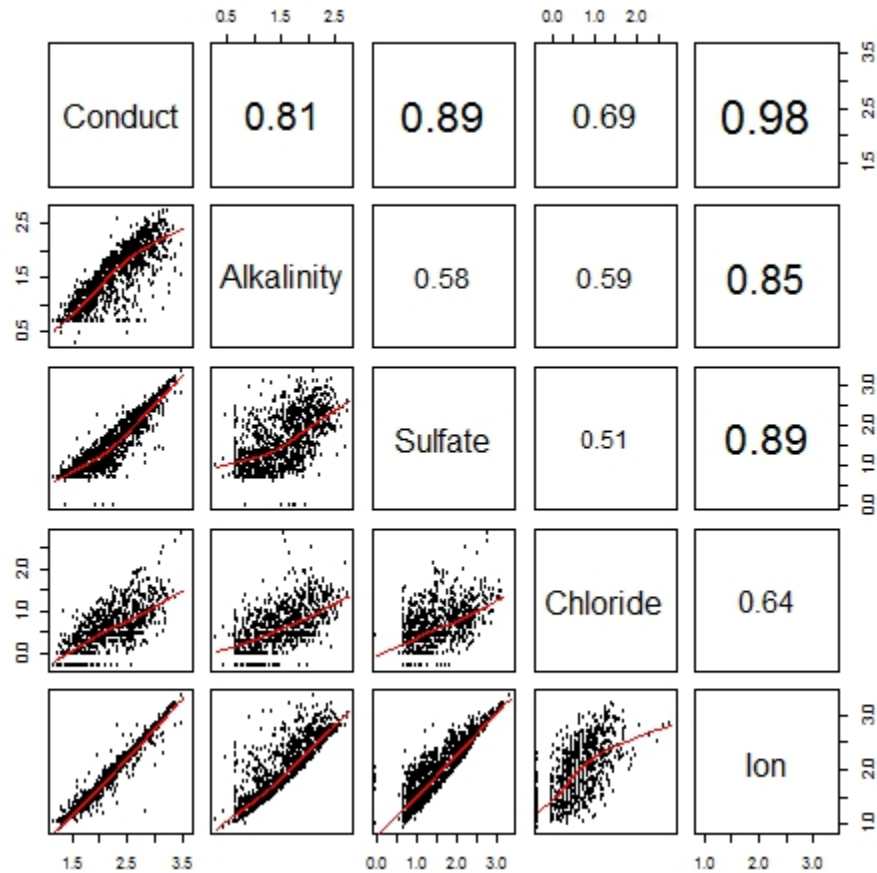
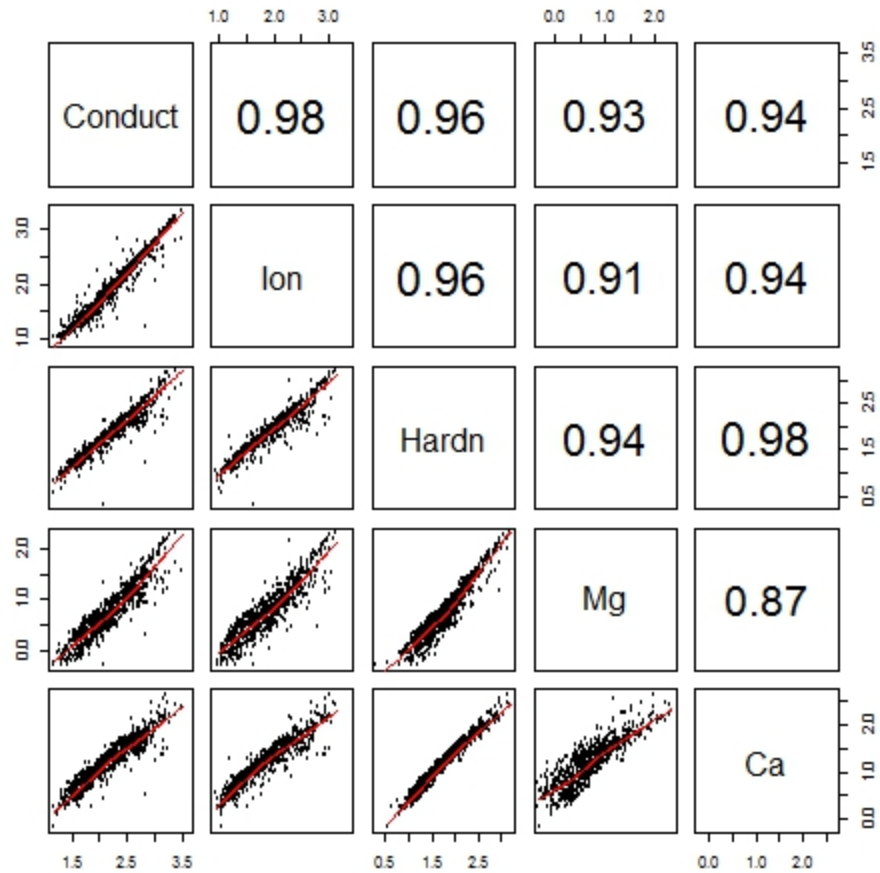


## APPENDIX A. CASE STUDY I SUPPORTING MATERIALS

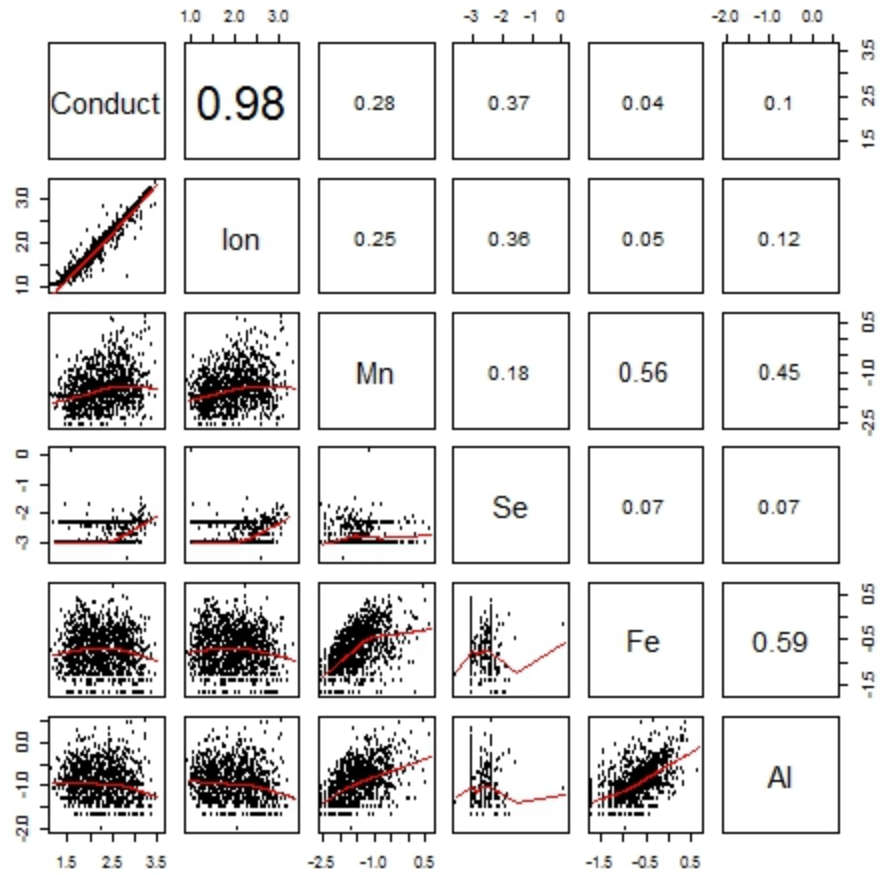
### A.1. CASE STUDY I: MATRICES OF SCATTER PLOTS AND ABSOLUTE SPEARMAN CORRELATION COEFFICIENTS



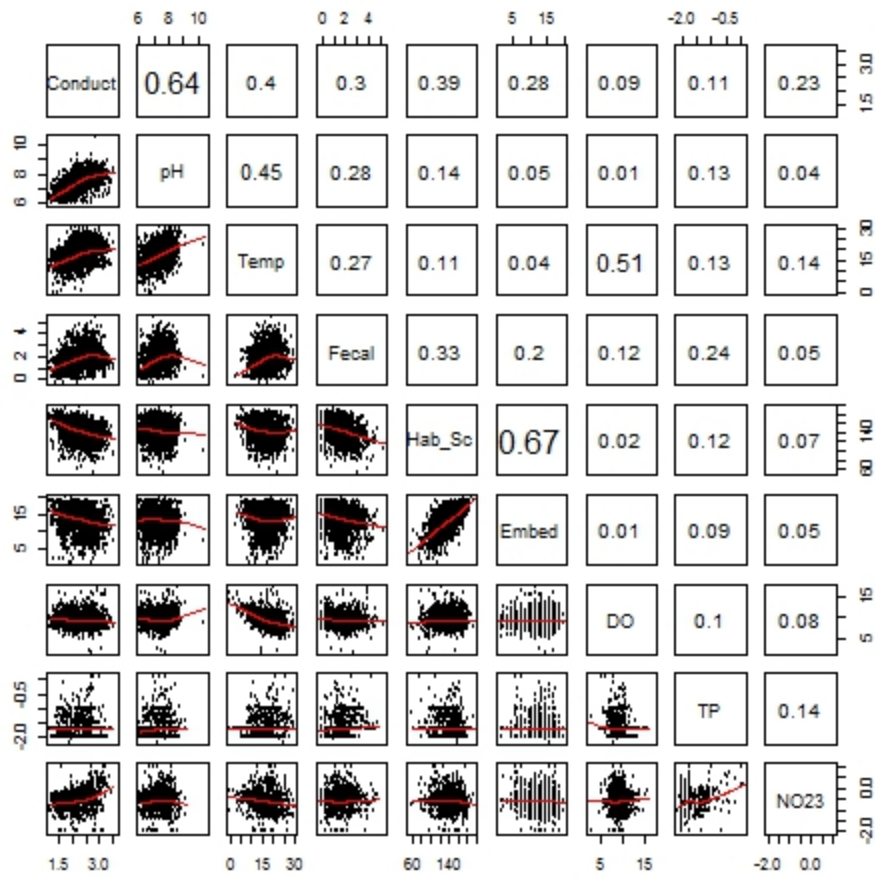
**Figure A-1. Anions.** Matrix of scatter plots and absolute Spearman correlation coefficients between specific conductivity ( $\mu\text{S}/\text{cm}$ ), alkalinity ( $\text{mg}/\text{L}$ ), sulfate ( $\text{mg}/\text{L}$ ), chloride ( $\text{mg}/\text{L}$ ), and ion ( $[\text{HCO}_3^- + \text{SO}_4^{2-}]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



**Figure A-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 the streams in Case Study I. All variables are logarithm transformed. The smooth lines are the locally weighted scatterplot smoothing (LOWESS) lines (span = 2/3).



**Figure A-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 the streams in Case Study I. All variables are logarithm transformed. The smooth lines represent the locally weighted scatterplot smoothing (LOWESS) lines (span = 2/3).



**Figure A-4. Other water-quality parameters.** Matrix of scatter plots and absolute Spearman correlation coefficients between environmental variables in Case Study I. The smooth lines are locally weighted scatterplot smoothing (LOWESS) lines (span = 2/3). 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); total phosphorus (TP) is logarithm transformed total phosphorus (mg/L); NO<sub>23</sub> is logarithm-transformed nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) (mg/L).

## **A.2. CASE STUDY I: 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, 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 were required to address confounding.

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

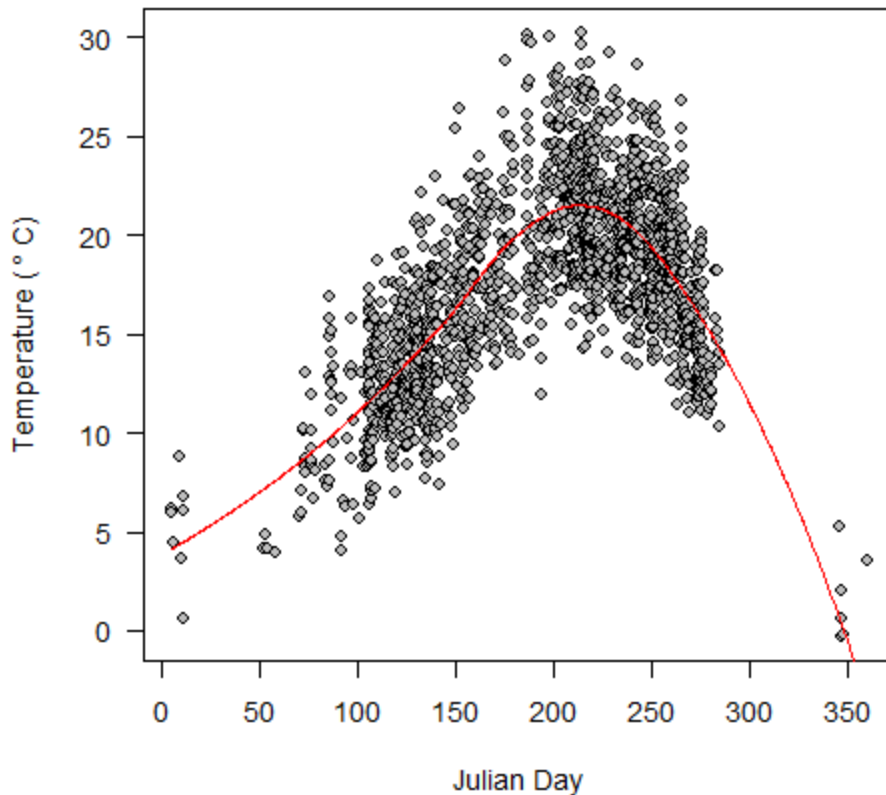
### **A.2.1. Multivariate Analysis**

Potential confounding of the model for Case Study I was reassessed for habitat (total RBP score), embeddedness (RBP subscore), temperature, and organic enrichment (fecal coliform) using a 2-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 A-1). However, to ensure that they were not influential, their combined effect on the hazardous concentration ( $HC_{05}$ ) was determined (see Section A.2.2). The most influential parameter other than SC was temperature (slope =  $-0.252$ , spearman SE 0.22). 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 A-5), further analyses were performed to evaluate temperature/sampling date related to the  $HC_{05}$  (see Section A.2.3).

**Table A-1. An output table for two generalized linear models.** The first is the simple model predicting the number of salt-intolerant genera from specific conductivity. The second is a multivariate model with the additional covariates 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 conductivity were first log<sub>10</sub> 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 is appropriate for counts of occurrences.

Parameter	Estimate	Standard error
<b>Univariate model</b>		
Intercept	0.792	0.020
Specific conductivity slope	-0.722	0.017
<b>Multivariate model</b>		
Intercept	0.759	0.020
Specific conductivity slope	-0.539	0.020
RBP slope	0.068	0.018
Temperature slope	-0.252	0.018
Fecal coliform slope	-0.121	0.019

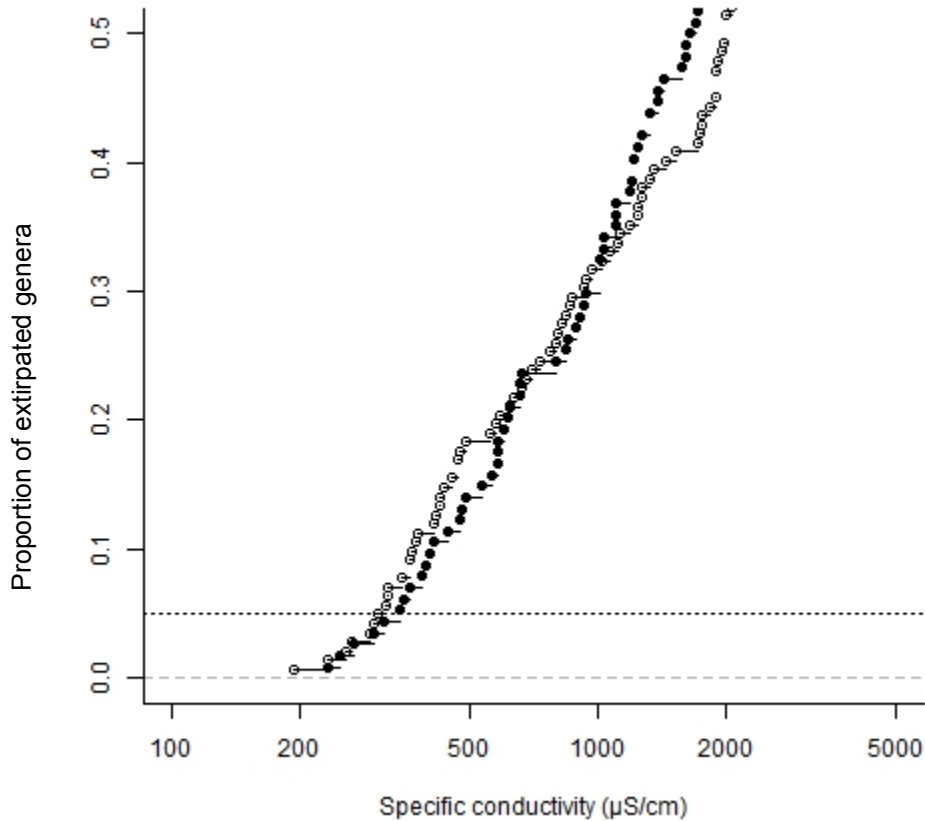


**Figure A-5. Scatter plot showing inter-relatedness of stream temperature and sampling date.** The fitted line is a locally weighted scatterplot smoothing fit (LOWESS, quadratic polynomial, span = 0.75).

