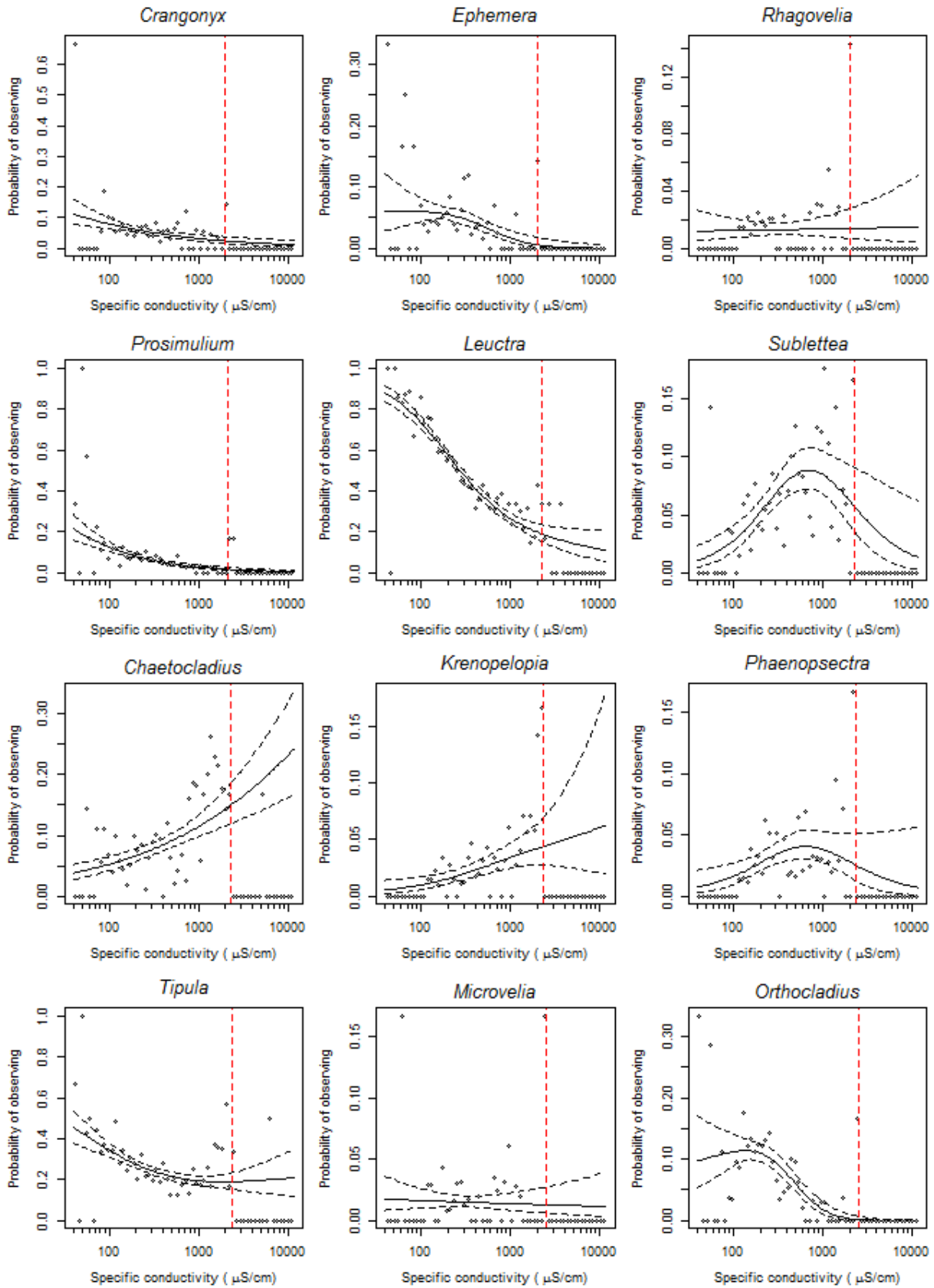


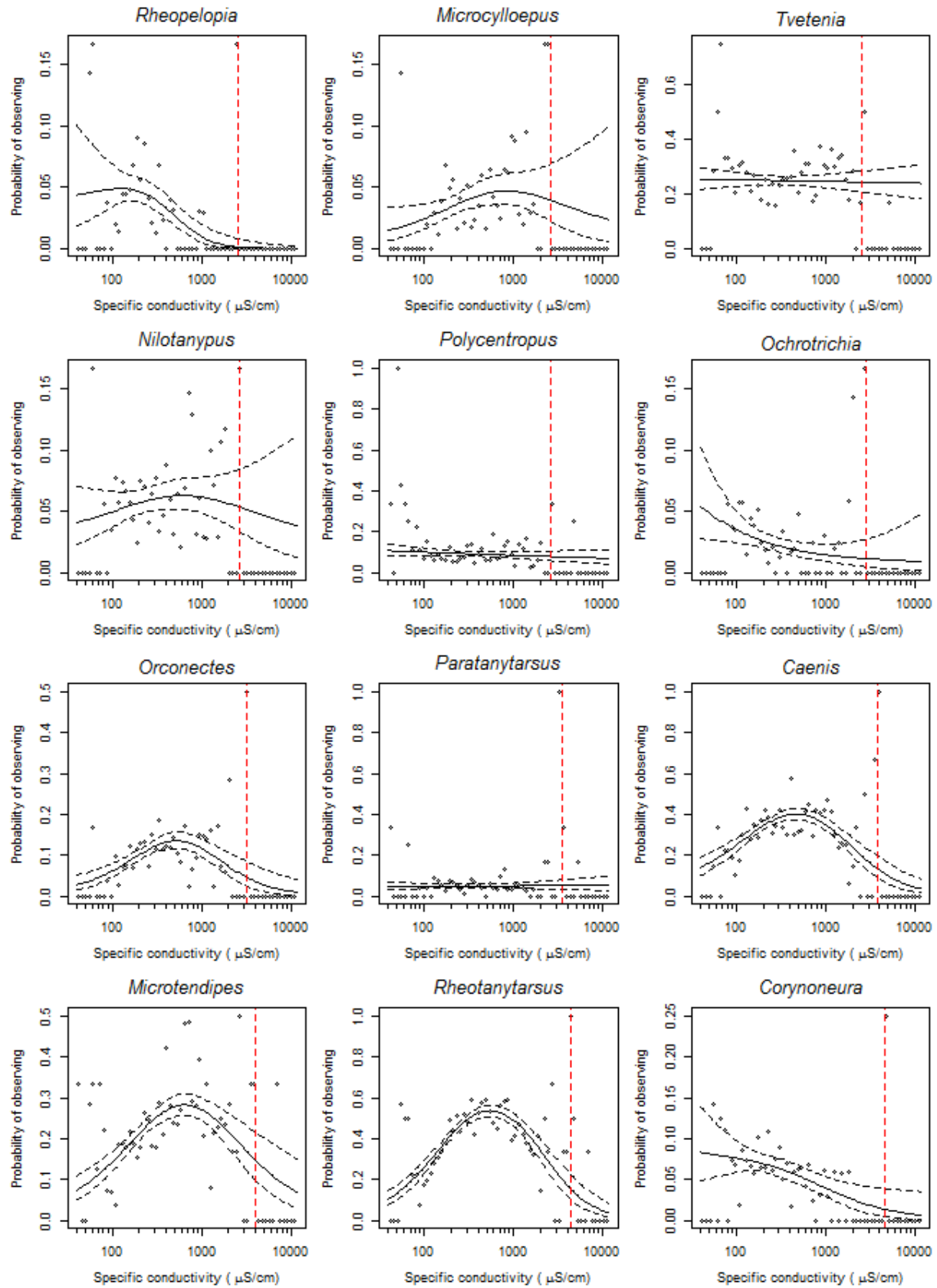


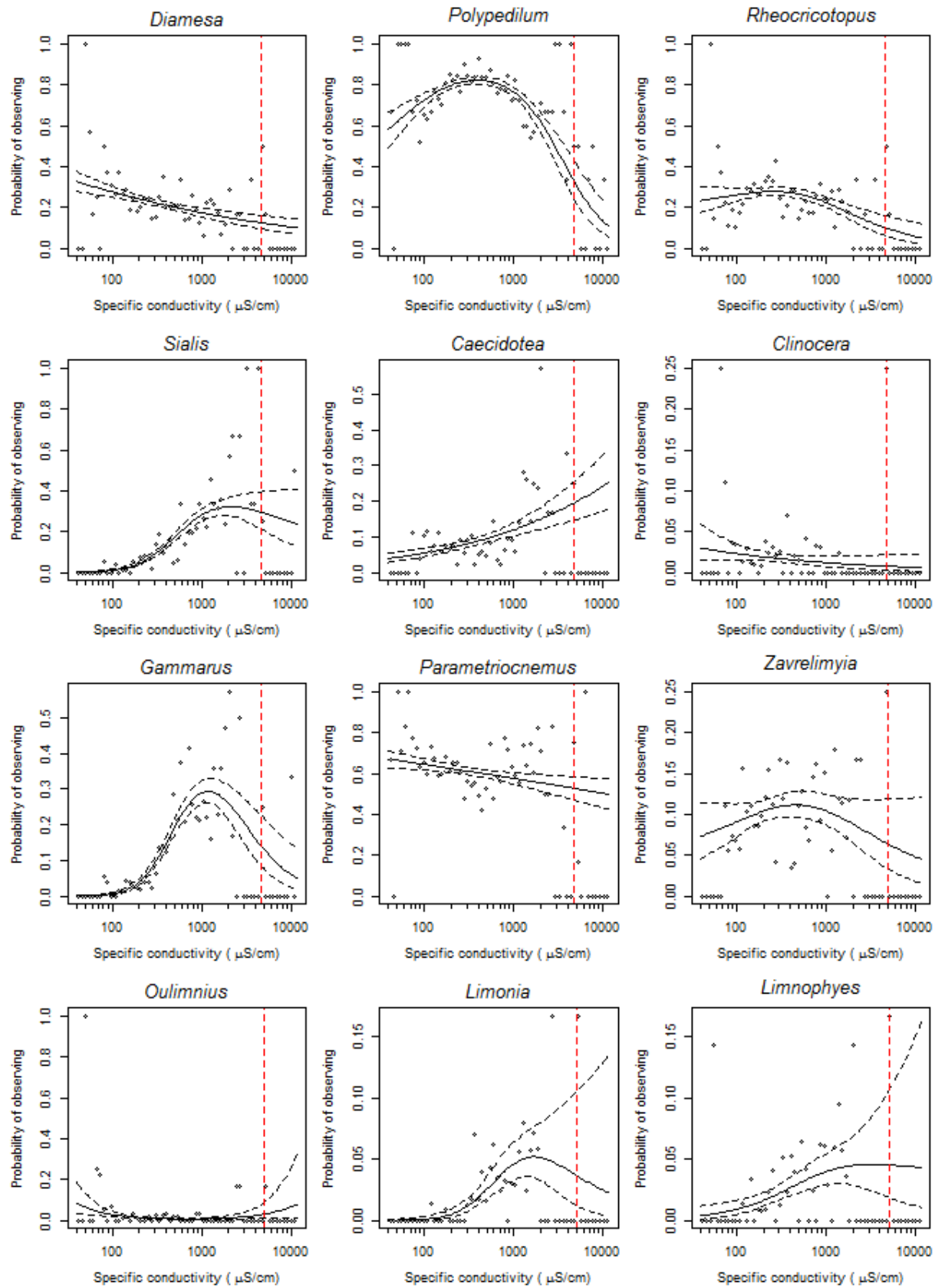


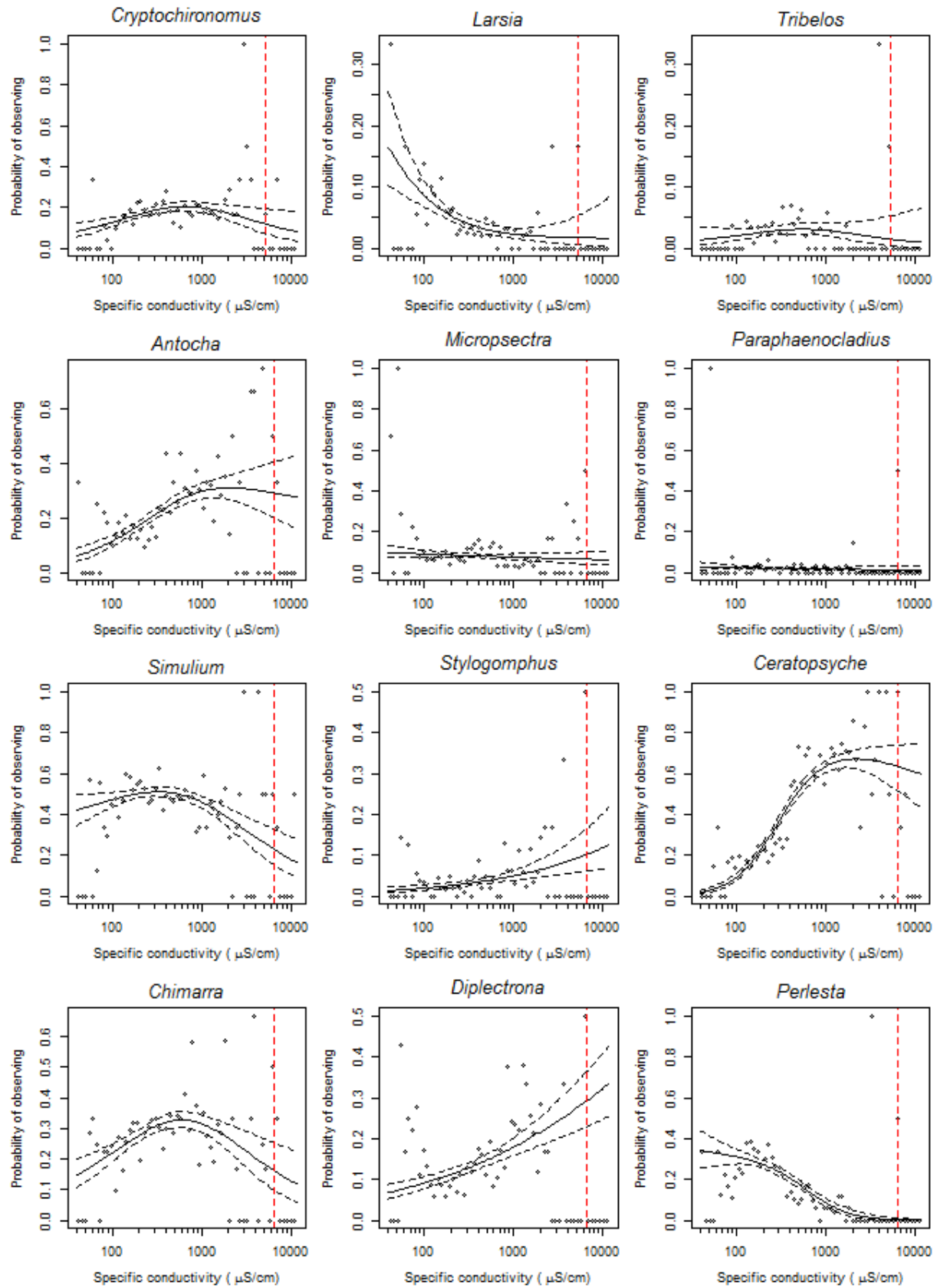