### **A.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 <135 total, pH <6 and fecal coliform ≤400 colonies/100 mL. The threshold of RBP <135 was selected as an upper bound on acceptable habitat by Gerritsen, et al. (2010) that also provided an adequate sample size (relevant  $n = 922$ ). This threshold of RBP <135 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 A-6). With this constrained data set the HC<sub>05</sub> was 336  $\mu\text{S}/\text{cm}$  (95% confidence interval (CI) 233–351  $\mu\text{S}/\text{cm}$ ). The confidence interval overlaps with the HC<sub>05</sub> for the example criterion continuous concentration (CCC) (305  $\mu\text{S}/\text{cm}$  95% CI 233–329  $\mu\text{S}/\text{cm}$ ). Therefore, no correction was made for habitat quality or organic enrichment.



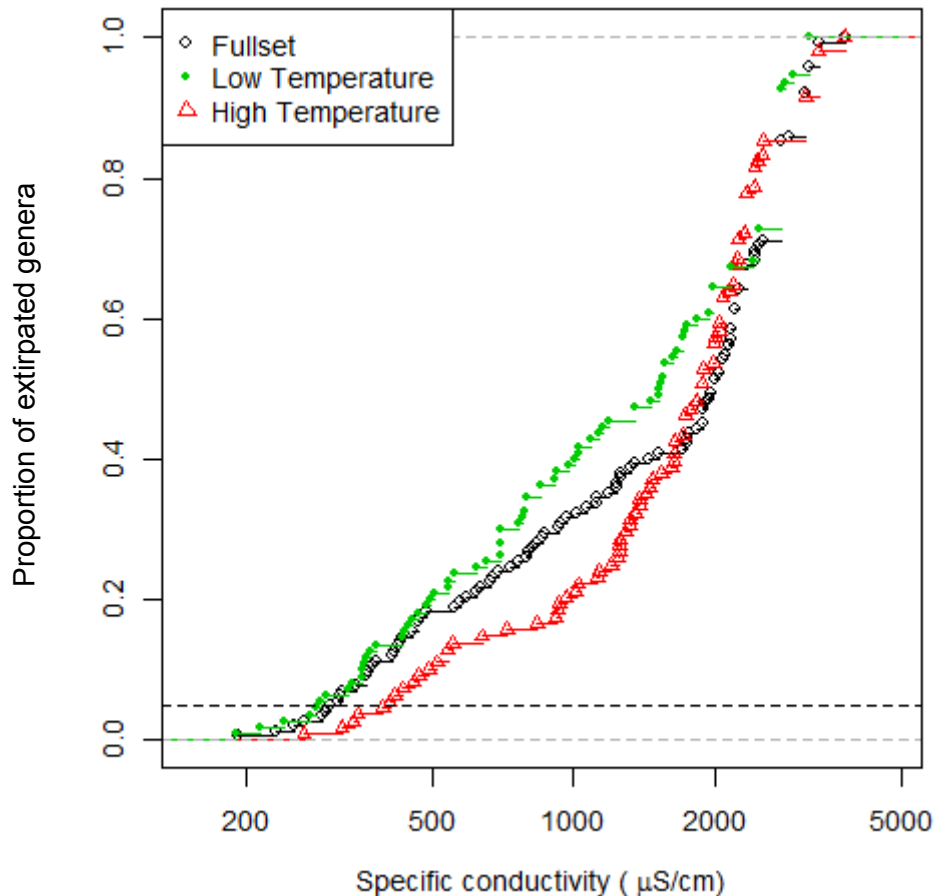
**Figure A-6. Lower portion of genus extirpation concentration distribution with and without removal of sites with poor habitat and organic enrichment.** For both data sets the pH >6, Rapid Bioassessment Protocol score is >135 and fecal coliform is <400 colonies/100 mL. The full (unconstrained, open circles) data set has 142 genera and the constrained data set has 114 genera (closed circles). Habitat disturbance and organic enrichment have little influence; the hazardous concentration (HC<sub>05</sub>) for the constrained data set is 336  $\mu\text{S}/\text{cm}$  (95% CI, 233–351  $\mu\text{S}/\text{cm}$ ). ([RBP scores: example criterion [unconstrained] data set [53–195]; constrained data set [135–195].])



### **A.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 (0–30°C) was constrained to samples with pH <6 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 temperature upper 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 to 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 increased the HC<sub>05</sub> (see Figure A-7). With the data set constrained to temperatures  $\geq 17^\circ\text{C}$ , the HC<sub>05</sub> was 400  $\mu\text{S}/\text{cm}$  (108 genera, relevant  $n = 940$  samples, 95% CI 284–408  $\mu\text{S}/\text{cm}$ ) (see Figure A-7). This is opposite the direction that is expected to occur if temperature is a confounder. Removal of warmer samples from the data set lowered the HC<sub>05</sub> (see Figure A-7). With the data set constrained to temperatures  $< 17^\circ\text{C}$ , the HC<sub>05</sub> was 287  $\mu\text{S}/\text{cm}$  (110 genera, relevant  $n = 721$  samples, 95% CI 226–334  $\mu\text{S}/\text{cm}$ ). This is the opposite the direction that is expected to occur if high temperature is a confounder. Hence, the results are logically inconsistent with temperature acting as a cause of extirpation. The confidence intervals for the  $\geq 17^\circ\text{C}$  HC<sub>05</sub> and the  $< 17^\circ\text{C}$  HC<sub>05</sub> overlap with the unconstrained example HC<sub>05</sub> (305  $\mu\text{S}/\text{cm}$ ; 95% CI 233–329  $\mu\text{S}/\text{cm}$ ). Therefore, no correction was made for temperature.

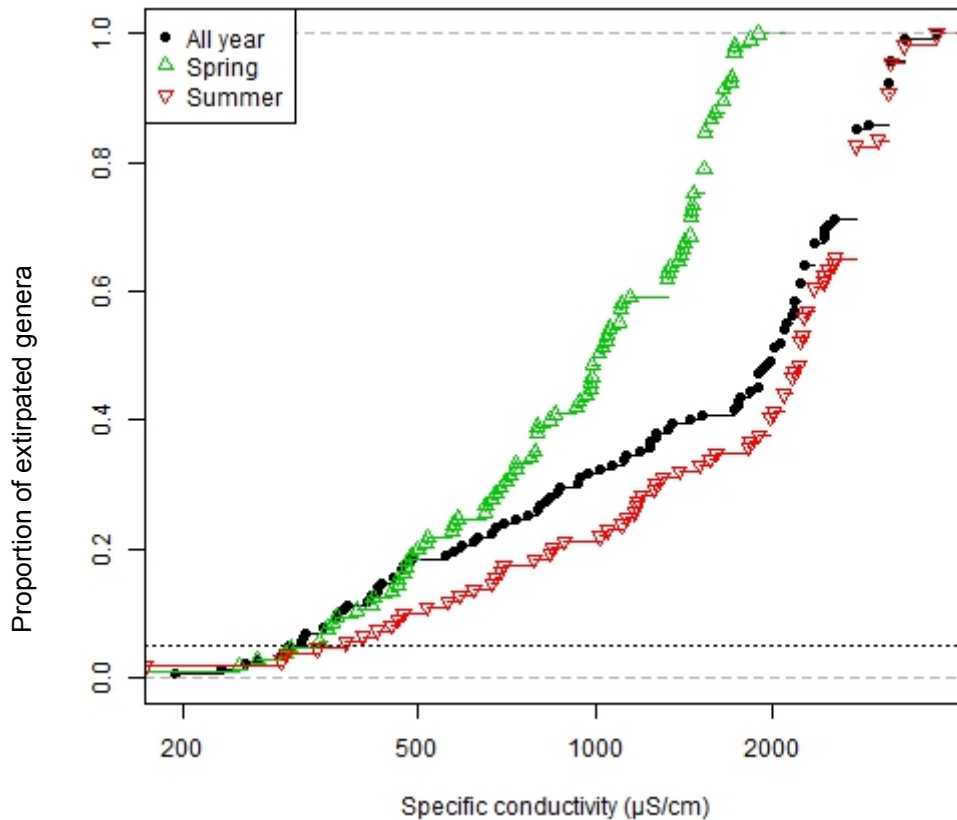


**Figure A-7. Genus extirpation concentration distributions for example criterion data set and temperature constrained data sets.** Samples with  $\text{pH} \leq 6$  and  $(\text{SO}_4^- + \text{HCO}_3^-)/\text{Cl}^- \leq 1$  were removed from all data sets. The example criterion (unconstrained, 0–35°C) data set has extirpation concentration ( $\text{XC}_{95}$ ) values for 142 genera (open black diamonds). The  $\leq 17^\circ\text{C}$  constrained data set has 110 genera (closed green diamonds ( $n = 720$ )). The  $\geq 17^\circ\text{C}$  constrained data set has 108 genera (open red triangles ( $n = 940$ )).

#### A.2.4. Potential Influence of Sampling Date on the Hazardous Concentration ( $\text{HC}_{05}$ )

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 capture of apparently salt-intolerant genera. First, the  $\text{HC}_{05}$  using the spring (March–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 spring  $\text{HC}_{05}$  confidence bounds overlap with the confidence bounds of the full-year data set (see Figure A-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.

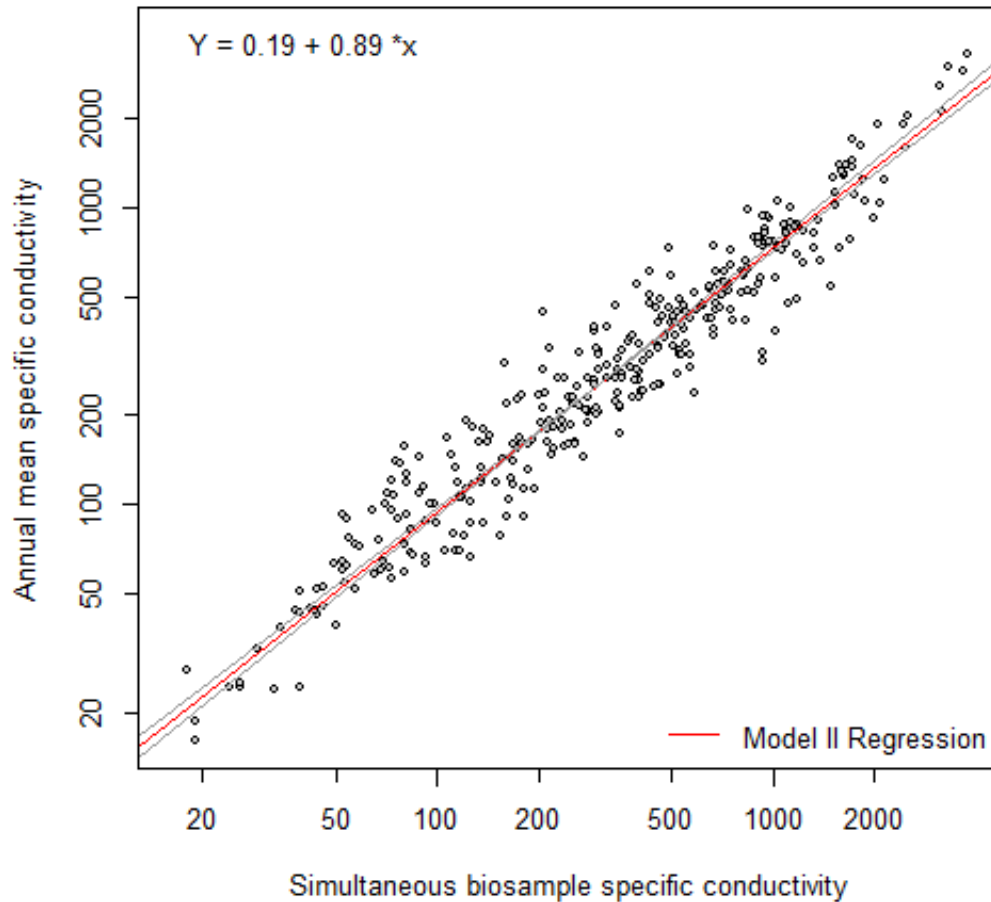


**Figure A-8. Comparison of genus extirpation concentration distributions (XCD) full data set and subsets in different months.** Example criterion data set (black circles) and subsets of March to June (spring, inverted green triangles) and July to October (summer, red triangles) collected samples from the Case Study I Criterion-data set. The all year XCD has 142 genera, the spring XCD has 105 genera ( $N = 627$ ), summer XCD has 109 genera ( $N = 1,016$ ). The horizontal dotted line is the 5<sup>th</sup> centile. The spring and summer hazardous concentration ( $HC_{05}$ ) confidence bounds overlap with 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 A-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 342 sites with paired SC and biological data meeting these additional data requirements (see Figure A-9). 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 304  $\mu\text{S}/\text{cm}$  on the biological sampling date, the regression prediction for an annual mean SC for the same site is 256  $\mu\text{S}/\text{cm}$ .

### Model II Regression and 95% confidence Intervals



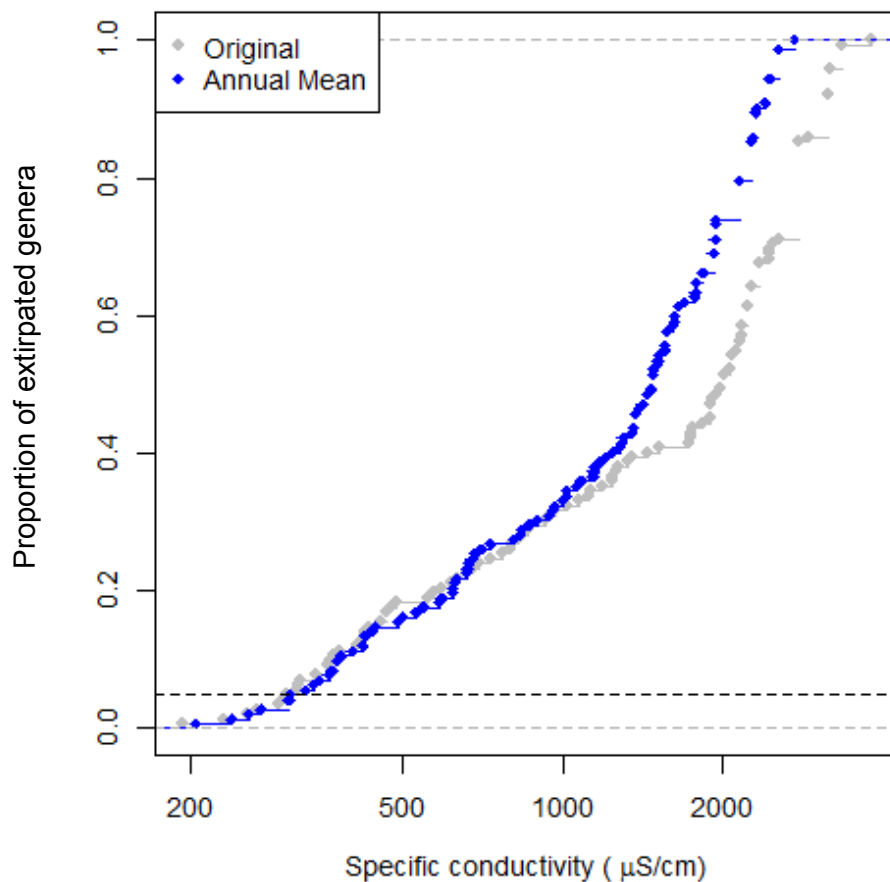
**Figure A-9. Relationship between specific conductivity sample at the time of biological sampling and annual mean specific conductivity (using 6–12 intra-annual samples) in the Case Study I data set (1999–2011).** Though the relationship is nearly 1:1, some variability may be attributable to different seasonal conductivity regimes. Model II Regression with 95% confidence intervals. Specific conductivity is expressed as  $\mu\text{S}/\text{cm}$  on a log10 scale; therefore,  $x$  and  $y$  are log10 expressions in the regression formula.