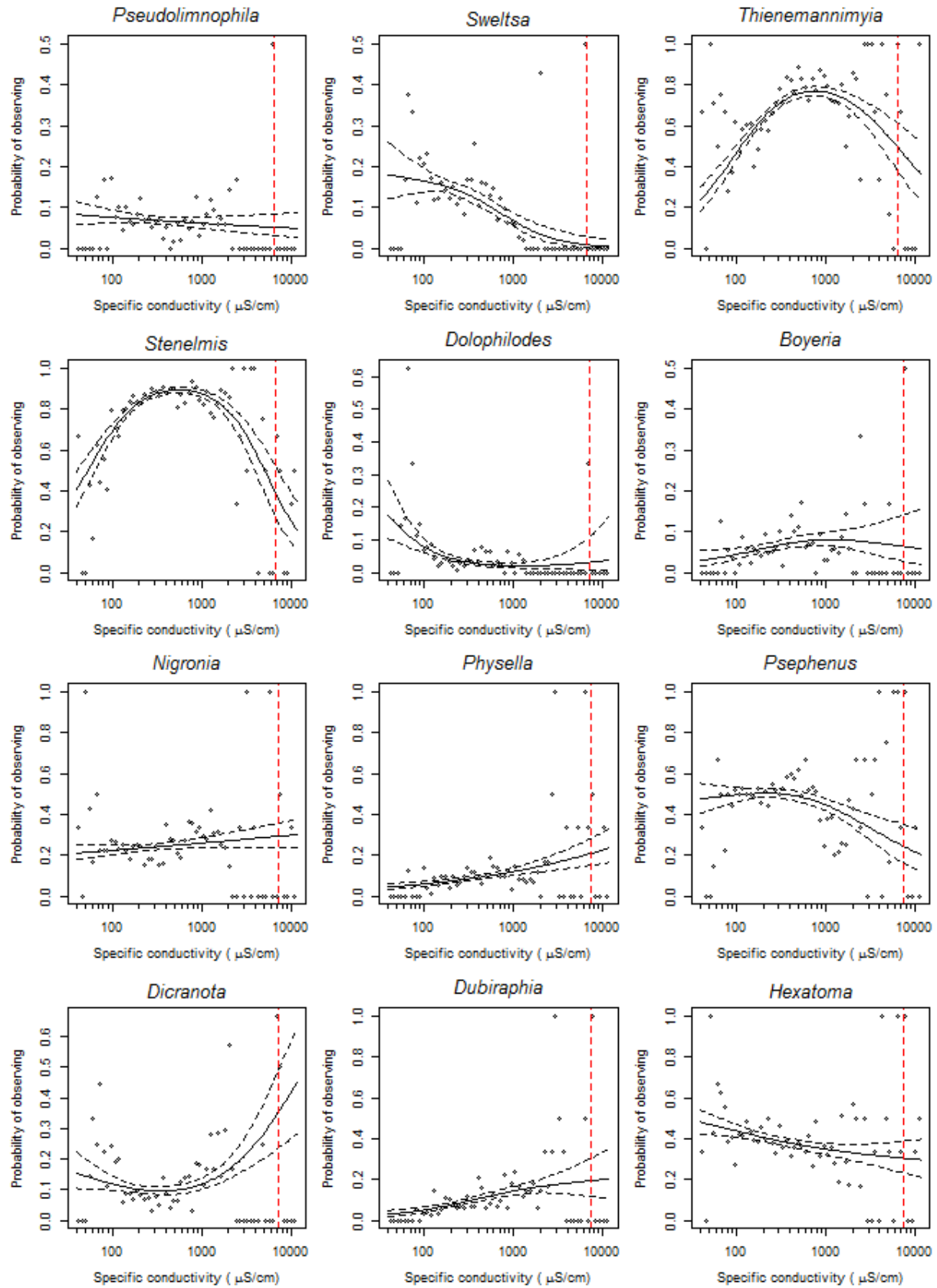


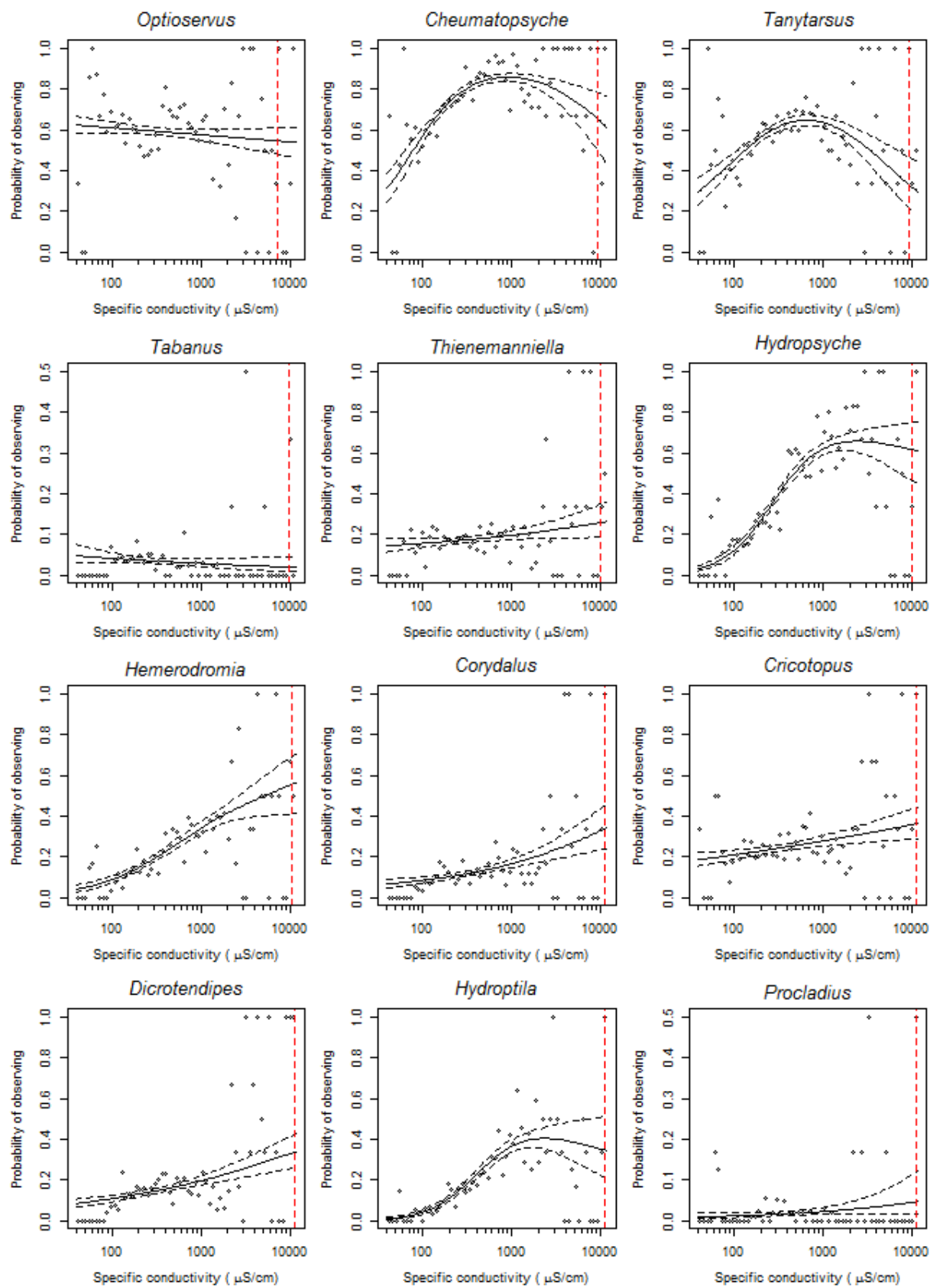


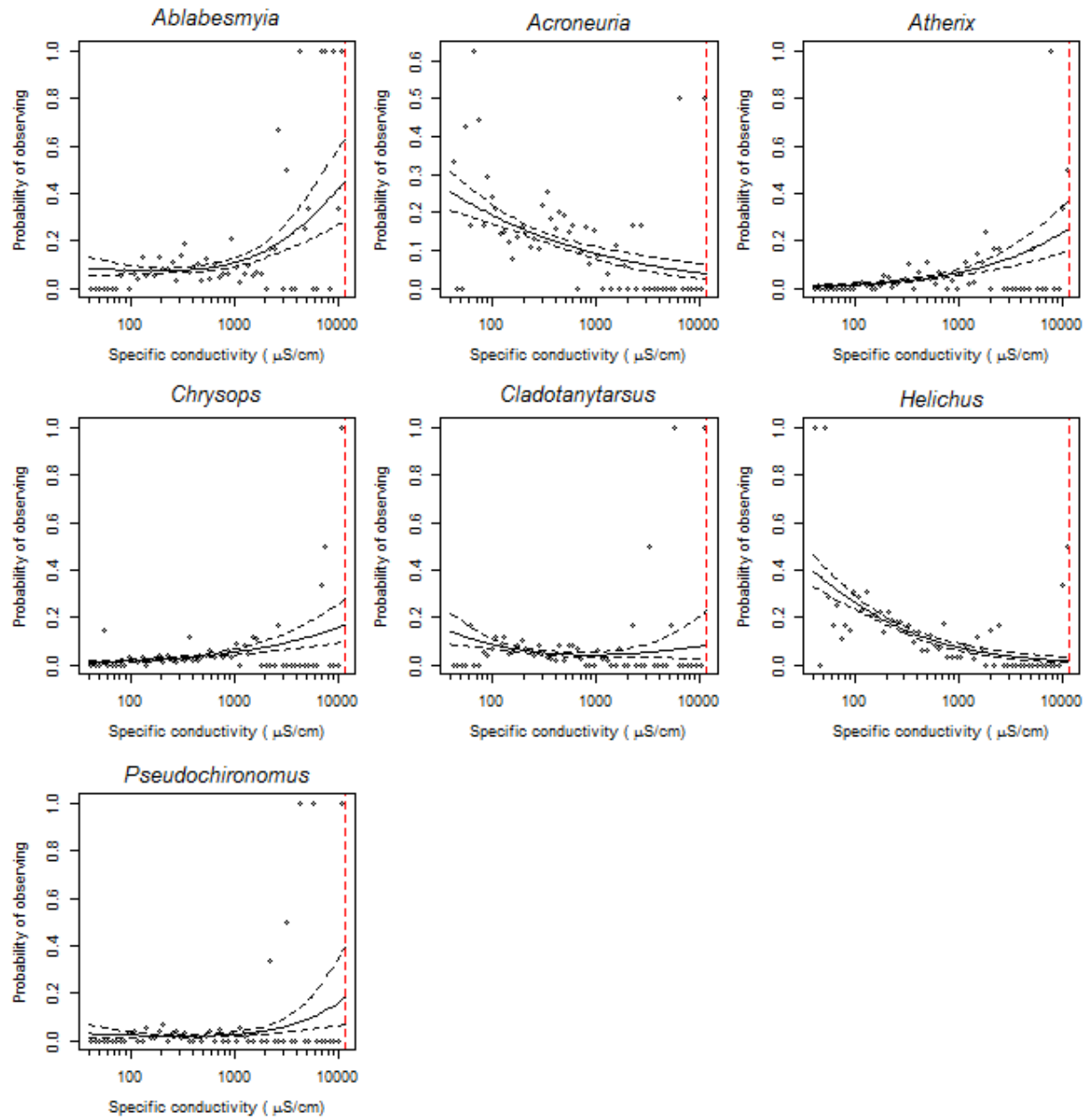






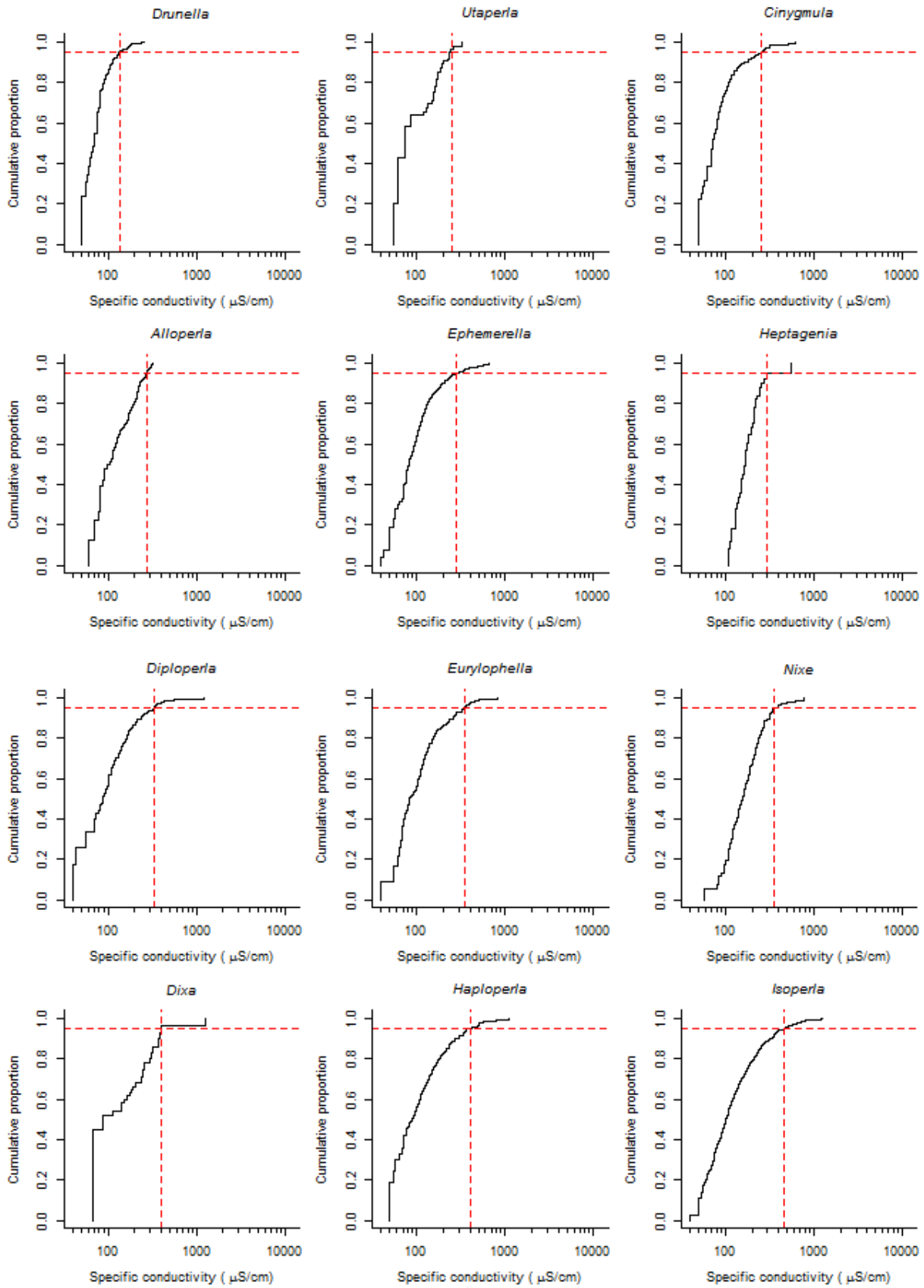