Lastly, to account for the seasonal variability, conductivity 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 0.95 to 1.05 (see Table A-2). June through October SC values are slightly higher than the annual average, so the weighting factors are generally lower, while November through May weighting factors are generally higher. The  $\text{HC}_{05}$  calculated with weighted SC measurements is  $311 \mu\text{S}/\text{cm}$

(CI 242–326  $\mu\text{S}/\text{cm}$ ) (see Figure A-10). These three analyses suggest that sampling date is at most a minor confounder. Correction for minor confounding may increase error in the estimated  $\text{HC}_{05}$ ; therefore, no correction was made for sampling date.

**Table A-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	1.03	1.03	1.05	1.02	0.99	0.98	0.95	0.96	0.97	1.01	1.02



**Figure A-10. Case Study I comparison of annual weighted and original extirpation concentrations distributions (XCD).** Genus XCD of unweighted extirpation concentration ( $\text{XC}_{95}$ ) values (gray) and XCD of  $\text{XC}_{95}$  derived from specific conductivity normalized to an annual geometric mean (blue). Hazardous concentration ( $\text{HC}_{05}$ ) values are 305, and 311  $\mu\text{S}/\text{cm}$ , respectively.

### **A.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, lack of headwaters, size of catchment area, settling ponds, dissolved oxygen, and metals.

The additional analyses described in this Appendix (see Section A.2) using data from Ecoregion 69 indicate that SC remains the strongest influence in the multivariate model of genera with low  $XC_{95}$  values (see Table A-1). Organic enrichment (estimated based on fecal coliform counts) and habitat score showed a minor effect in the multivariate model, but recalculating the  $HC_{05}$  using a data set removing fecal coliform  $\geq 400$  and RBP score  $< 135$  samples 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 with this data set confounds the  $HC_{05}$  of the genus XCD for the example criteria. Therefore, the example criterion data set was not altered and no corrections were made for habitat, temperature, or sample date.

### **A.3. CASE STUDY I: 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 69 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.

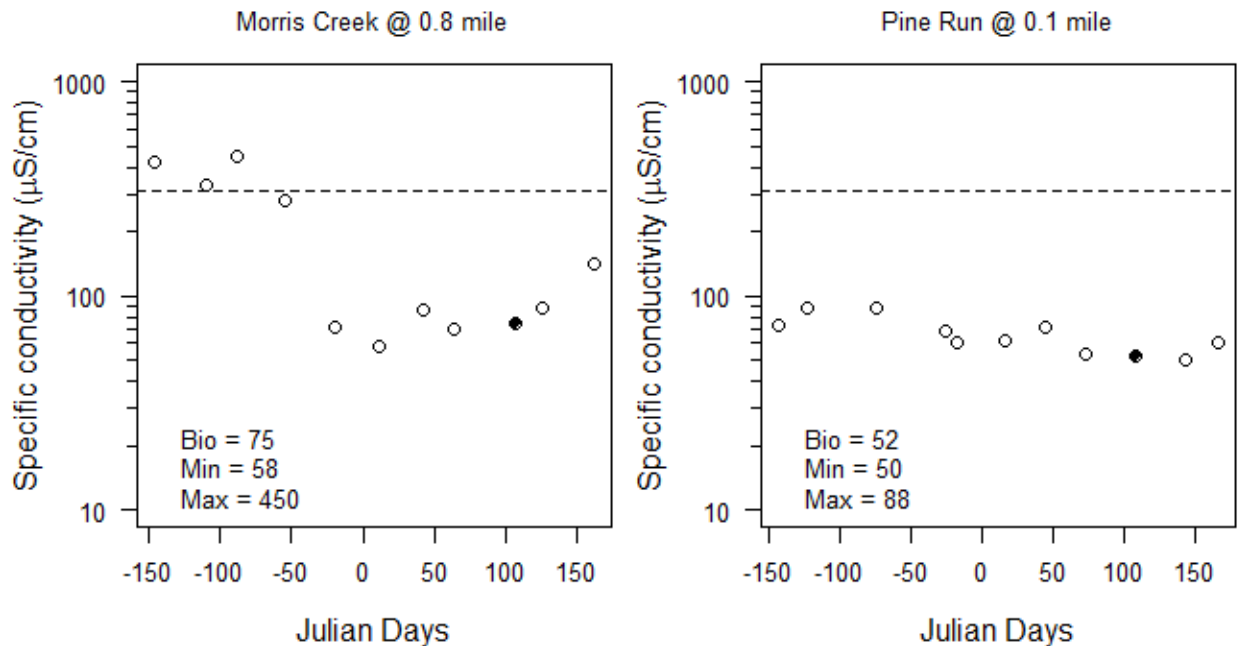
Owing to the moderate number of biological samples with multiple seasonal sampling of water chemistry available for Ecoregion 69, it was possible to estimate a maximum SC that could occur and salt-intolerant genera had survived until the following year shortly before emergence

as winged adults. Salt-intolerant genera tend to be more commonly observed when they are larger and nearing emergence usually in April through June. The maximum SC of streams in Ecoregion 69 usually occurs between August and September.

A data set was constructed from the Ecoregion 69 criterion-data set. For a site to be included, it required a minimum of six water chemistry samples taken over the course of the year prior to biological sampling, which was defined as a rotating year. Of the 564 sites sampled in the data set, only 110 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 among SC and the presence of most salt-intolerant taxa were inspected for each of the 111 sites meeting the criteria for inclusion in the data set. Salt-intolerant taxa are those taxa with an  $XC_{95} \leq 310 \mu\text{S}/\text{cm}$ . Figure A-11 depicts two plots where the annual average SC is well below  $310 \mu\text{S}/\text{cm}$  and the CMEC calculated for the case study. Pine Run has two of the salt-intolerant taxa and low SC year round. Morris Creek has two salt-intolerant taxa and a maximum grab sample SC of  $450 \mu\text{S}/\text{cm}$ .

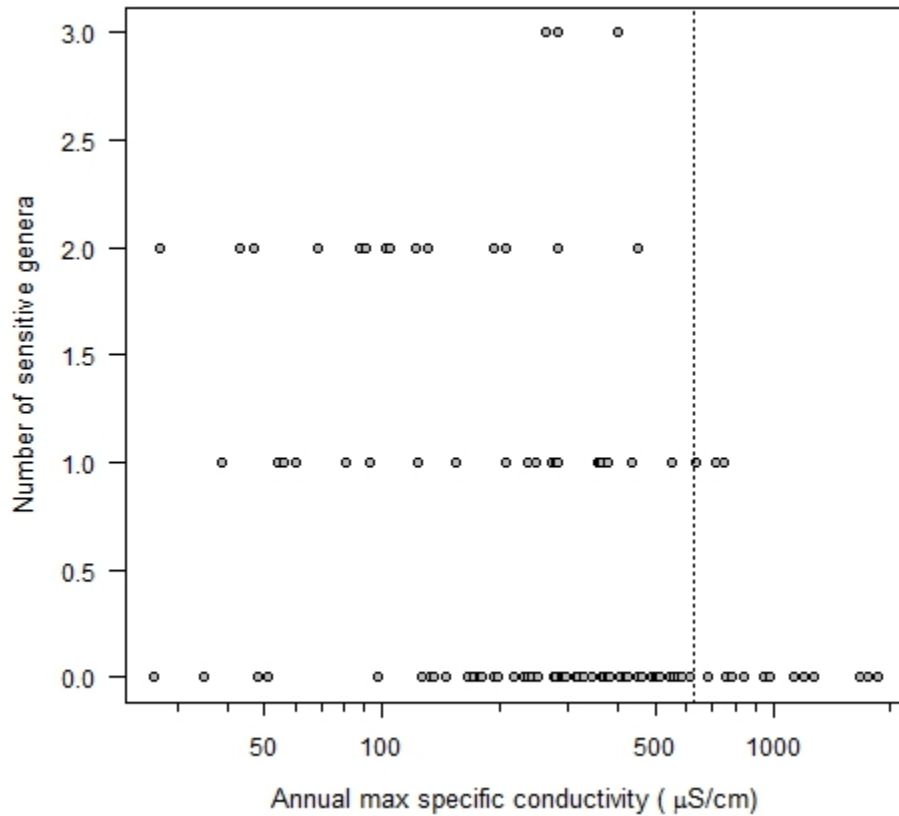




**Figure A-11. Specific conductivity and temperature variations in stations with multiple observations.** Julian day, 0 = January, is on the x-axis. Specific conductivity is on the left y-axis with water chemistry samples as open circles; a filled circle is date of biological sampling. Dashed line is at 310 µS/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. Two of the 7 most salt-intolerant genera ( $XC_{95} < 310 \mu\text{S/cm}$ ) were observed at both stream stations.

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 with  $XC_{95} < \text{CCC}$ , and maximum SC that occurred in the year prior to biological sampling (see Figure A-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 at  $>630 \mu\text{S/cm}$ , the CMEC calculated from chemistry only data. The chemistry only analysis used a much more representative sample of sites comprised of 564 rotation years from 536 unique stations, with at least one sample from July to October and one from March to June, and at least six samples within a rotation year (see Table 4-3). Because the CMEC analysis is based on a much larger

and more representative sample, the CMEC of 630  $\mu\text{S}/\text{cm}$  was retained. However, as data becomes more available, the method using biological samples may become preferable.



**Figure A-12. Scatter plot of count of most salt-intolerant genera ( $\text{XC}_{95} < 310 \mu\text{S}/\text{cm}$ ) and maximum conductivity in preceding year.** Very few salt-intolerant genera are observed at sites ( $N = 110$ ) with a specific conductivity greater than the criterion maximum exposure concentration (CMEC) of 630  $\mu\text{S}/\text{cm}$  (vertical dashed line). Specific conductivity expressed as  $\mu\text{S}/\text{cm}$ .

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

Order	Family	Genus	Symbol	XC <sub>95</sub>	95% CI	N
Ephemeroptera	Leptophlebiidae	<i>Leptophlebia</i>		193	153–276	56
Plecoptera	Perlodidae	<i>Remenus</i>		232	127–532	53
Trichoptera	Limnephilidae	<i>Pycnopsyche</i>		255	166–363	67
Ephemeroptera	Leptophlebiidae	<i>Paraleptophlebia</i>		266	224–340	275
Diptera	Ceratopogonidae	<i>Bezzia</i>		293	151–608	33
Plecoptera	Chloroperlidae	<i>Alloperla</i>		298	184–438	60
Ephemeroptera	Ameletidae	<i>Ameletus</i>		303	243–418	146
Ephemeroptera	Heptageniidae	<i>Epeorus</i>		317	279–394	449
Diptera	Chironomidae	<i>Conchapelopia</i>		320	266–419	34
Diptera	Chironomidae	<i>Stempellina</i>		322	210–713	37
Ephemeroptera	Heptageniidae	<i>Heptagenia</i>		345	250–374	72
Ephemeroptera	Heptageniidae	<i>Cinygmula</i>		363	168–453	104
Ephemeroptera	Ephemeridae	<i>Ephemera</i>		363	293–747	130
Ephemeroptera	Baetidae	<i>Dipheter</i>		366	302–655	116
Ephemeroptera	Heptageniidae	<i>Leucrocuta</i>		373	297–409	152
Diptera	Chironomidae	<i>Demicryptochironomus</i>		380	210–912	54
Trichoptera	Lepidostomatidae	<i>Lepidostoma</i>	~	411	196–1,890	155
Ephemeroptera	Ephemerellidae	<i>Ephemerella</i>		418	340–490	367
Ephemeroptera	Heptageniidae	<i>Stenacron</i>		425	357–500	234
Ephemeroptera	Ephemerellidae	<i>Drunella</i>		427	292–1,890	217
Trichoptera	Uenoidae	<i>Neophylax</i>		434	251–1,831	138
Diptera	Chironomidae	<i>Platysmittia</i>	~	454	220–637	31
Plecoptera	Chloroperlidae	<i>Haploperla</i>	~	468	384–595	233
Plecoptera	Capniidae	<i>Paracapnia</i>	>	468	327–604	45
Ephemeroptera	Ephemerellidae	<i>Serratella</i>		474	381–956	132
Plecoptera	Peltoperlidae	<i>Tallaperla</i>	~	488	247–784	119
Ephemeroptera	Baetidae	<i>Procloeon</i>	~	558	436–615	58
Ephemeroptera	Ephemerellidae	<i>Eurylophella</i>	~	574	432–765	143
Diptera	Simuliidae	<i>Prosimulium</i>	~	591	335–1,172	130
Trichoptera	Psychomyiidae	<i>Lype</i>	~	620	312–741	28
Plecoptera	Perlodidae	<i>Isoperla</i>	~	633	447–896	327
Plecoptera	Perlodidae	<i>Diploperla</i>	~	664	221–803	49
Plecoptera	Perlodidae	<i>Yugus</i>	>	676	363–866	129
Diptera	Tipulidae	<i>Pseudolimnophila</i>	>	697	520–1,322	79
Coleoptera	Elmidae	<i>Promoresia</i>	>	735	380–1,257	115