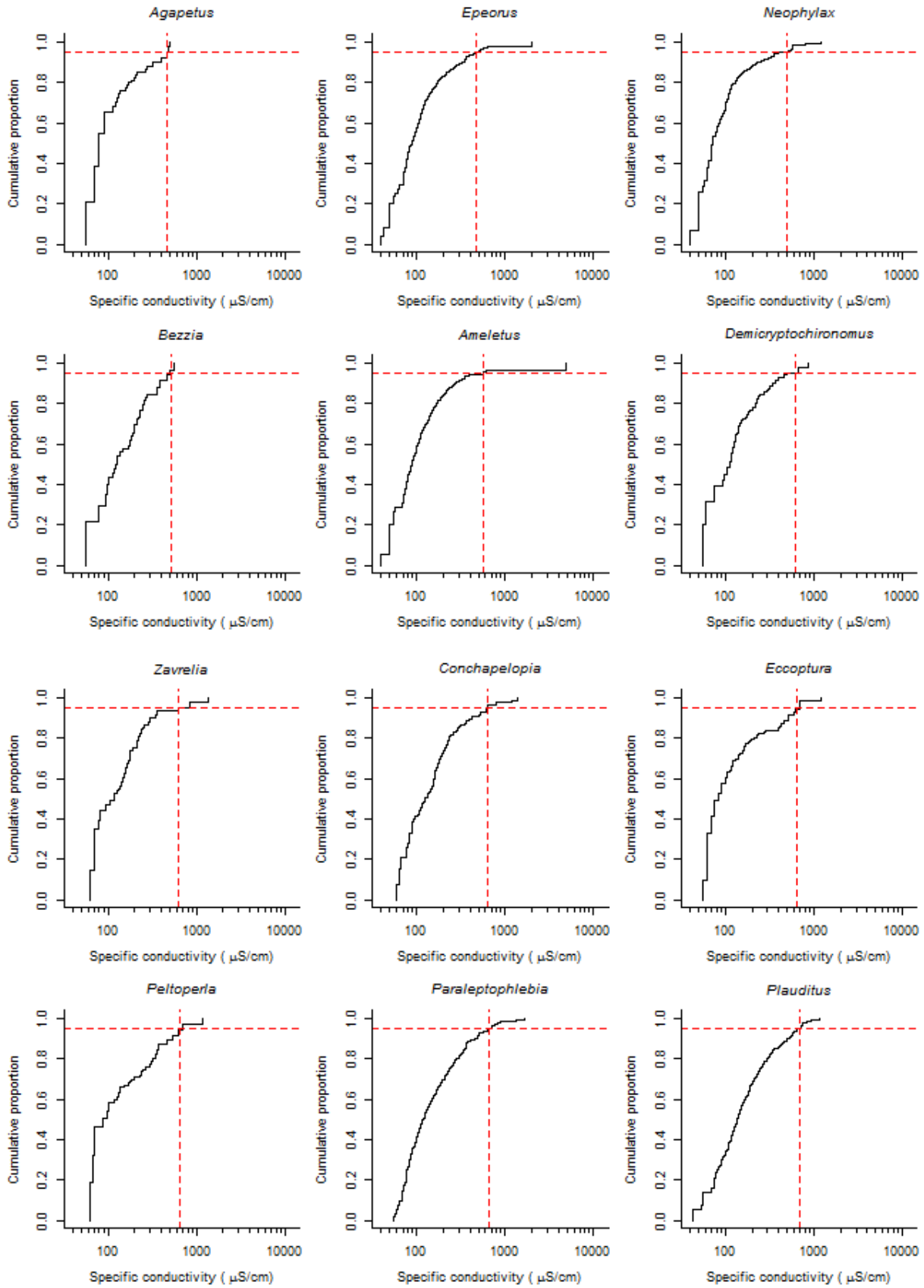


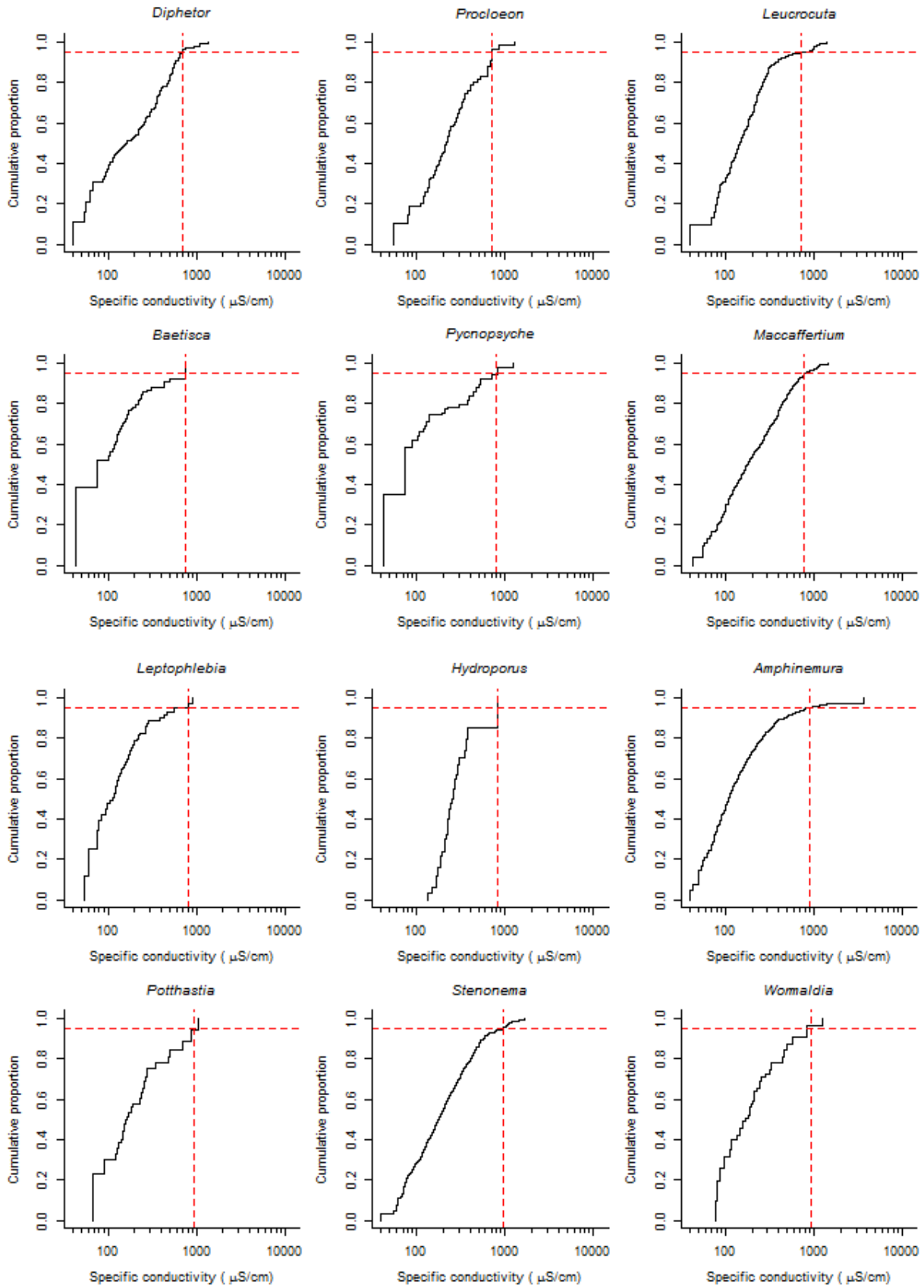


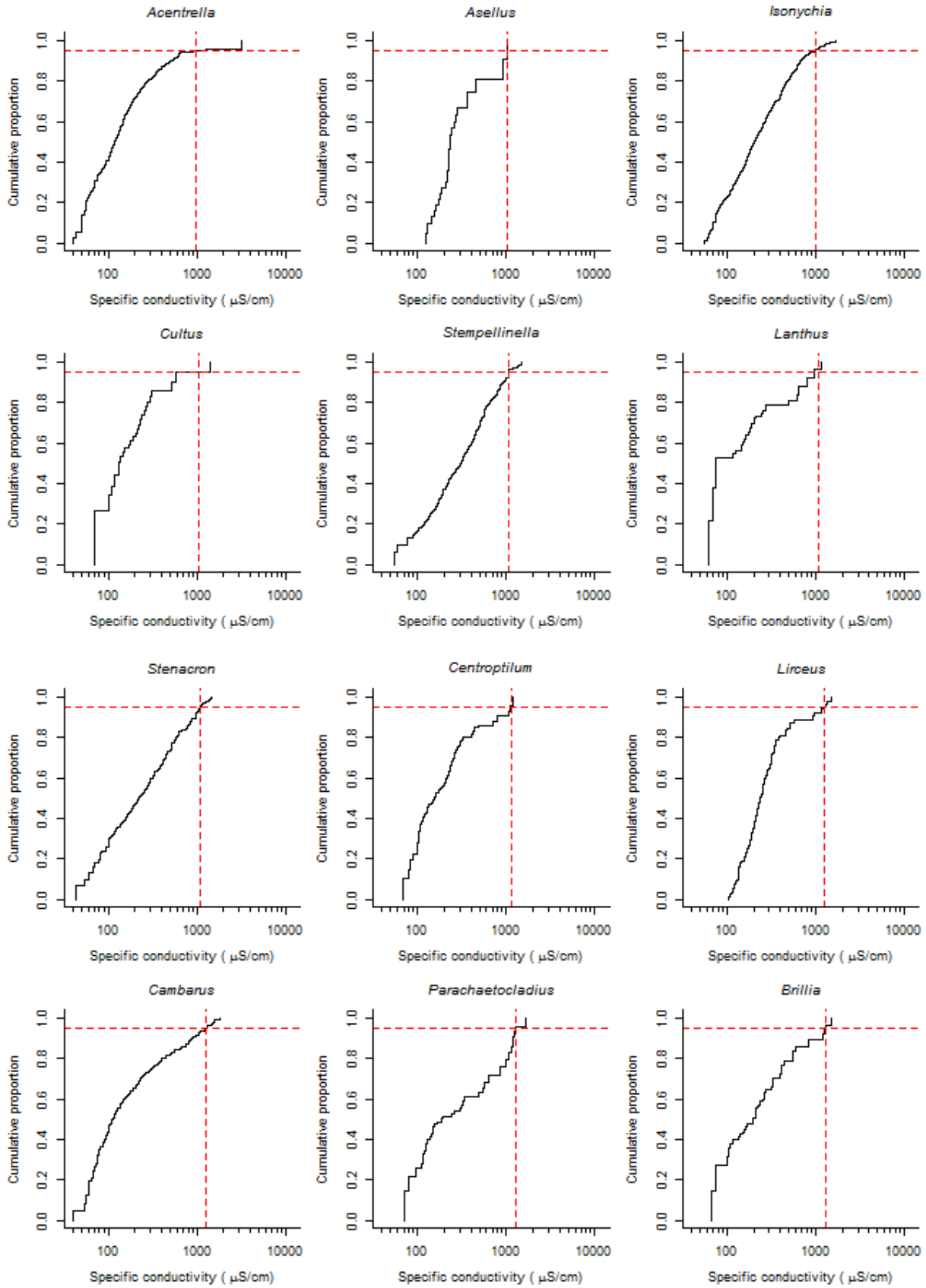
## **B.6. CASE STUDY II: CUMULATIVE DISTRIBUTION FUNCTION (CDF) PLOTS**

The CDFs used to derive the  $XC_{95}$  values are shown in this Appendix Section B.6. Conductivity is reported as specific conductivity. The red, dashed vertical line is the  $XC_{95}$  value for the genus (see Section B.4) obtained from each plotted CDF in Appendix Section B.6. Plots are arranged from the lowest to the highest  $XC_{95}$  value.