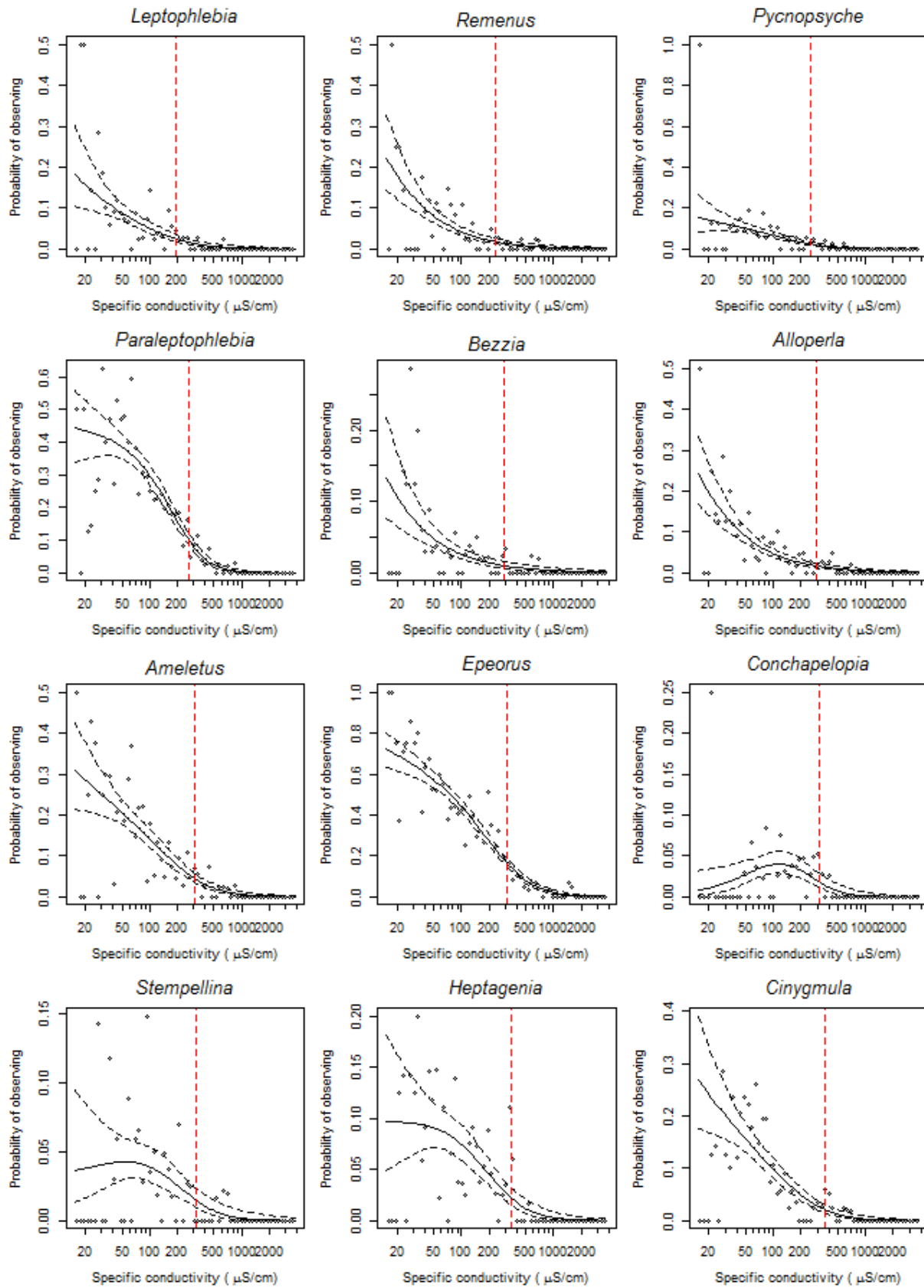
Order	Family	Genus	Symbol	XC <sub>95</sub>	95% CI	N
Plecoptera	Perlidae	<i>Eccoptura</i>	>	768	358–1,123	48
Ephemeroptera	Baetidae	<i>Acerpenna</i>	~	795	402–1,036	40
Plecoptera	Nemouridae	<i>Amphinemura</i>	>	805	598–1,343	436
Diptera	Chironomidae	<i>Pagastia</i>	>	824	439–1,104	36
Ephemeroptera	Heptageniidae	<i>Maccaffertium</i>	>	838	702–1,013	353
Plecoptera	Taeniopterygidae	<i>Taeniopteryx</i>	~	863	236–1,076	25
Plecoptera	Perlodidae	<i>Malirekus</i>	>	870	342–974	34
Ephemeroptera	Heptageniidae	<i>Stenonema</i>	>	931	820–1,066	604
Ephemeroptera	Baetiscidae	<i>Baetisca</i>	~	942	551–1,016	36
Ephemeroptera	Baetidae	<i>Centroptilum</i>	>	969	337–1,163	32
Ephemeroptera	Baetidae	<i>Acentrella</i>	>	1,018	836–1,620	724
Diptera	Chironomidae	<i>Cladotanytarsus</i>	>	1,069	777–1,165	57
Diptera	Chironomidae	<i>Micropsectra</i>	>	1,119	585–2,553	217
Diptera	Chironomidae	<i>Microtendipes</i>	>	1,125	773–1,471	361
Hemiptera	Veliidae	<i>Rhagovelia</i>	>	1,188	337–1,191	25
Diptera	Chironomidae	<i>Rheopelopia</i>	>	1,236	484–1,543	62
Trichoptera	Psychomyiidae	<i>Psychomyia</i>	>	1,239	638–1,388	51
Ephemeroptera	Caenidae	<i>Caenis</i>	>	1,263	1,007–1,500	292
Ephemeroptera	Isonychiidae	<i>Isonychia</i>	>	1,270	1,147–1,500	649
Ephemeroptera	Baetidae	<i>Plauditus</i>	>	1,323	846–2,257	351
Plecoptera	Peltoperlidae	<i>Peltoperla</i>	>	1,354	774–1,703	156
Ephemeroptera	Baetidae	<i>Baetis</i>	>	1,450	1,163–1,706	1,271
Diptera	Chironomidae	<i>Cryptochironomus</i>	>	1,520	998–1,702	76
Diptera	Chironomidae	<i>Chaetocladius</i>	>	1,720	759–1,725	100
Trichoptera	Philopotamidae	<i>Wormaldia</i>	>	1,746	591–1,979	82
Diptera	Dixidae	<i>Dixa</i>	>	1,747	515–1,831	70
Diptera	Chironomidae	<i>Paratanytarsus</i>	>	1,759	1,323–1,837	78
Diptera	Chironomidae	<i>Zavrelia</i>	~	1,837	260–1,837	33
Coleoptera	Elmidae	<i>Dubiraphia</i>	>	1,890	1,058–1,890	72
Diptera	Tipulidae	<i>Antocha</i>	>	1,896	1,487–2,257	491
Diptera	Chironomidae	<i>Parakiefferiella</i>	>	1,896	711–1,896	39
Diptera	Chironomidae	<i>Potthastia</i>	>	1,896	1,164–1,896	57
Diptera	Chironomidae	<i>Limnophyes</i>	>	1,912	1,548–1,979	76
Diptera	Chironomidae	<i>Eukiefferiella</i>	>	1,948	1,604–2,440	491
Decapoda	Cambaridae	<i>Cambarus</i>	>	1,974	1,006–2,244	351
Diptera	Chironomidae	<i>Dicrotendipes</i>	>	2,006	1,165–2,006	64

Order	Family	Genus	Symbol	XC <sub>95</sub>	95% CI	N
Plecoptera	Leuctridae	<i>Leuctra</i>	>	2,006	1,323–2,768	926
Diptera	Chironomidae	<i>Nilotanytus</i>	>	2,006	1,255–2,006	30
Trichoptera	Rhyacophilidae	<i>Rhyacophila</i>	>	2,054	829–3,794	519
Plecoptera	Perlidae	<i>Paragnetina</i>		2,087	247–2,087	71
Odonata	Gomphidae	<i>Lanthus</i>	>	2,087	1,234–2,087	45
Diptera	Chironomidae	<i>Sublettea</i>	>	2,087	1,543–2,440	184
Trichoptera	Glossosomatidae	<i>Glossosoma</i>	>	2,110	970–2,257	206
Diptera	Chironomidae	<i>Diamesa</i>	>	2,160	1,585–2,344	303
Coleoptera	Elmidae	<i>Macronychus</i>	>	2,160	1,347–2,160	36
Diptera	Chironomidae	<i>Rheotanytarsus</i>	>	2,168	1,702–2,485	683
Amphipoda	Crangonyctidae	<i>Crangonyx</i>	>	2,169	589–2,169	43
Coleoptera	Elmidae	<i>Stenelmis</i>	>	2,169	1,756–2,445	519
Decapoda	Cambaridae	<i>Orconectes</i>	>	2,226	1,120–2,226	79
Diptera	Chironomidae	<i>Phaenopsectra</i>	>	2,226	1,248–2,226	55
Diptera	Chironomidae	<i>Polypedilum</i>	>	2,226	1,496–2,768	1,151
Megaloptera	Sialidae	<i>Sialis</i>	>	2,226	1,970–2,539	124
Diptera	Ceratopogonidae	<i>Atrichopogon</i>	>	2,257	1,303–2,257	38
Diptera	Tipulidae	<i>Limonia</i>	>	2,257	1,502–2,257	33
Basommatophora	Physidae	<i>Physella</i>	>	2,257	1,437–2,257	48
Diptera	Chironomidae	<i>Thienemannimyia</i>	>	2,257	1,720–2,344	965
Diptera	Chironomidae	<i>Ablabesmyia</i>	>	2,344	1,332–2,344	69
Odonata	Cordulegastridae	<i>Cordulegaster</i>	>	2,344	765–2,344	28
Amphipoda	Gammaridae	<i>Gammarus</i>	>	2,344	876–2,344	85
Coleoptera	Dryopidae	<i>Helichus</i>	>	2,344	1,066–2,445	228
Plecoptera	Perlidae	<i>Perlesta</i>	>	2,344	585–2,344	65
Diptera	Simuliidae	<i>Simulium</i>	>	2,440	1,650–2,553	964
Trichoptera	Hydroptilidae	<i>Hydroptila</i>	>	2,443	1,845–2,539	229
Odonata	Gomphidae	<i>Stylogomphus</i>	>	2,445	1,175–2,445	91
Diptera	Chironomidae	<i>Parametriocnemus</i>	>	2,485	2,006–2,768	1,011
Isopoda	Asellidae	<i>Caecidotea</i>	>	2,553	765–2,553	68
Diptera	Tipulidae	<i>Limnophila</i>	~	2,768	228–2,768	50
Plecoptera	Pteronarcyidae	<i>Pteronarcys</i>	~	2,768	406–2,768	207
Diptera	Chironomidae	<i>Stempellinella</i>	~	2,768	329–2,768	162
Plecoptera	Perlidae	<i>Acroneuria</i>	>	2,768	1,303–3,174	515
Diptera	Chironomidae	<i>Brillia</i>	>	2,768	1,171–2,768	107
Diptera	Empididae	<i>Clinocera</i>	>	2,768	1,349–2,768	47

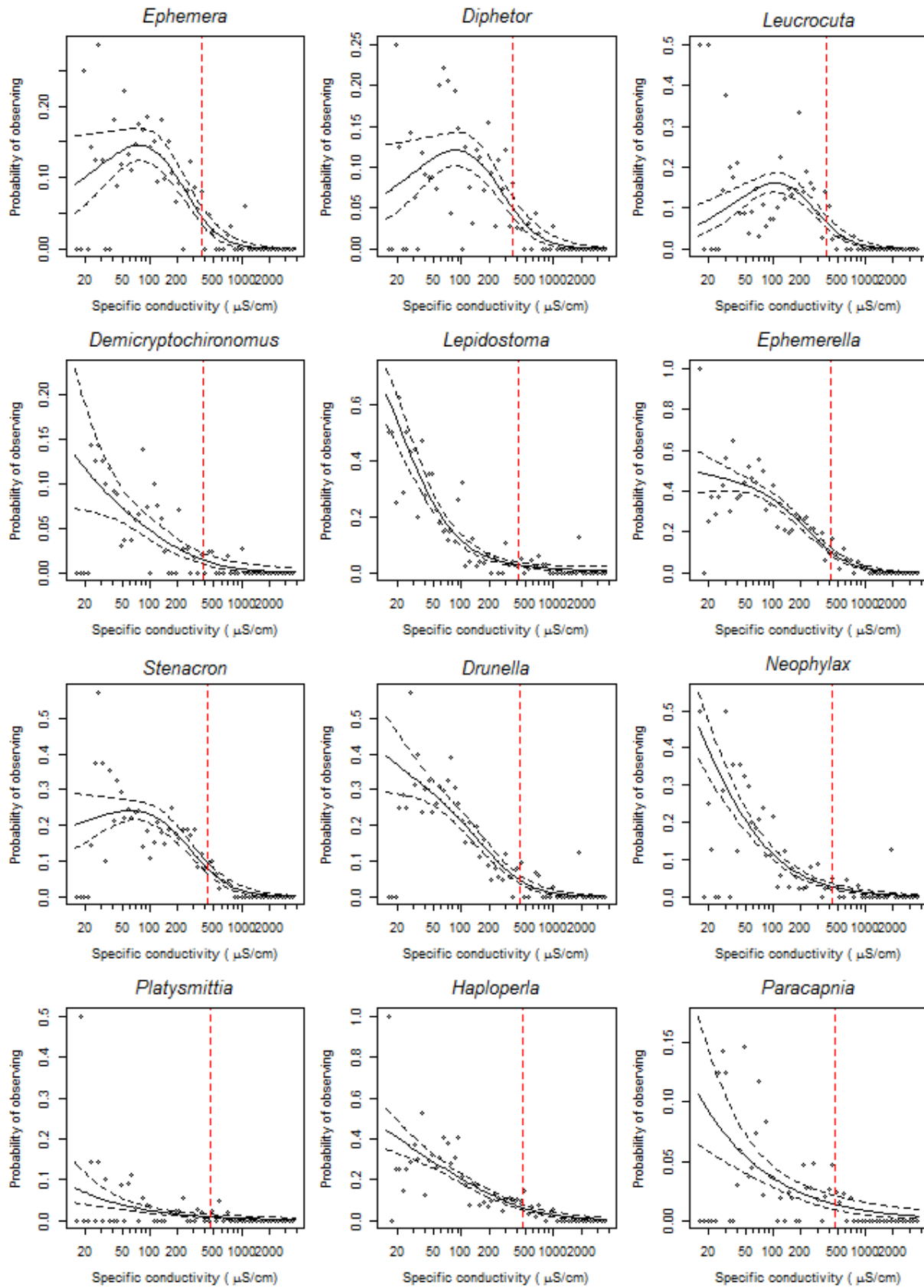
Order	Family	Genus	Symbol	XC <sub>95</sub>	95% CI	N
Diptera	Chironomidae	<i>Corynoneura</i>	>	2,768	1,324–2,768	86
Diptera	Tipulidae	<i>Dicranota</i>	>	2,768	915–2,768	296
Trichoptera	Hydropsychidae	<i>Diplectrona</i>	>	2,768	1,831–2,768	689
Trichoptera	Philopotamidae	<i>Dolophilodes</i>	>	2,768	741–2,768	479
Coleoptera	Psephenidae	<i>Ectopria</i>	>	2,768	1,136–2,768	287
Diptera	Chironomidae	<i>Heleniella</i>	>	2,768	356–2,768	56
Diptera	Empididae	<i>Hemerodromia</i>	>	2,768	2,226–3,341	438
Diptera	Tipulidae	<i>Hexatoma</i>	>	2,768	674–2,768	440
Coleoptera	Elmidae	<i>Oulimnius</i>	>	2,768	1,386–2,768	379
Diptera	Chironomidae	<i>Parachaetocladius</i>	>	2,768	533–2,768	192
Trichoptera	Polycentropodidae	<i>Polycentropus</i>	>	2,768	1,338–2,768	449
Diptera	Chironomidae	<i>Tanytarsus</i>	>	2,768	1,705–2,768	737
Diptera	Chironomidae	<i>Tvetenia</i>	>	2,768	1,896–2,768	660
Diptera	Chironomidae	<i>Zavreliomyia</i>	>	2,768	757–2,768	107
Plecoptera	Chloroperlidae	<i>Sweltsa</i>	>	2,904	563–3,174	224
Odonata	Aeshnidae	<i>Boyeria</i>	>	3,140	1,970–3,140	139
Trichoptera	Hydropsychidae	<i>Ceratopsyche</i>	>	3,140	2,160–3,140	853
Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i>	>	3,140	2,160–3,174	1,247
Trichoptera	Philopotamidae	<i>Chimarra</i>	>	3,140	2,296–3,174	453
Megaloptera	Corydalidae	<i>Corydalus</i>	>	3,140	1,739–3,341	342
Megaloptera	Corydalidae	<i>Nigronia</i>	>	3,140	2,257–3,140	669
Coleoptera	Psephenidae	<i>Psephenus</i>	>	3,140	1,266–3,140	503
Diptera	Chironomidae	<i>Rheocricotopus</i>	>	3,140	2,169–3,174	375
Diptera	Tipulidae	<i>Tipula</i>	>	3,140	1,979–3,140	435
Diptera	Athericidae	<i>Atherix</i>	>	3,174	1,550–3,174	198
Diptera	Chironomidae	<i>Cardiocladius</i>	>	3,174	1,534–3,174	254
Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	>	3,174	2,160–3,341	859
Coleoptera	Elmidae	<i>Optioservus</i>	>	3,174	2,186–3,341	1,134
Diptera	Chironomidae	<i>Thienemanniella</i>	>	3,174	1,931–3,174	216
Diptera	Empididae	<i>Chelifera</i>	>	3,341	1,831–3,341	160
Diptera	Chironomidae	<i>Cricotopus</i>	>	3,341	1,543–3,341	257
Diptera	Ceratopogonidae	<i>Dasyhelea</i>	>	3,341	1,610–3,341	61
Coleoptera	Elmidae	<i>Microcyloepus</i>	>	3,341	1,165–3,341	155
Diptera	Chironomidae	<i>Paraphaenocladius</i>	>	3,341	798–3,341	34
Diptera	Chironomidae	<i>Orthocladius</i>	>	3,794	1,193–3,794	174

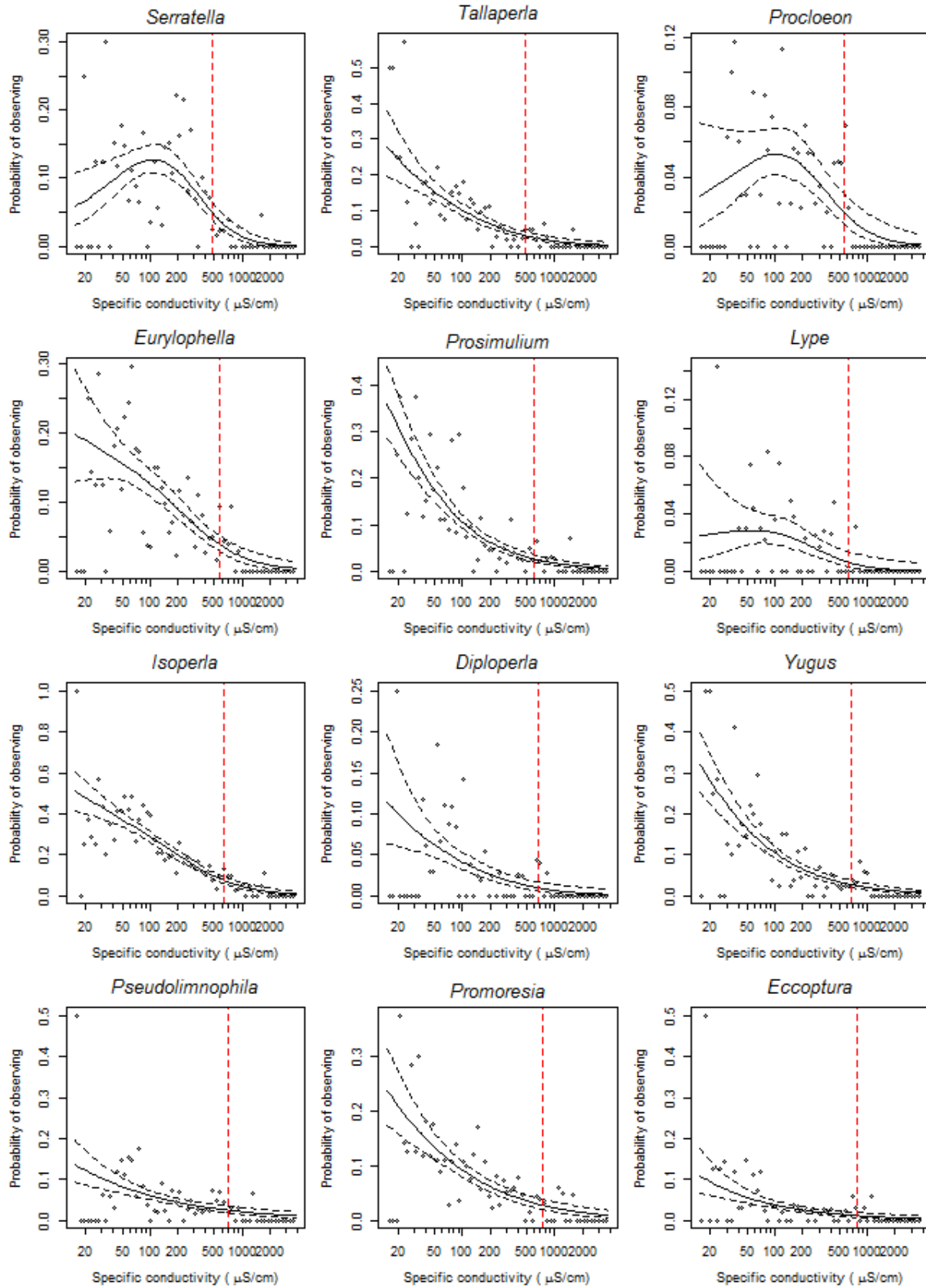
#### **A.5. CASE STUDY I: GENERALIZED ADDITIVE MODEL (GAM) PLOTS**

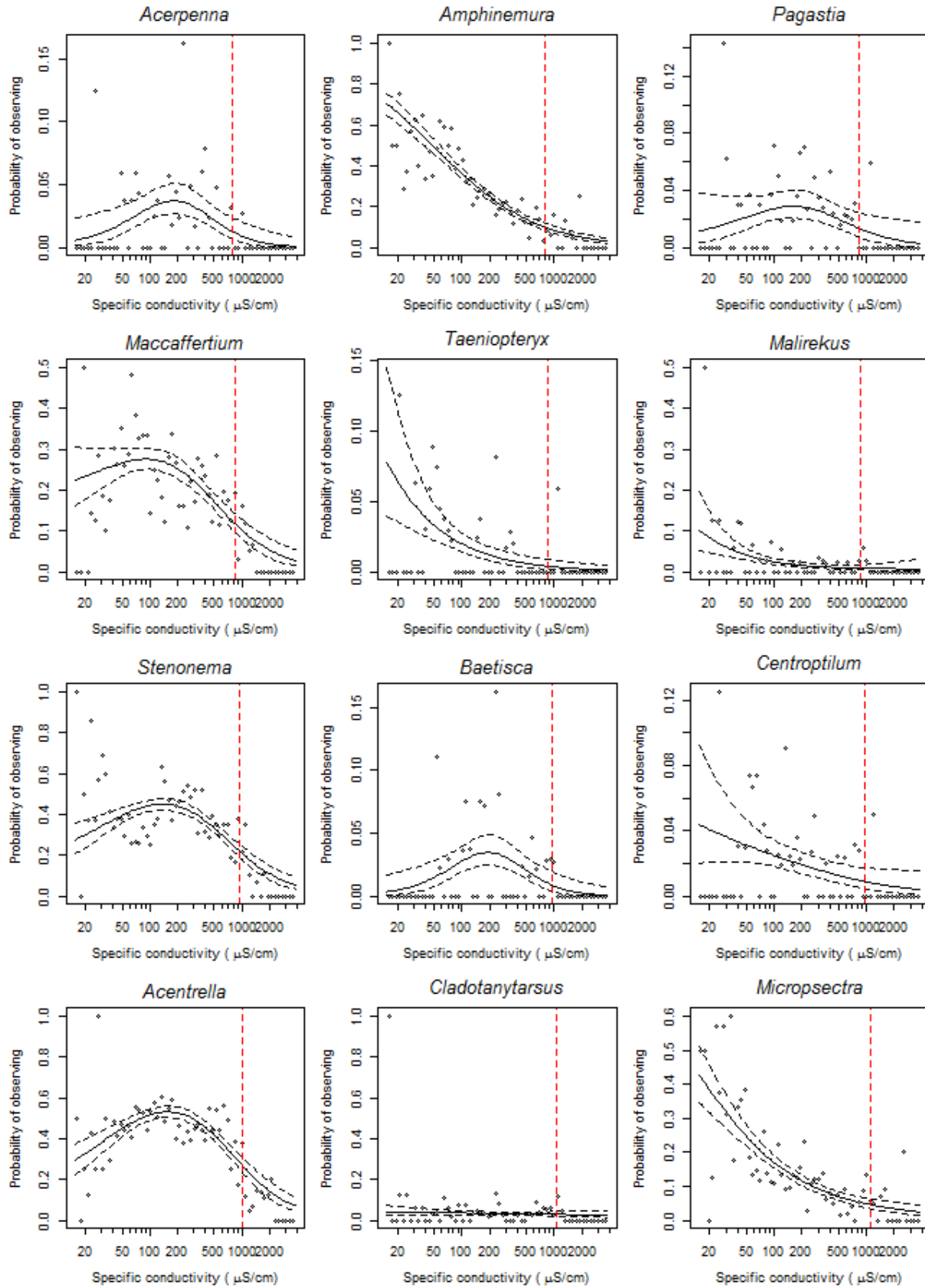
The GAM plots used to designate  $\sim$  and  $>$  values for those  $XC_{95}$  values are depicted in this Appendix (see Section A.5). In this example, the probability of observing a genus is the proportion of sampled stations in a conductivity bin with the genus present based on taxonomic identification 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 A.4) obtained from the plots of the cumulative distribution functions (CDFs) (see Section A.6. Plots are arranged from the lowest to the highest  $XC_{95}$  value.