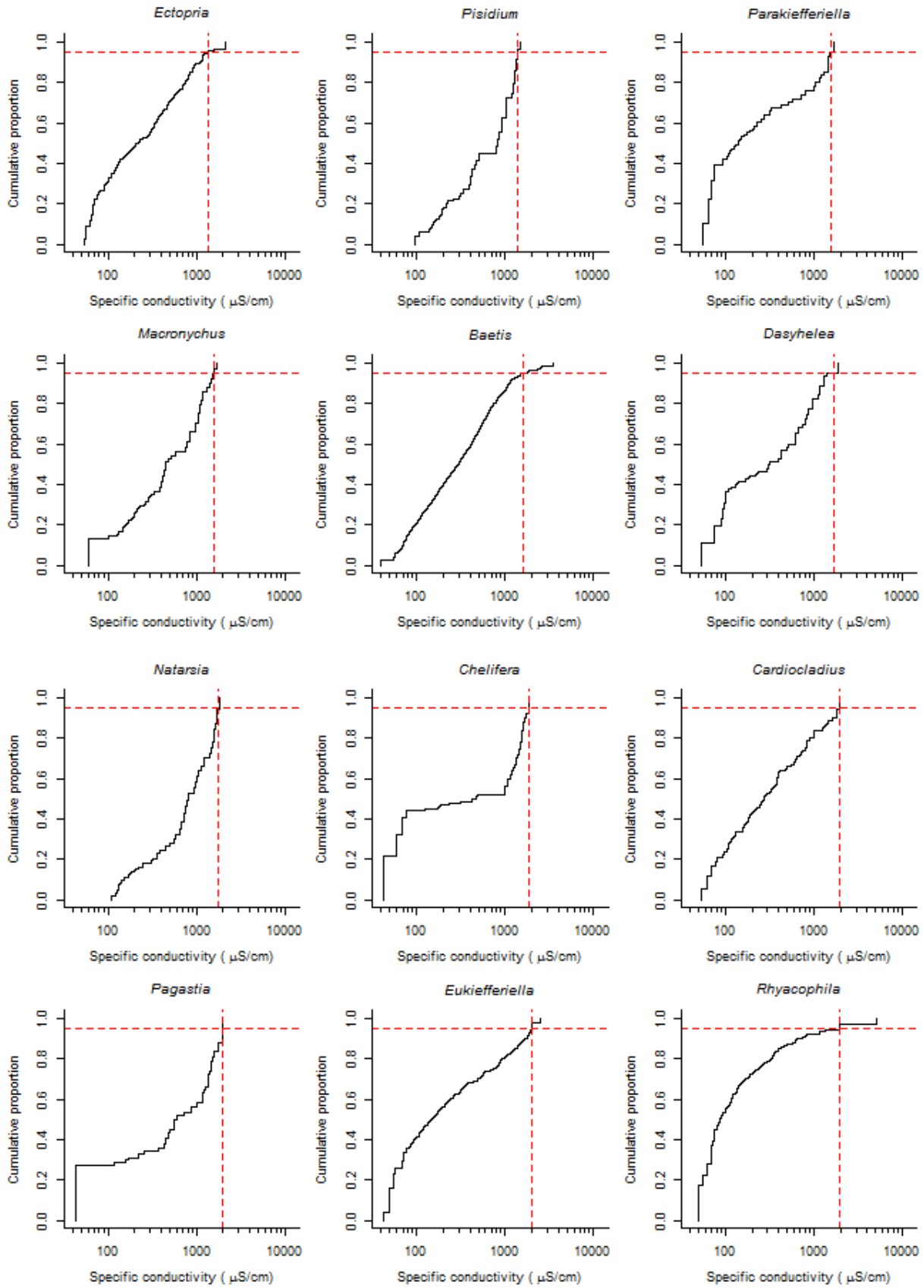


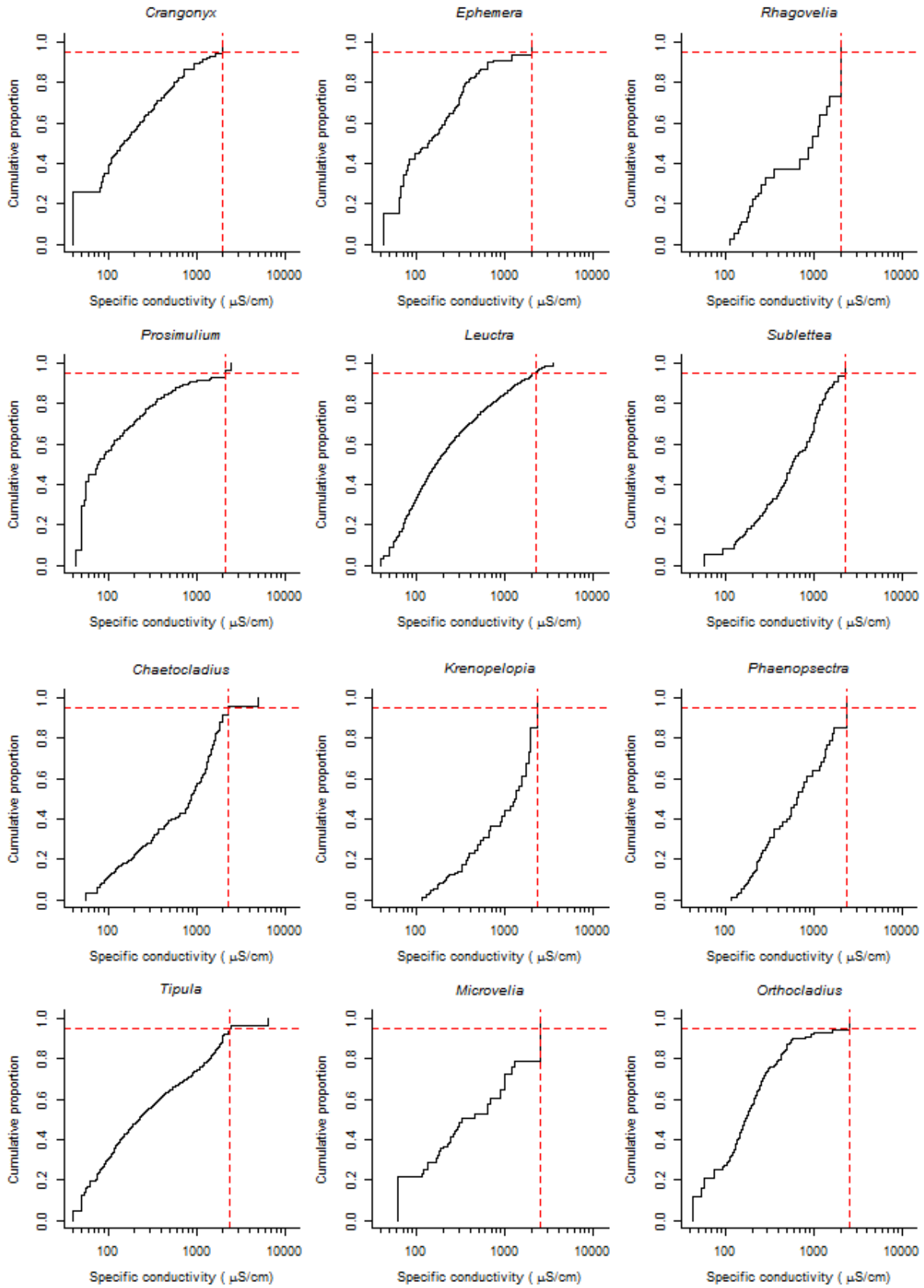


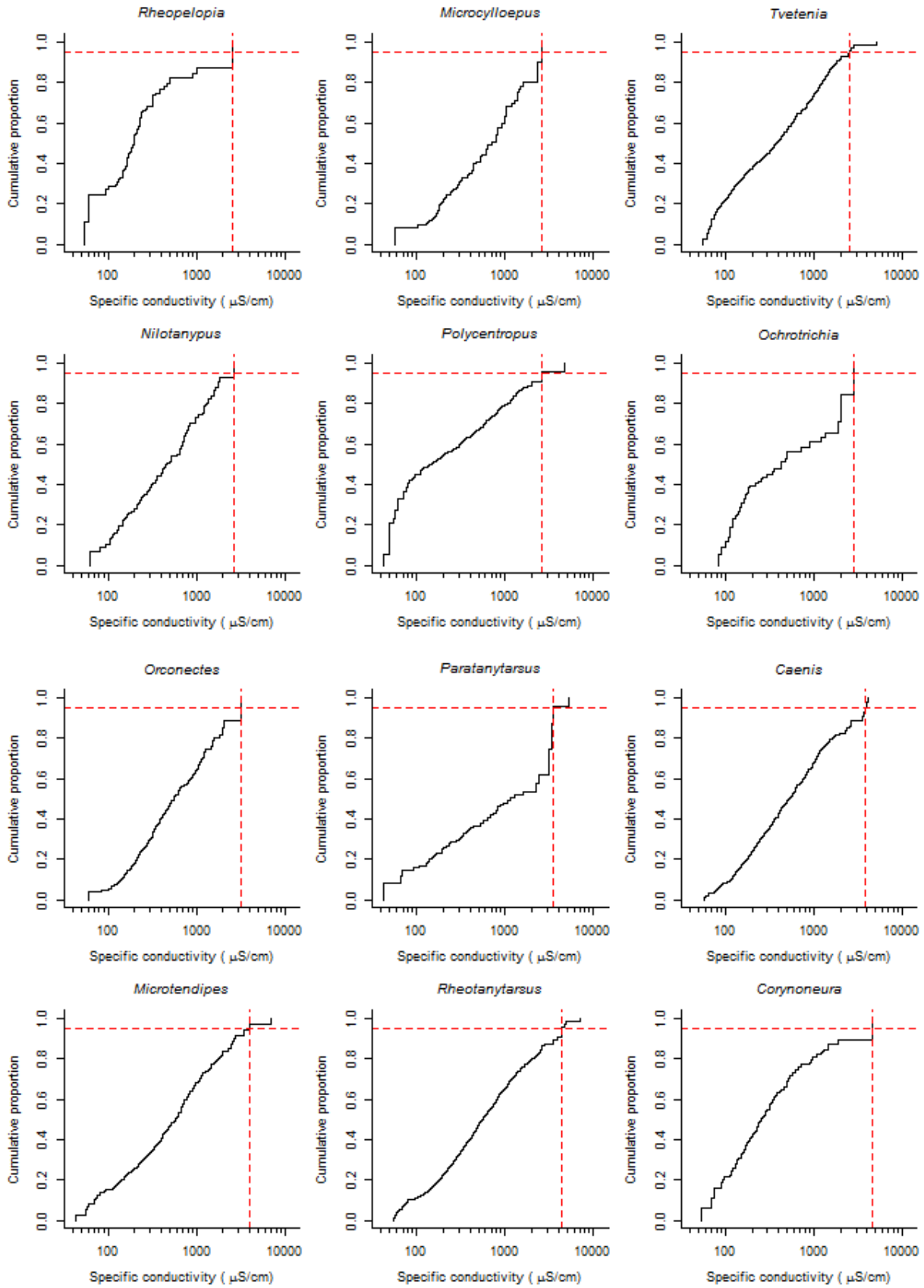