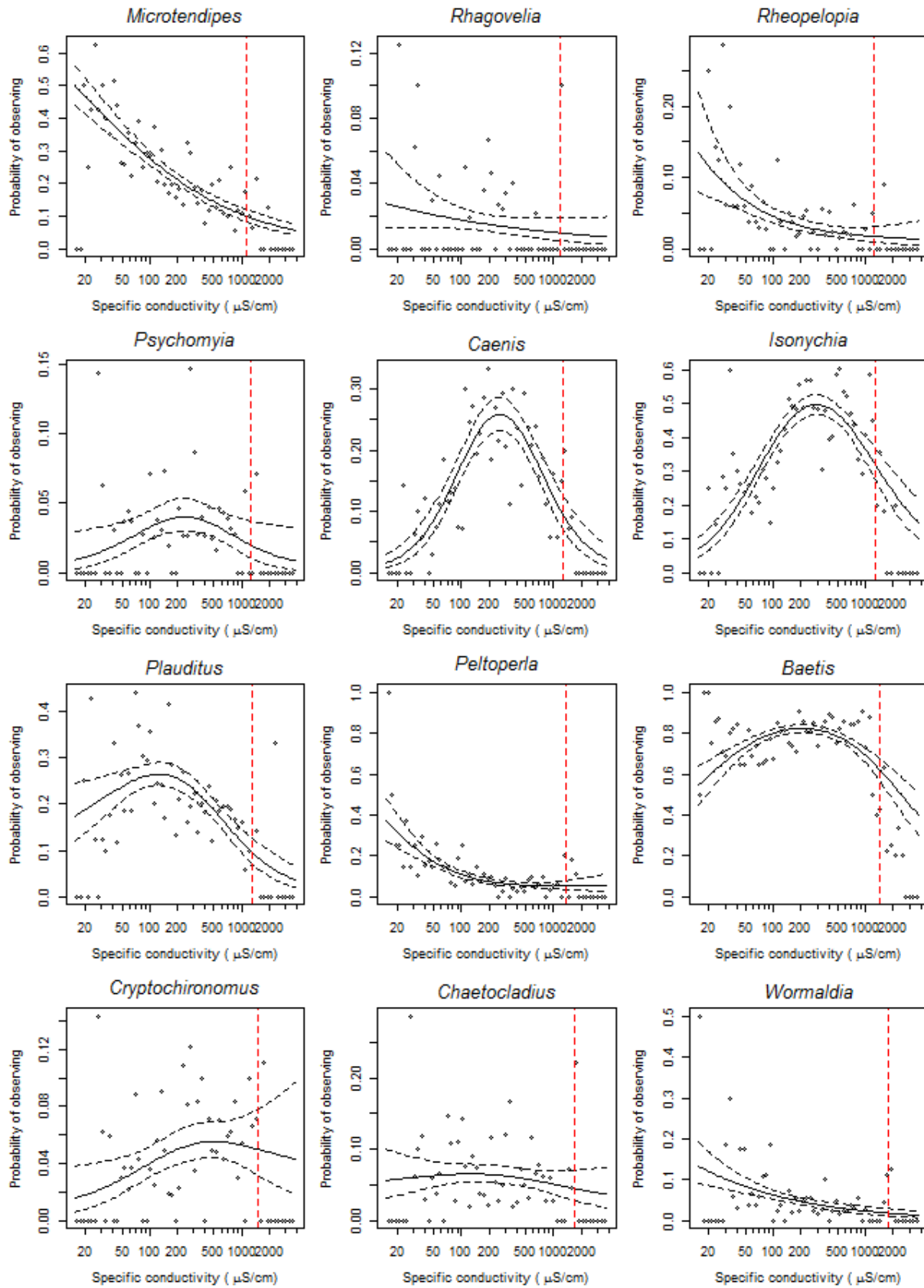


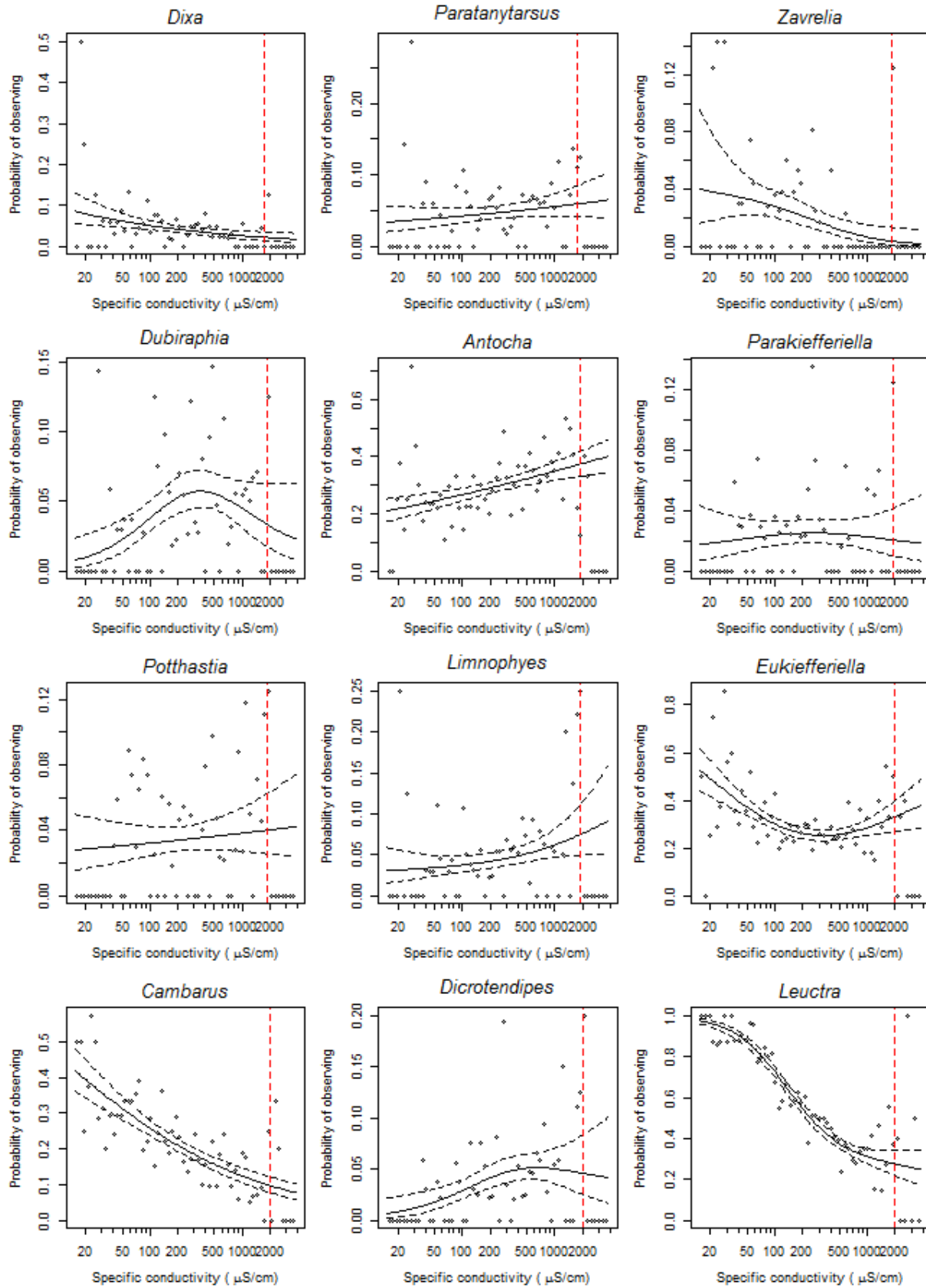


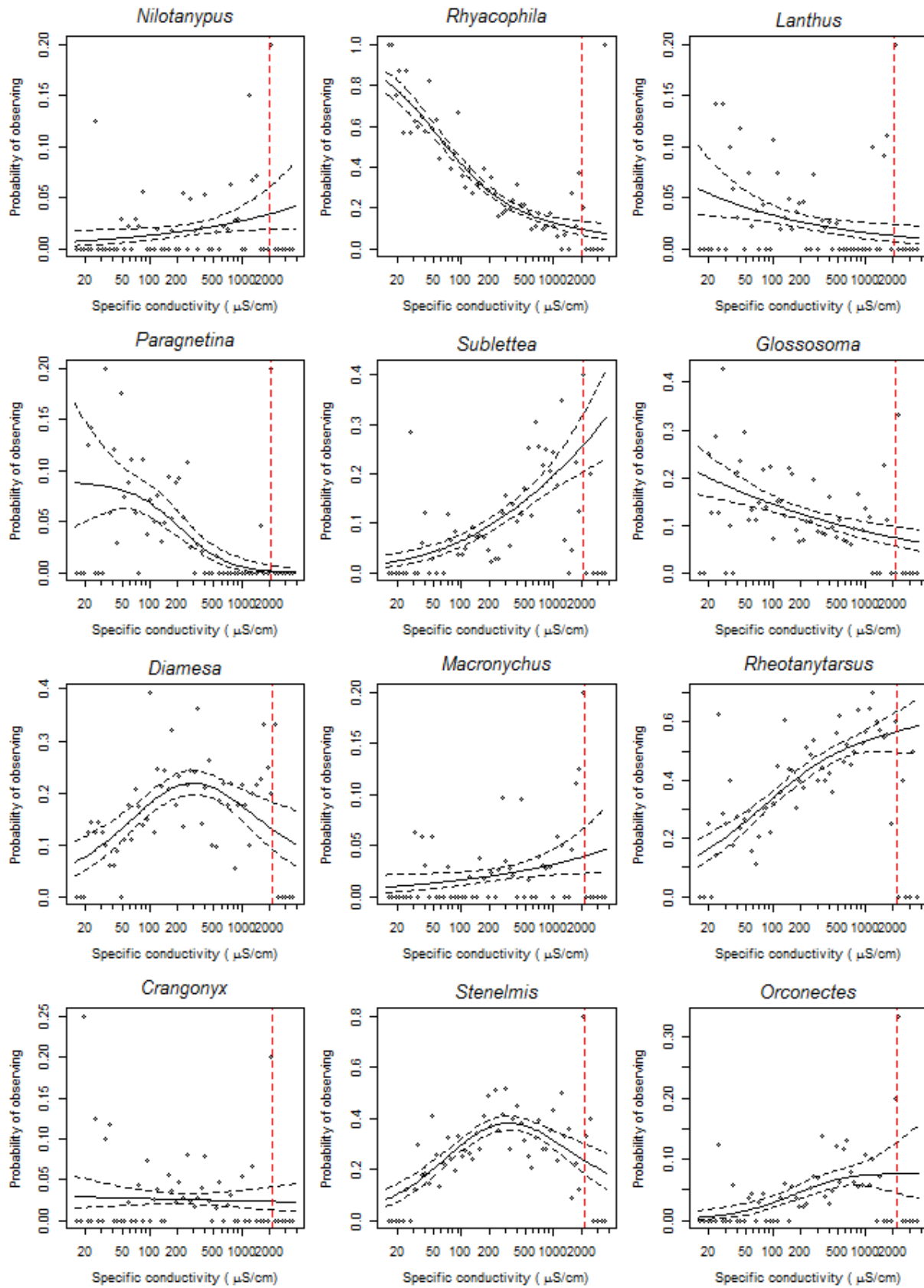


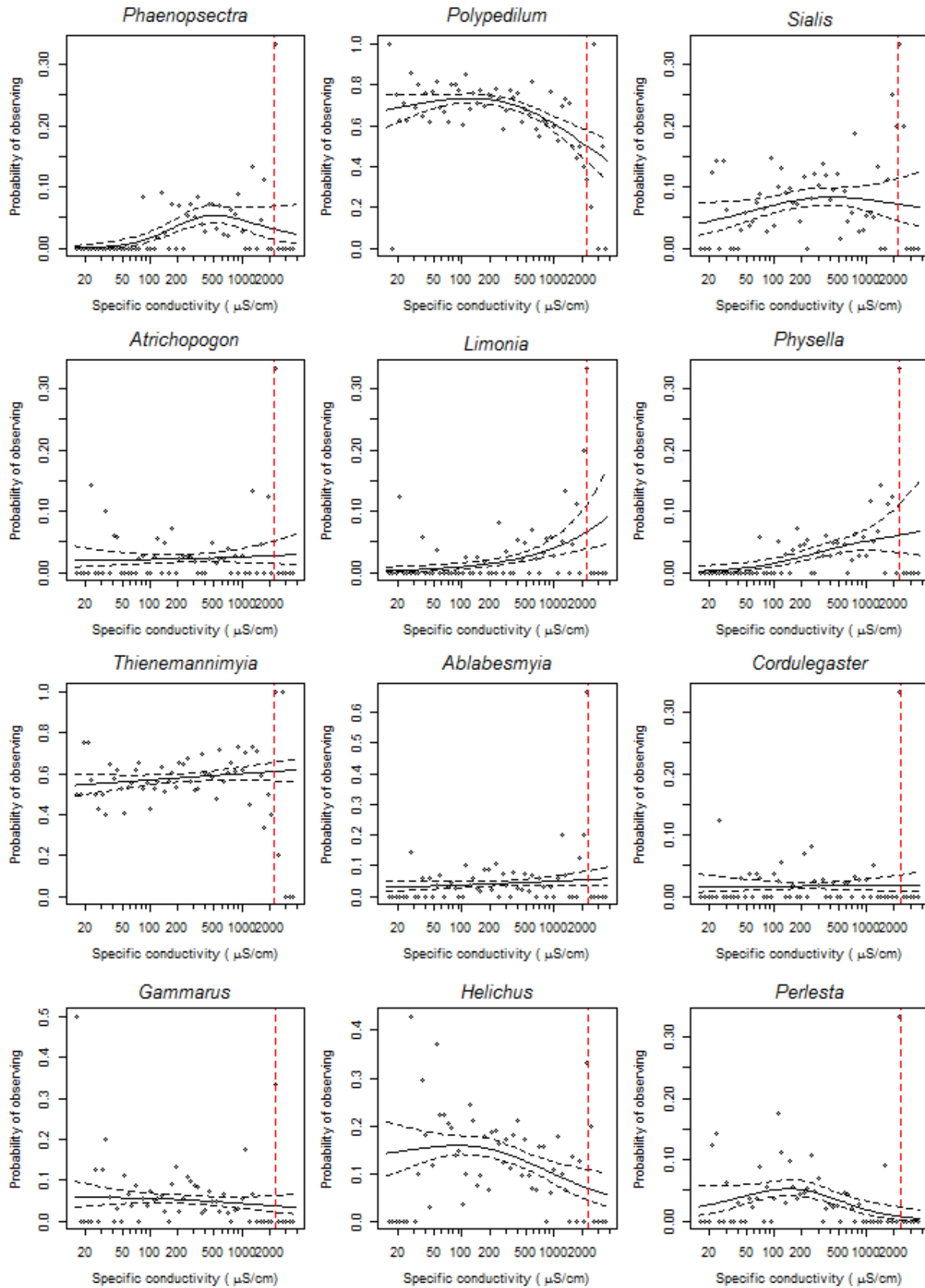




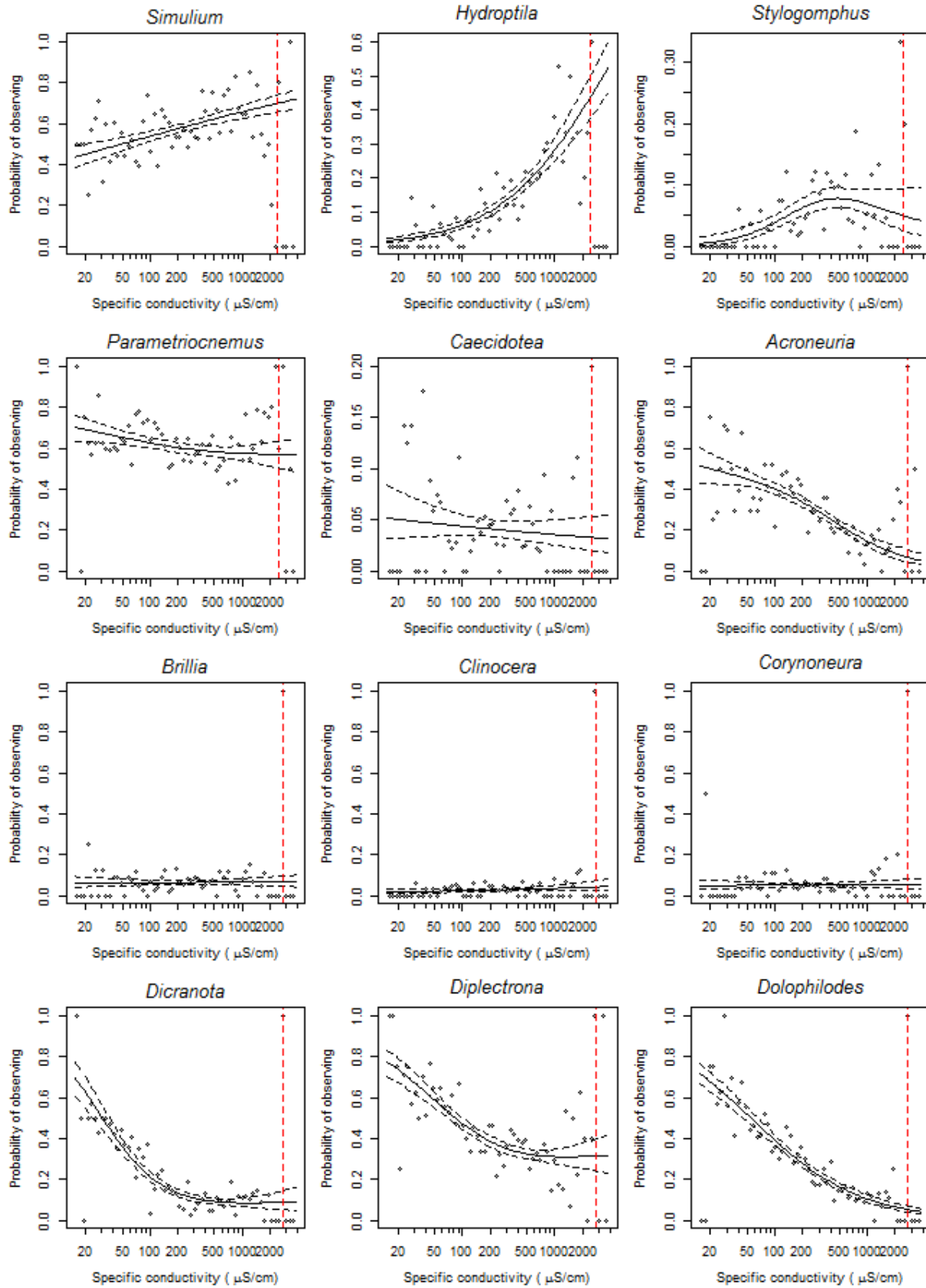




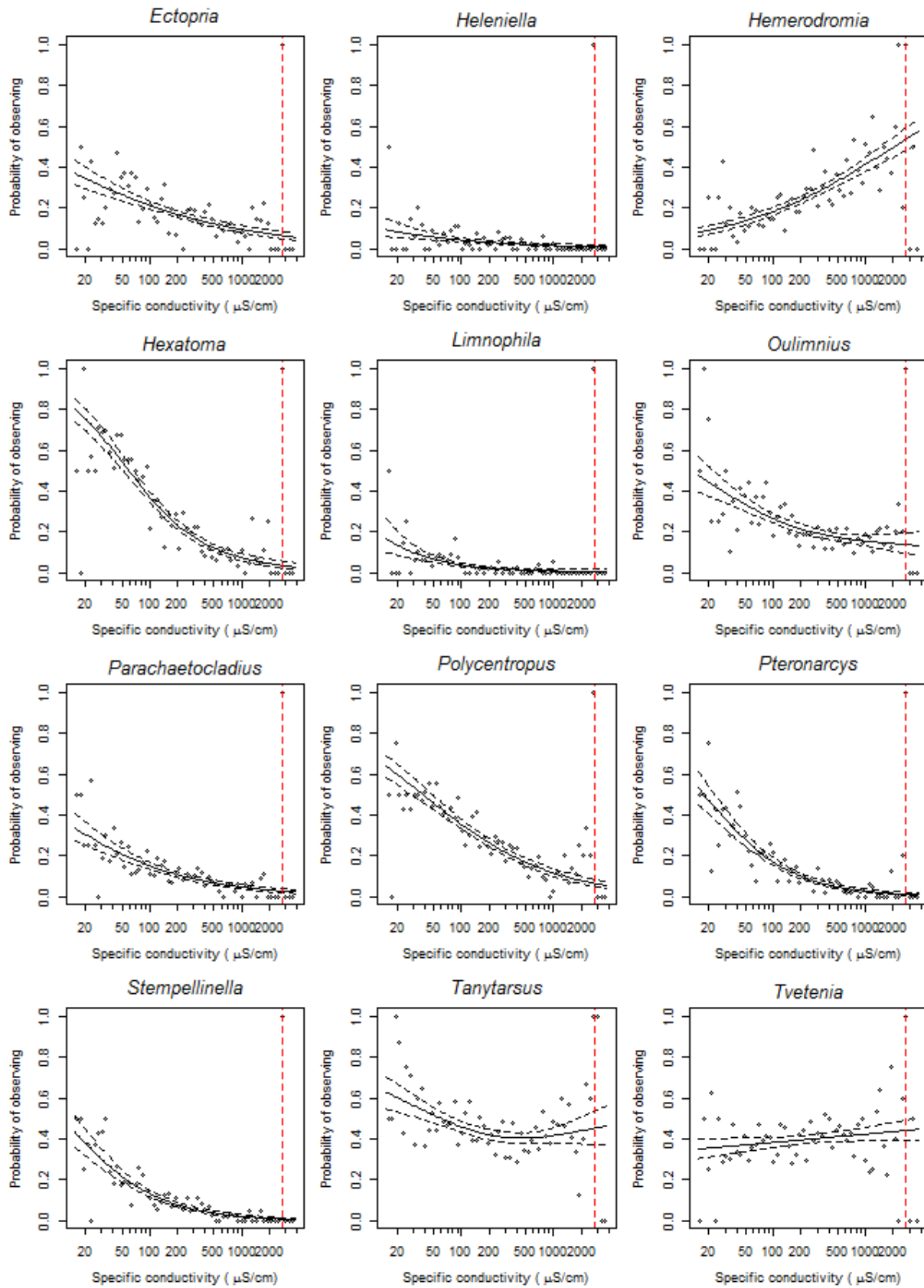