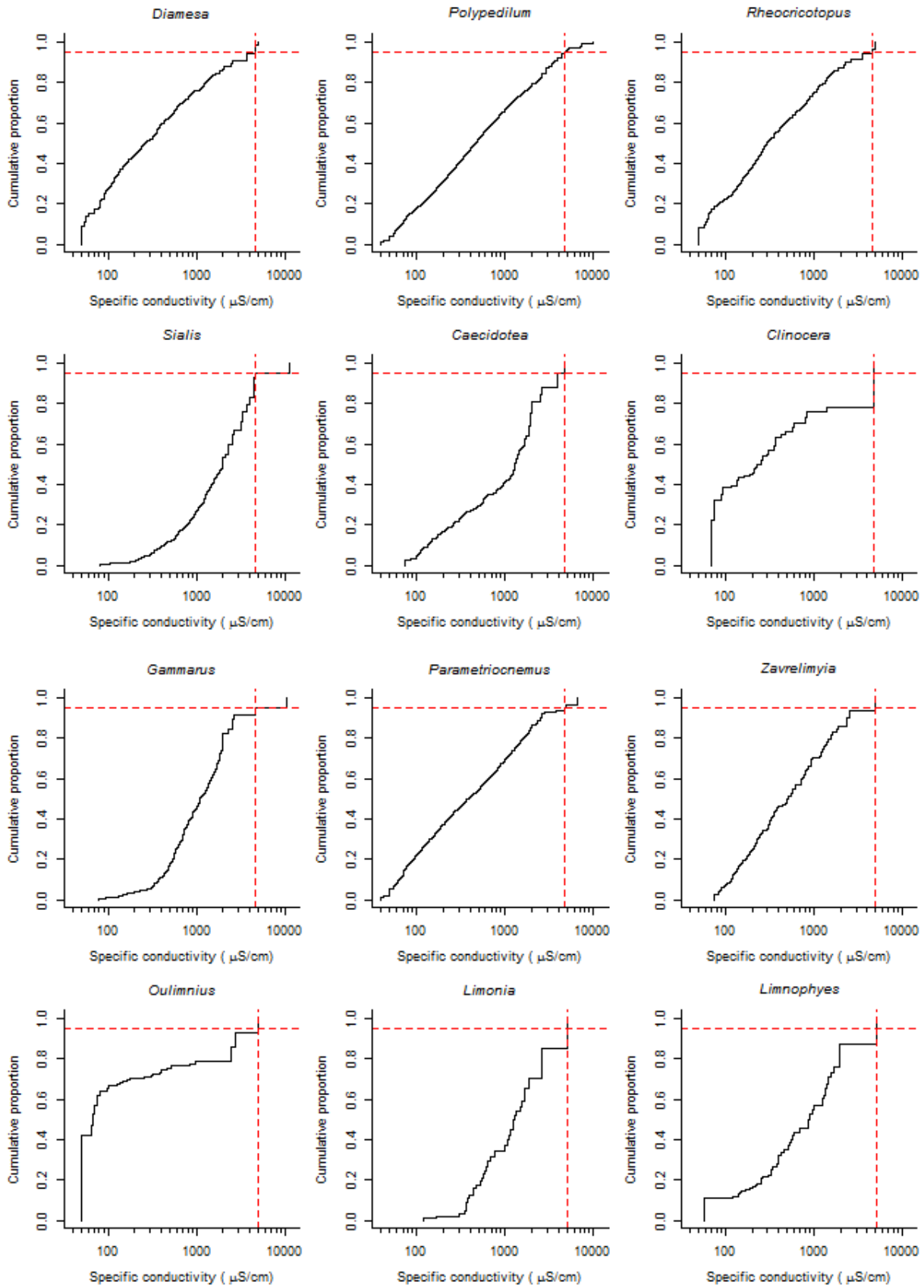


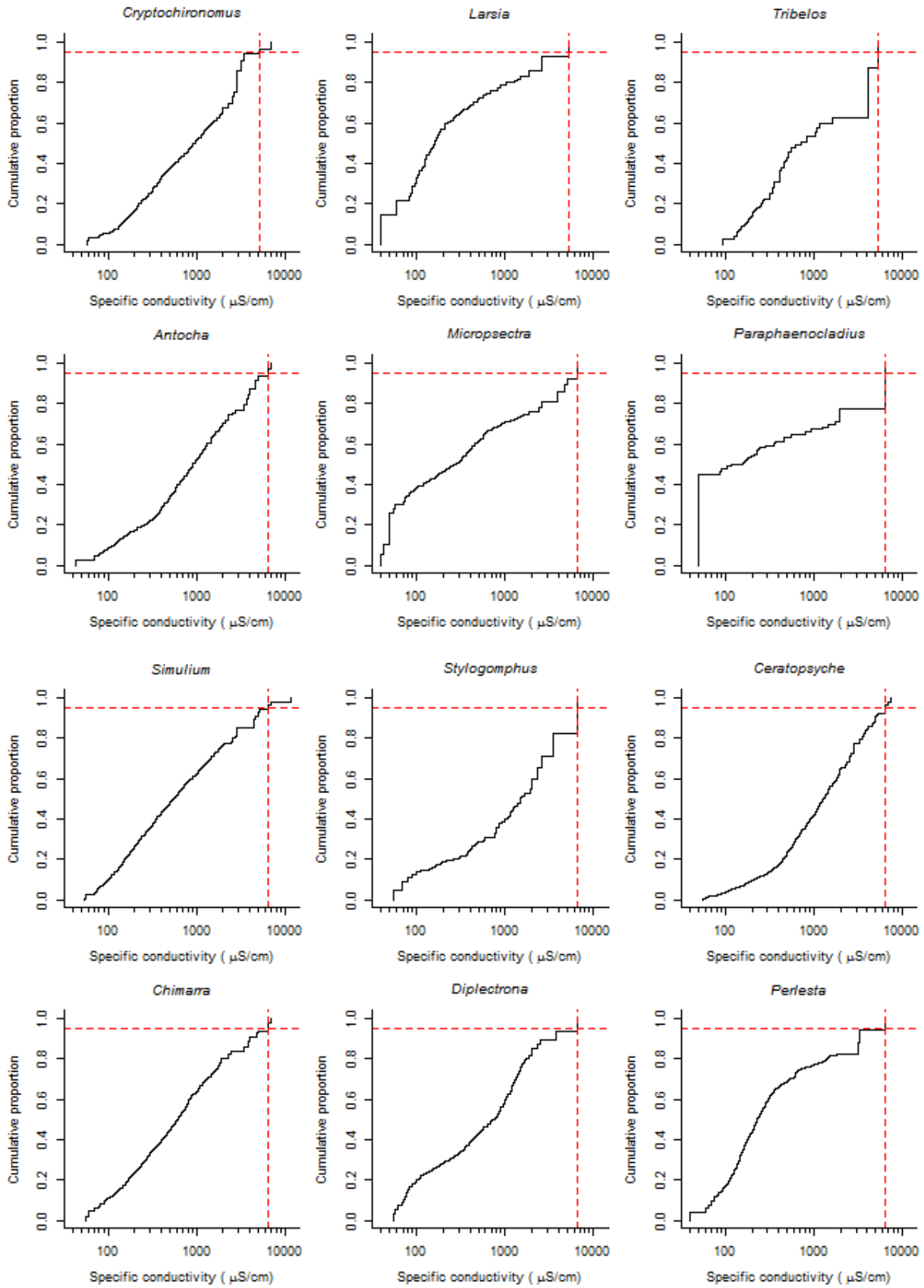


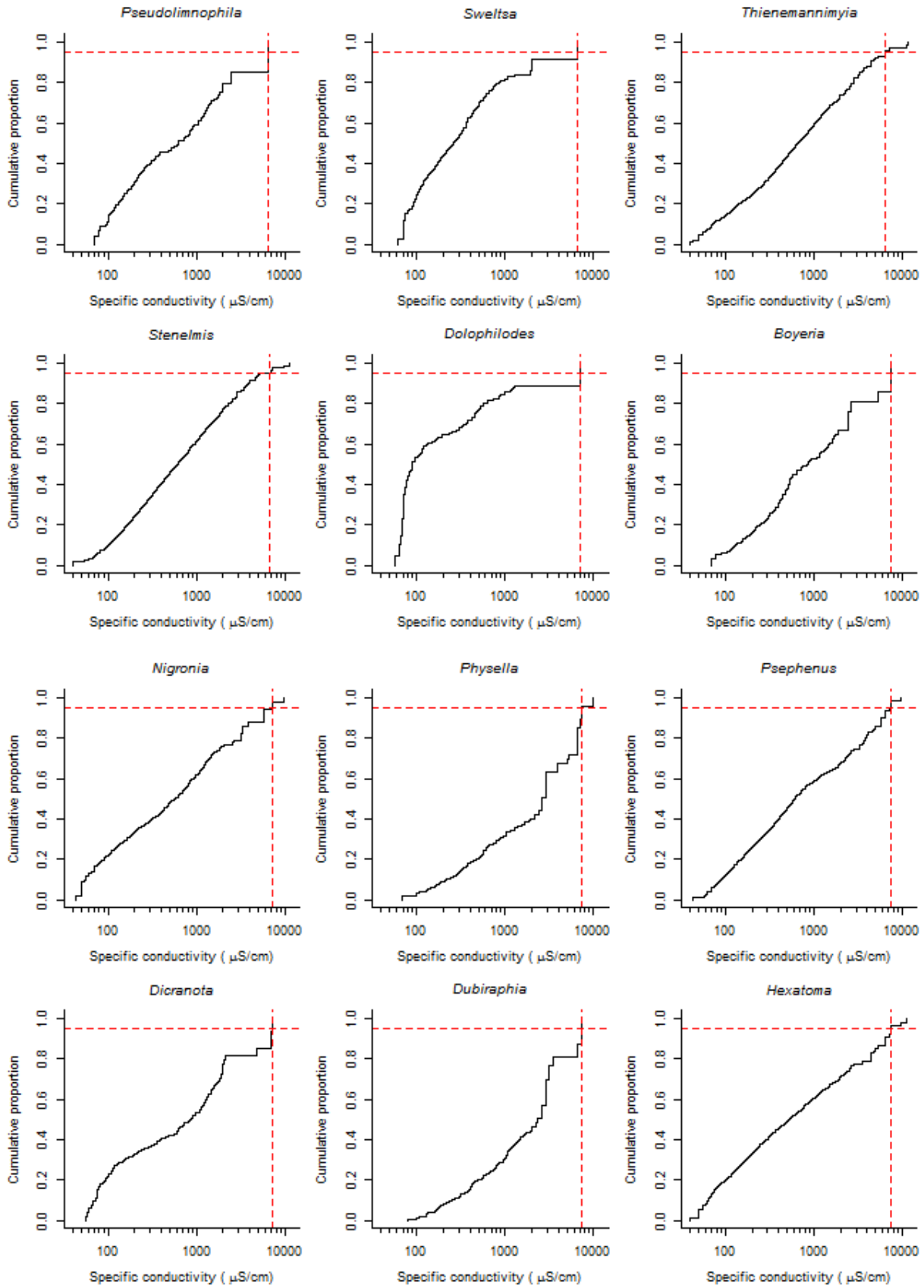