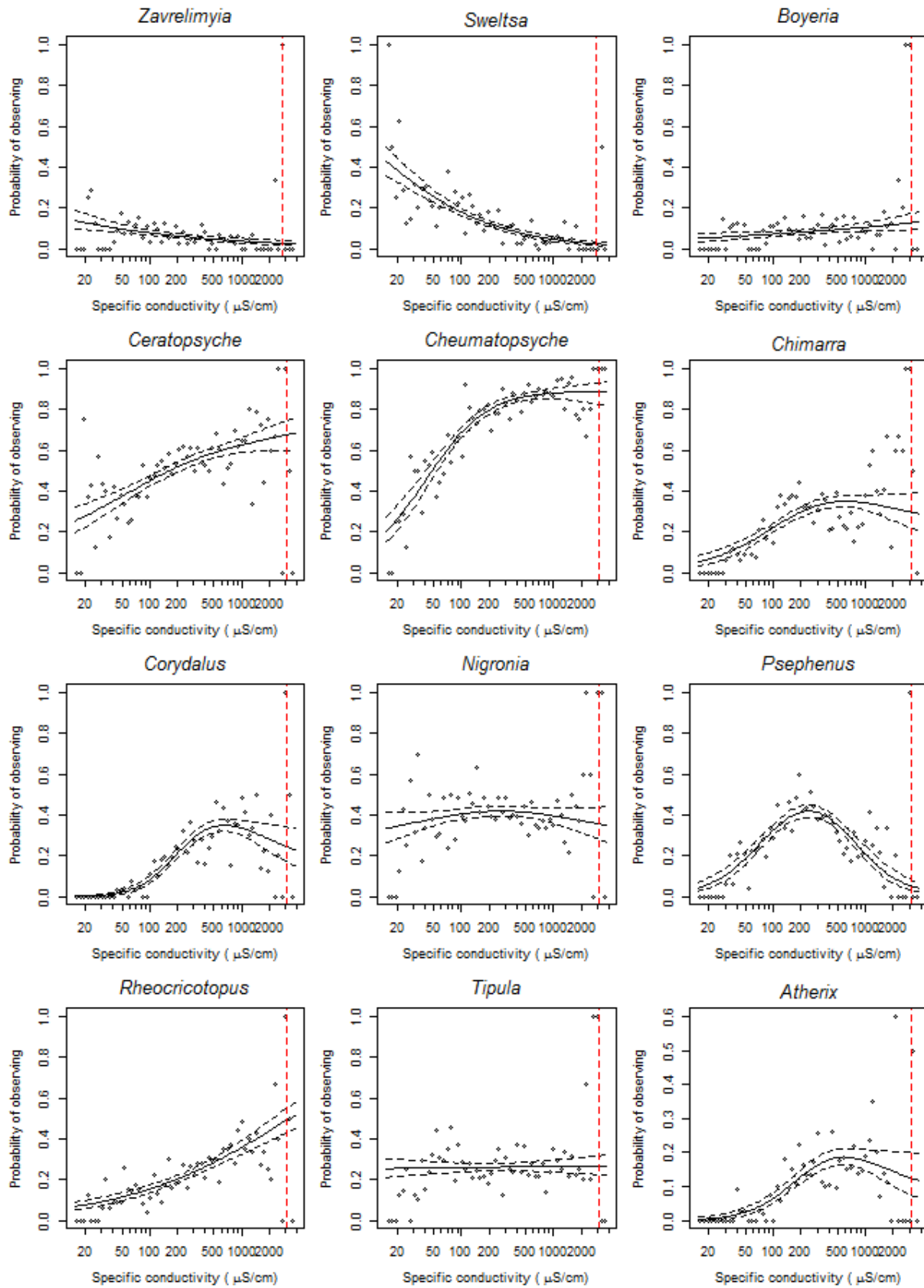


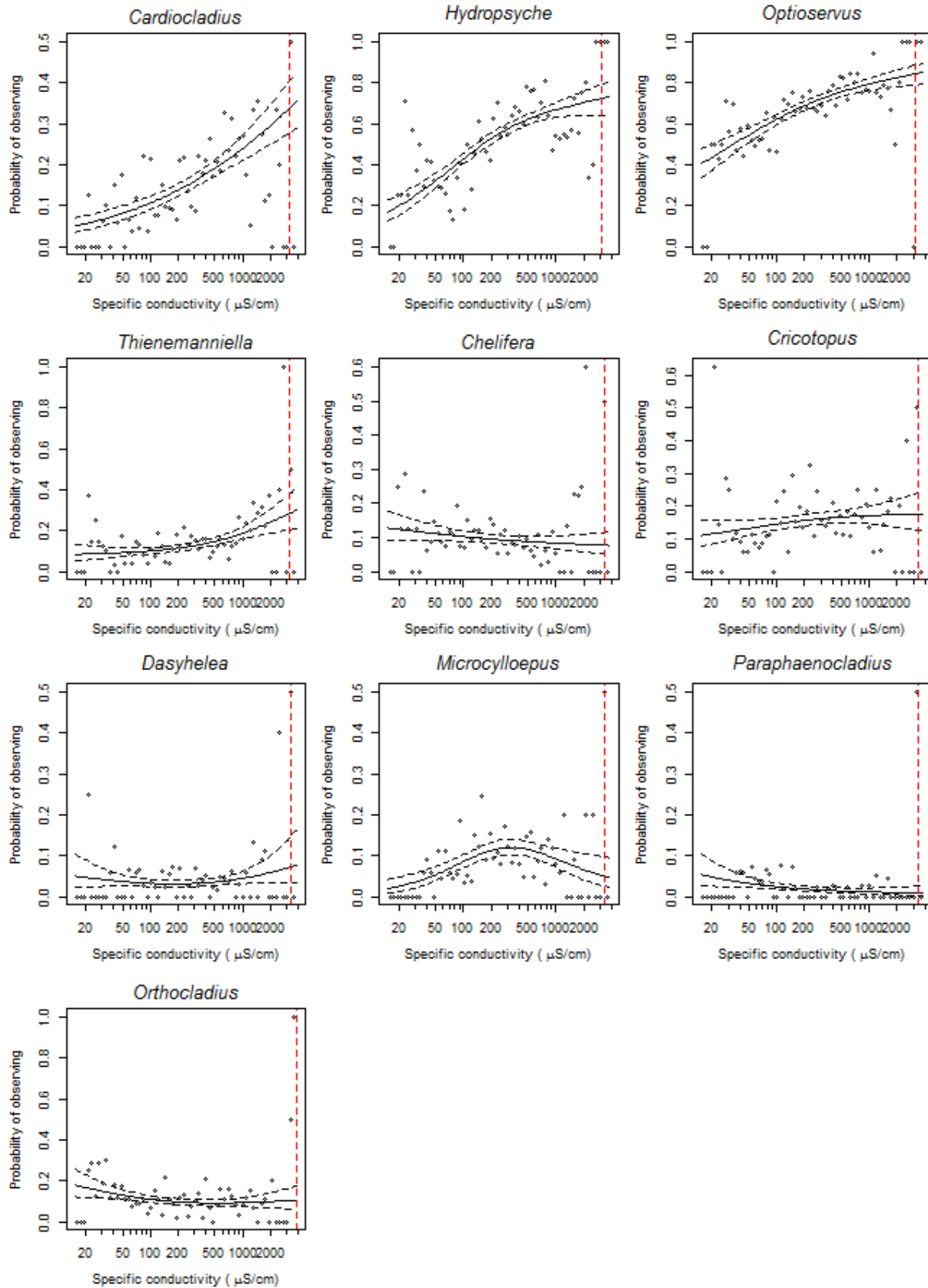






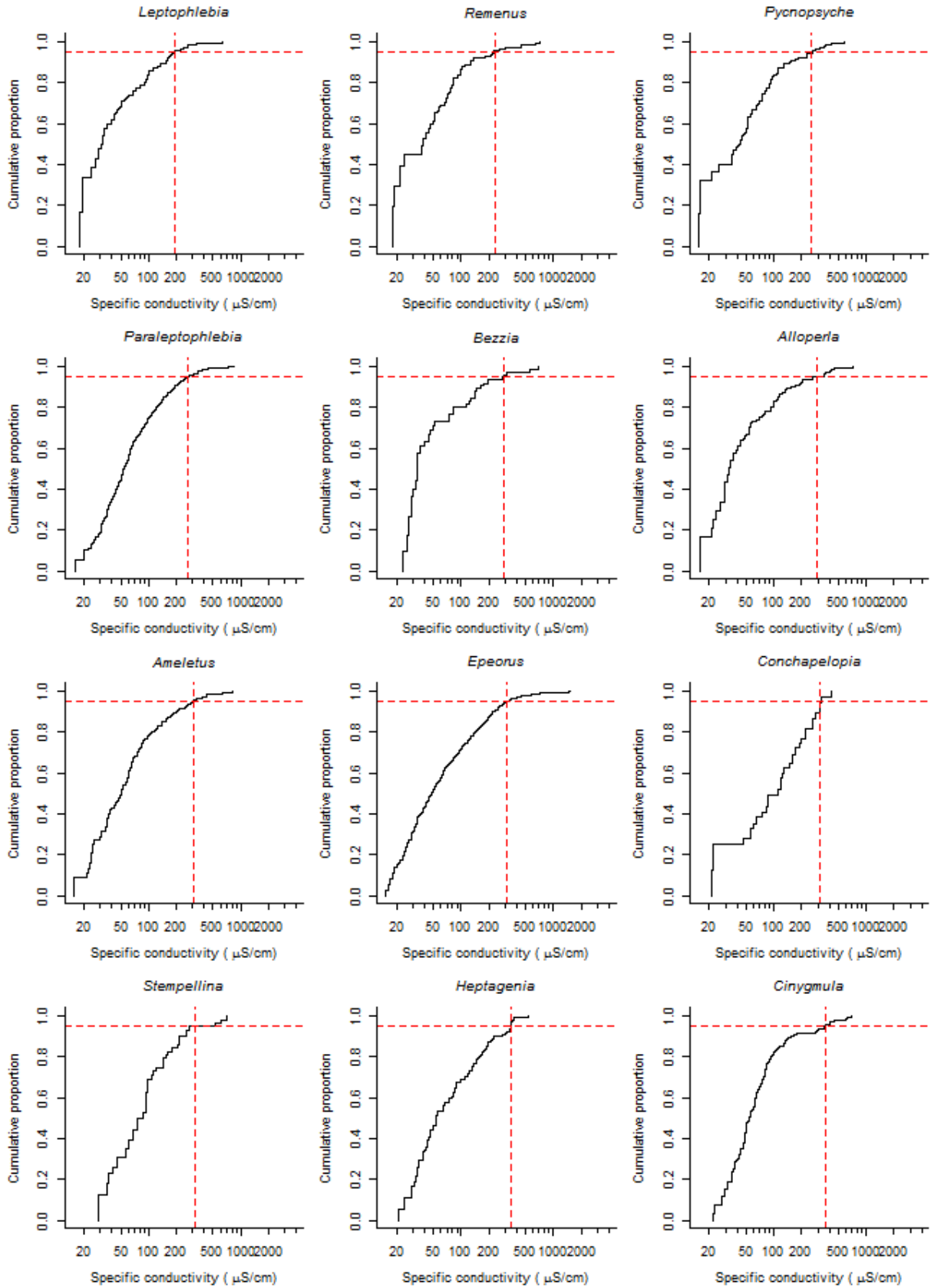


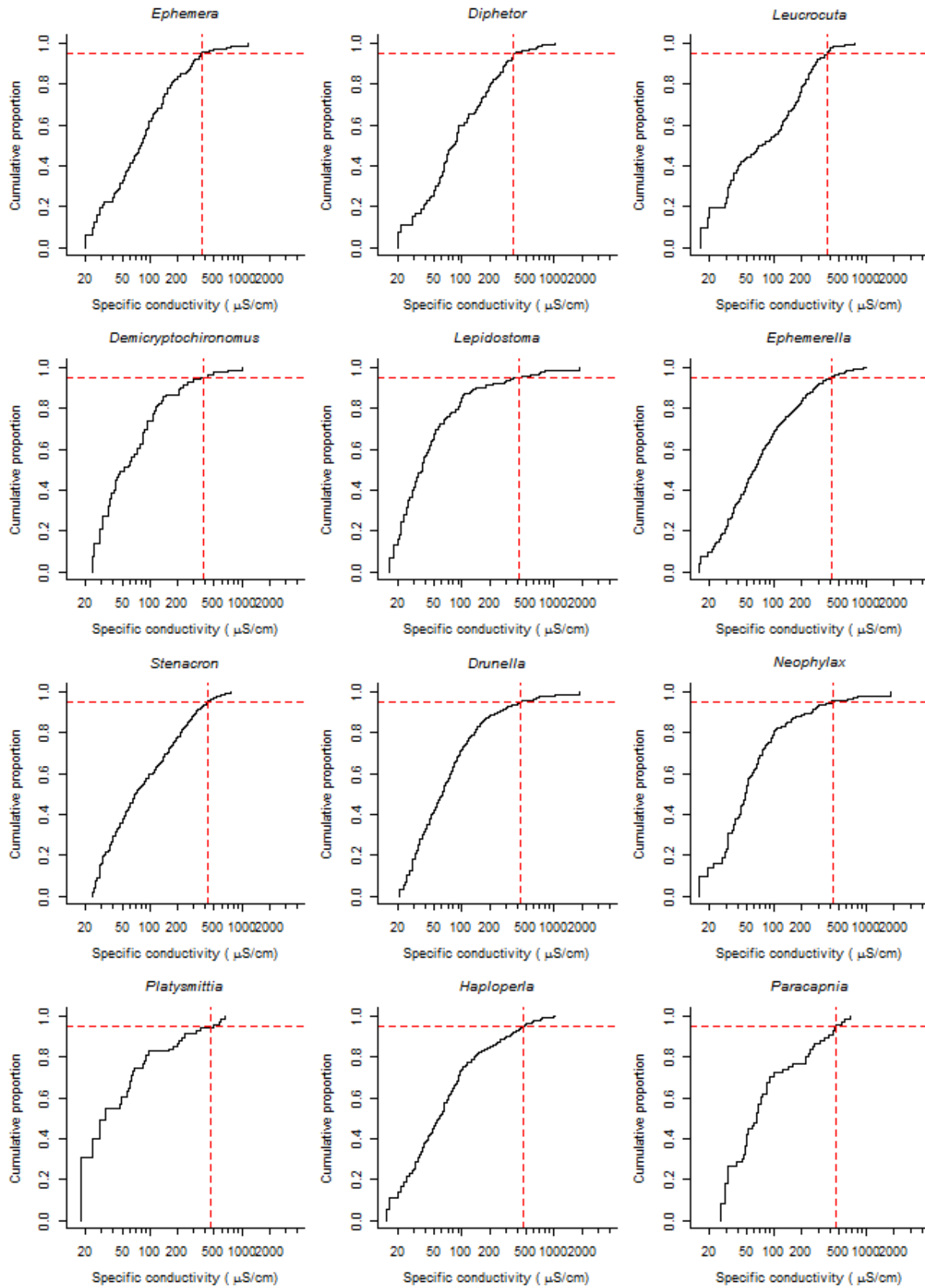


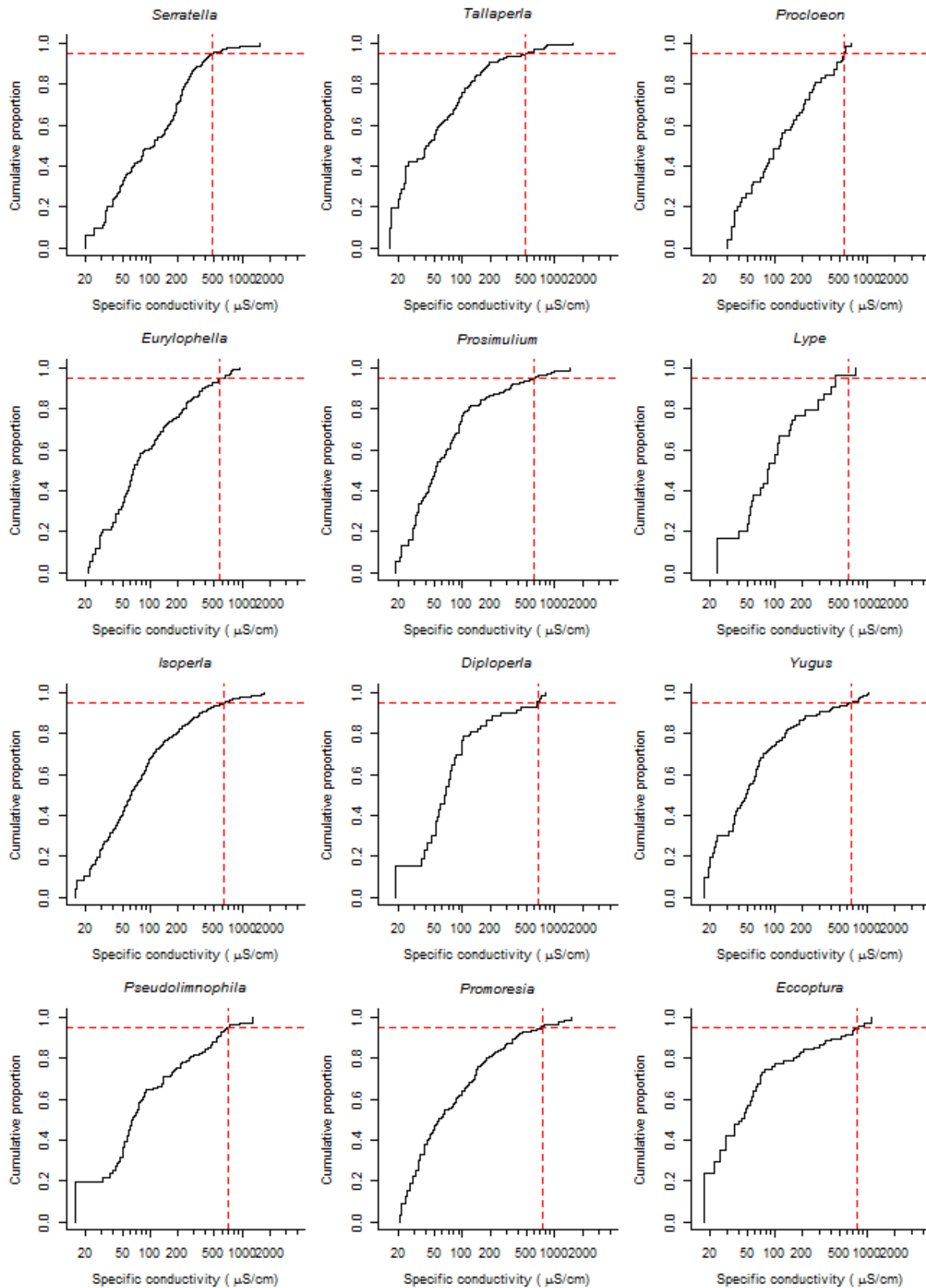


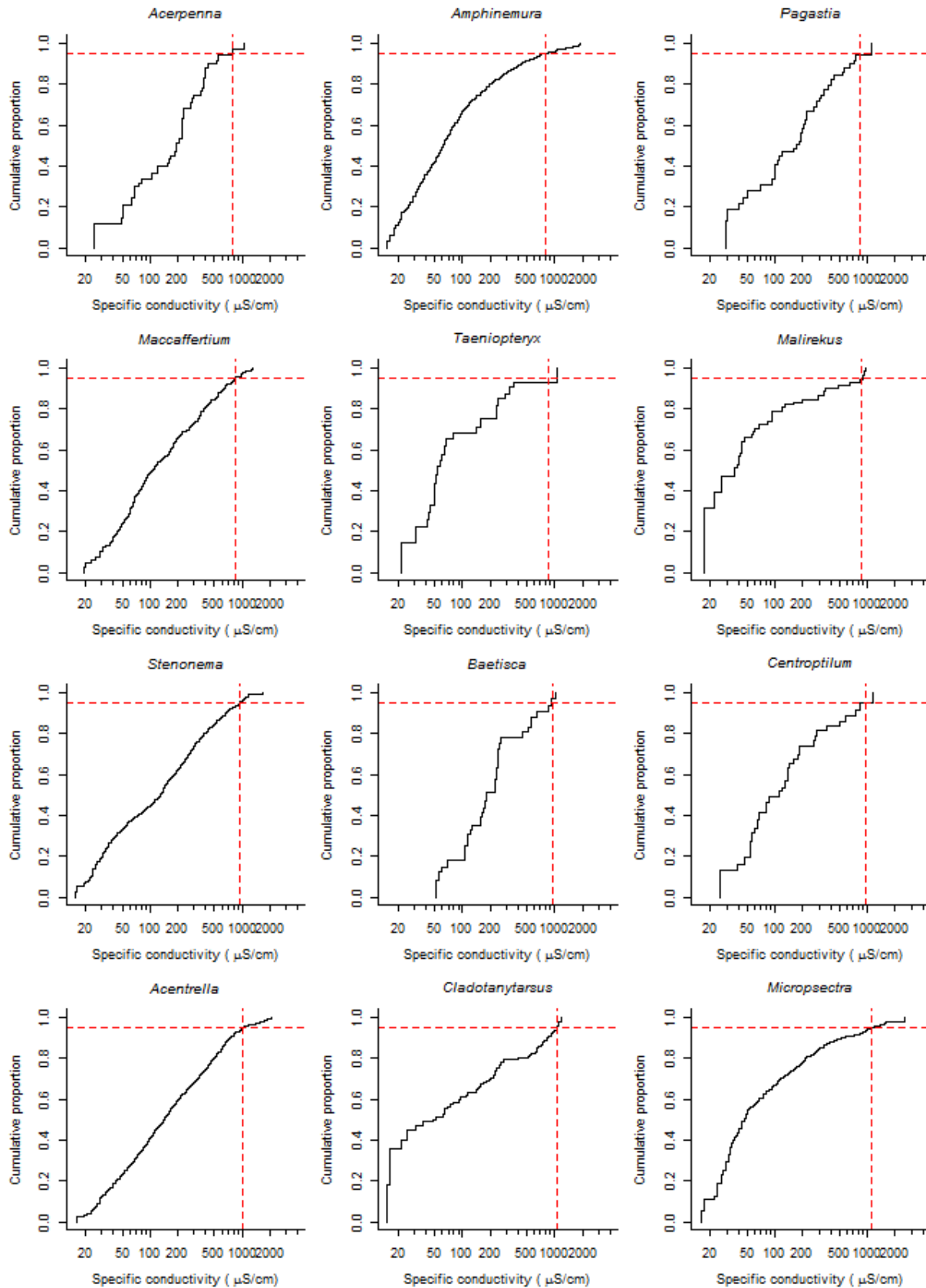