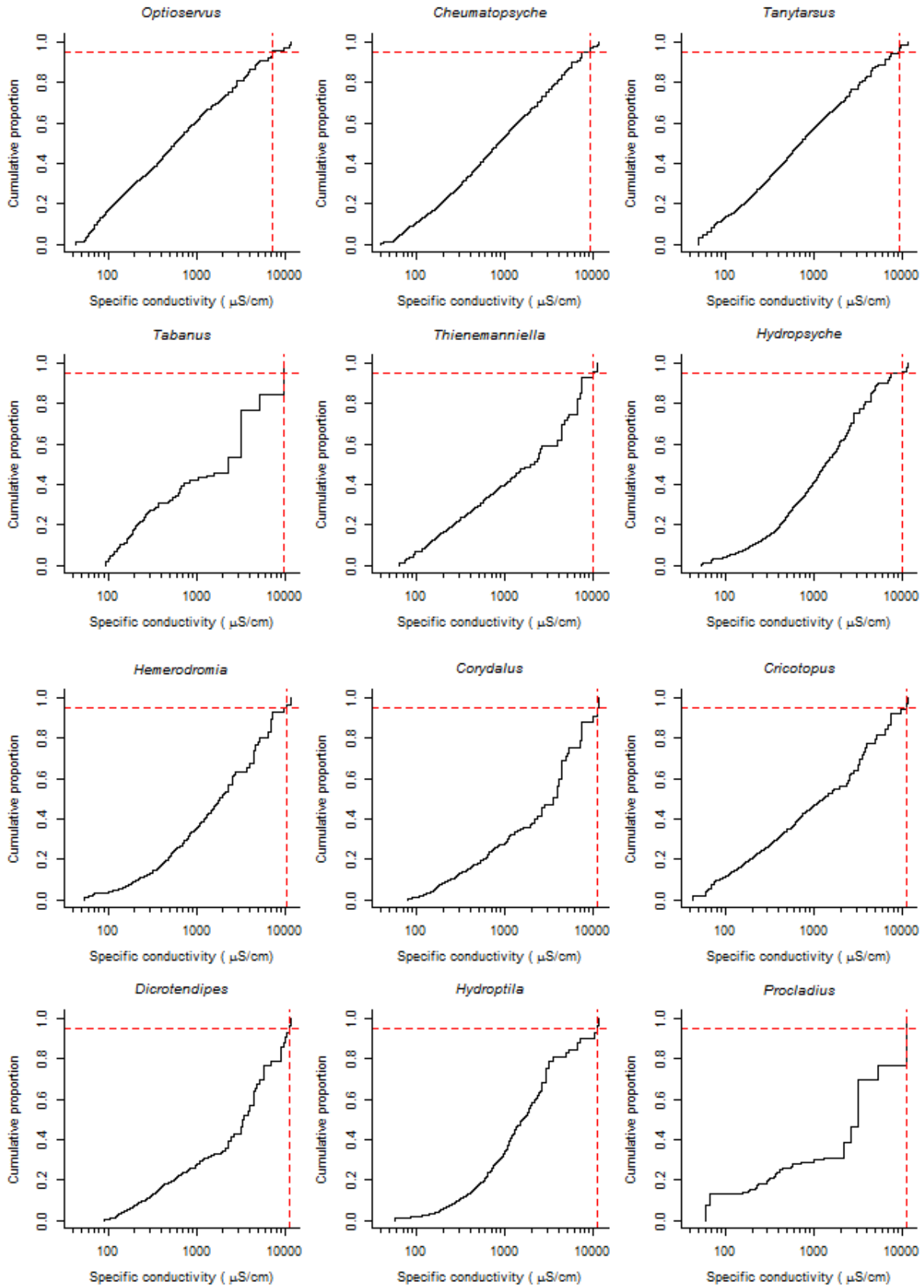


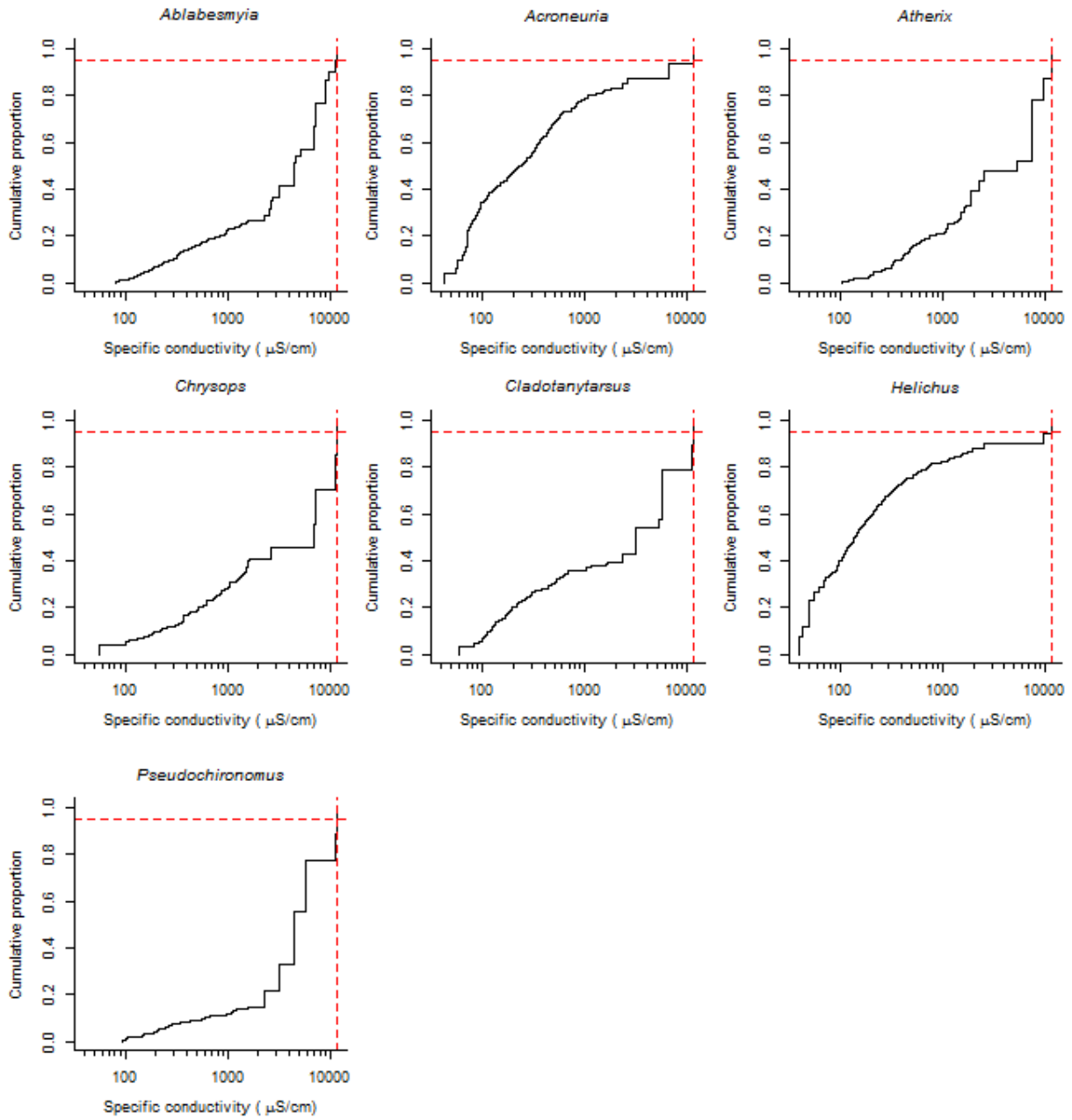














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