## **A.6. CASE STUDY I: CUMULATIVE DISTRIBUTION FUNCTION (CDF) PLOTS**

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

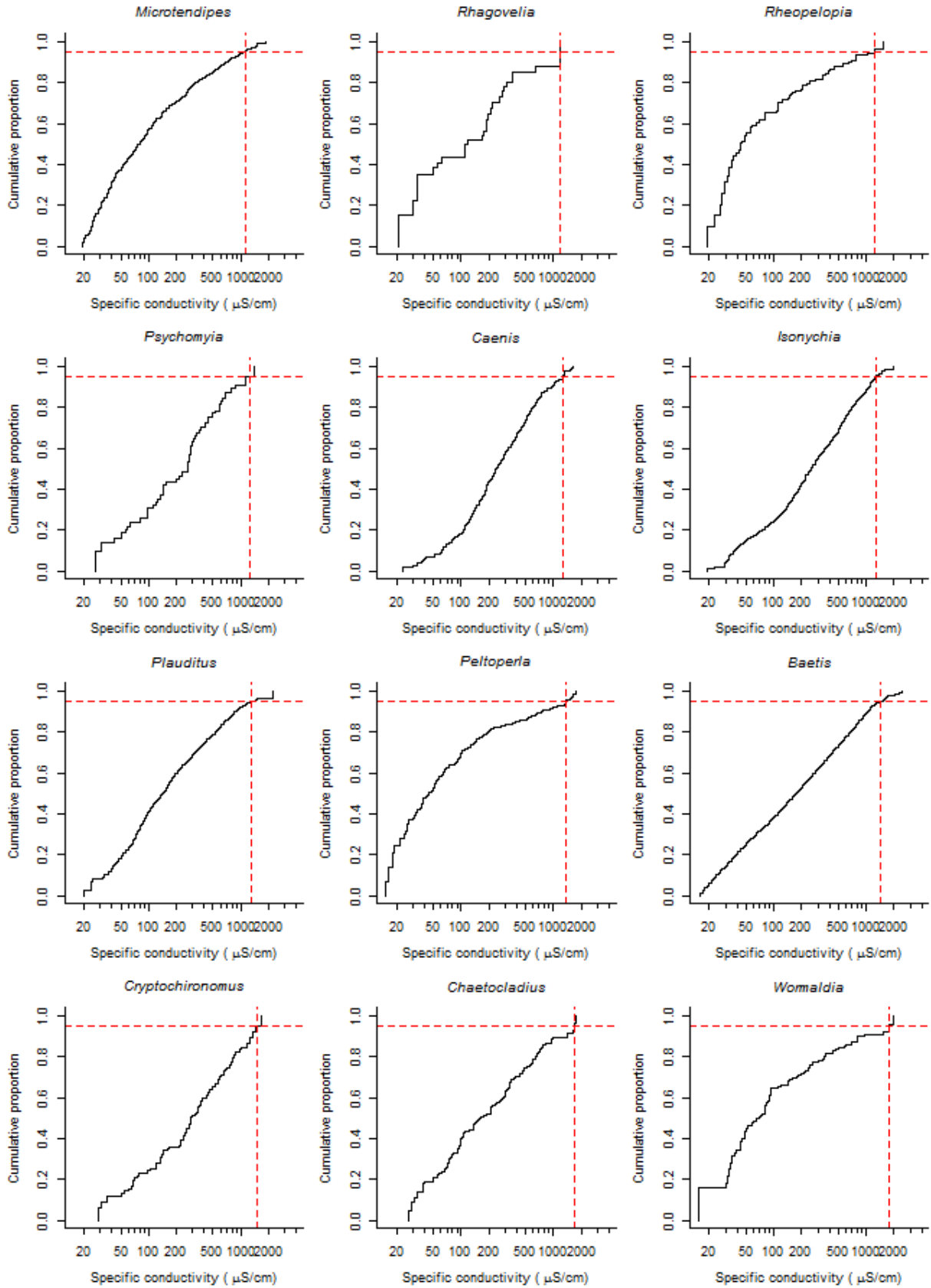


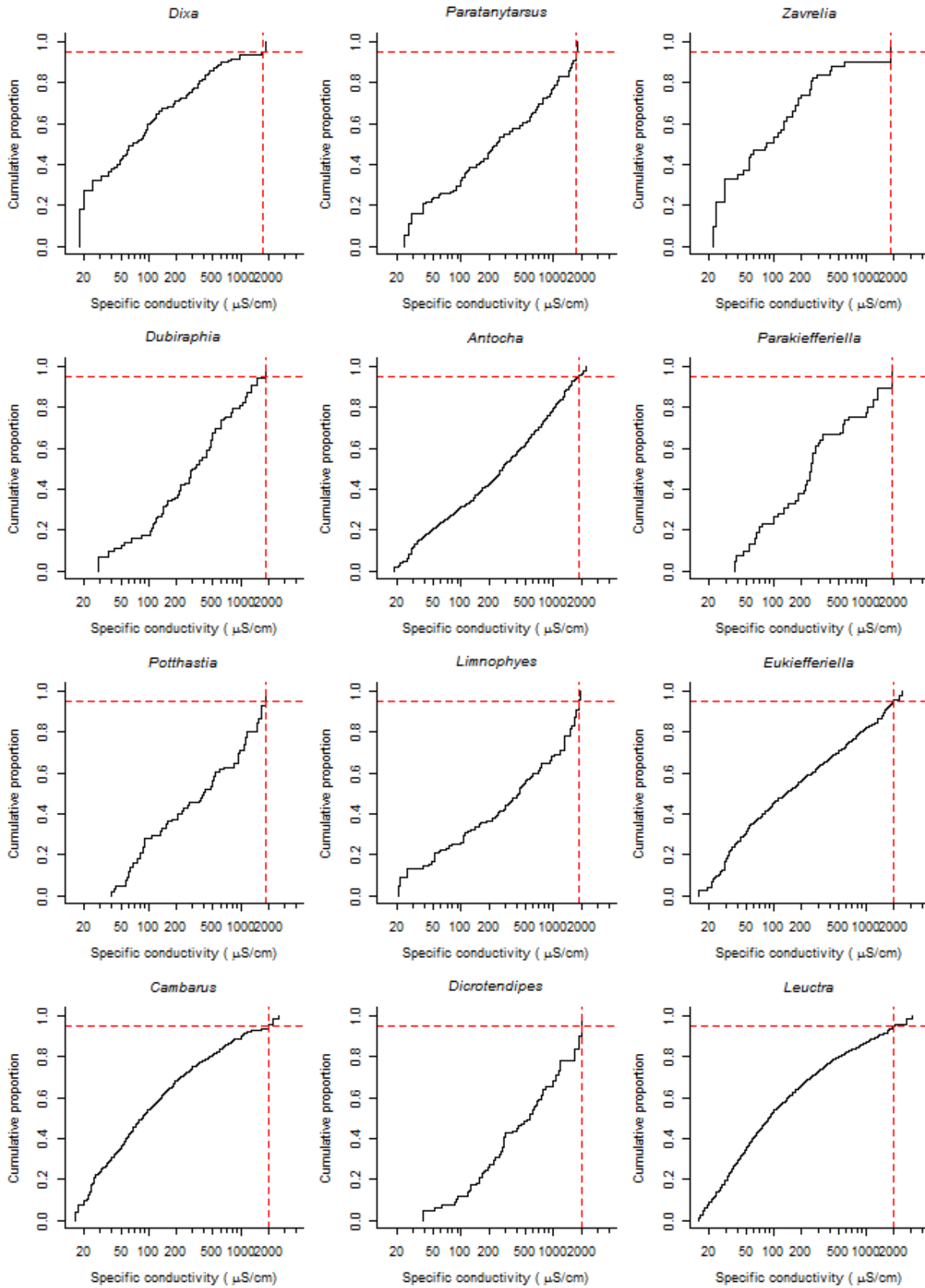


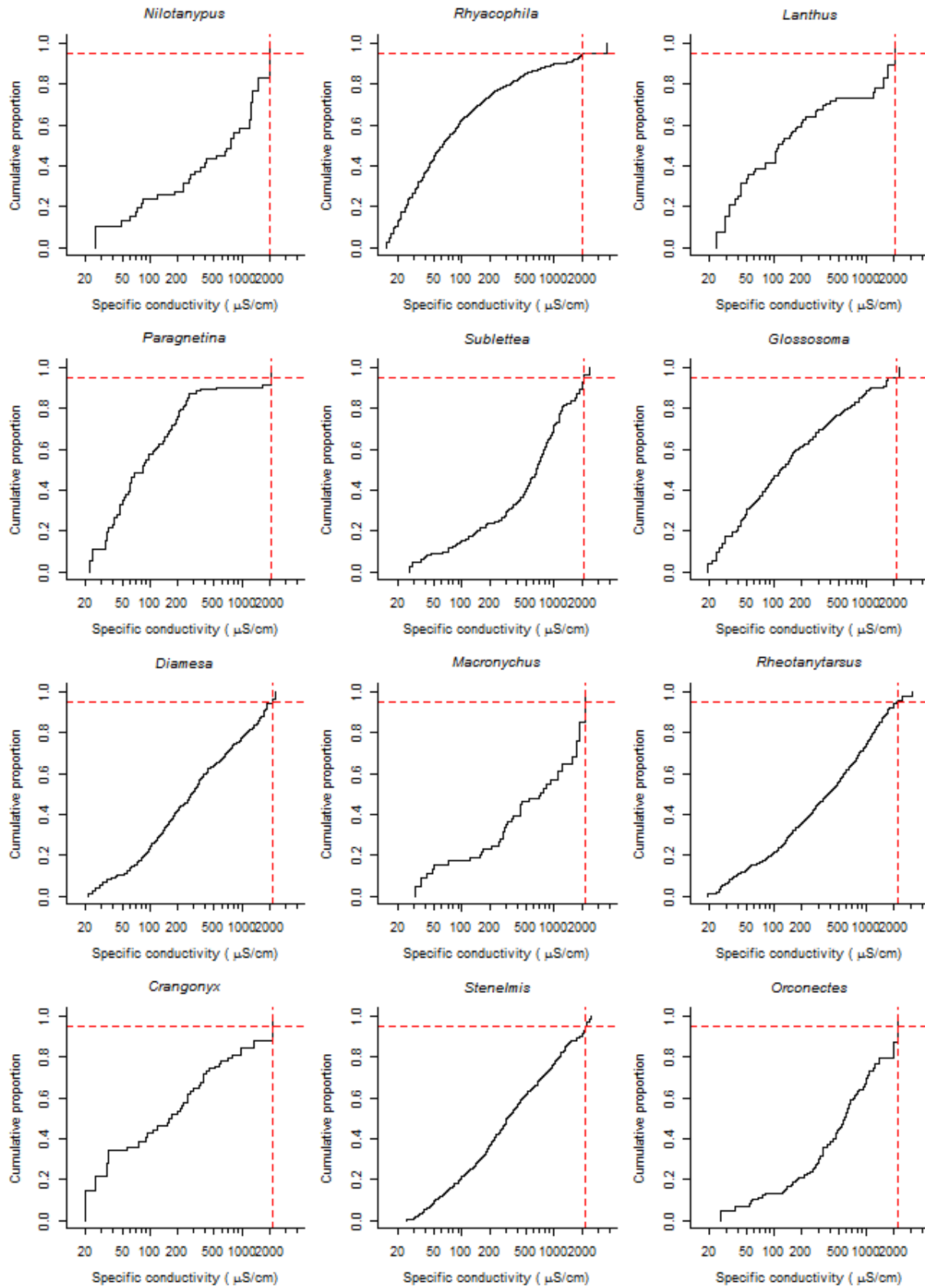


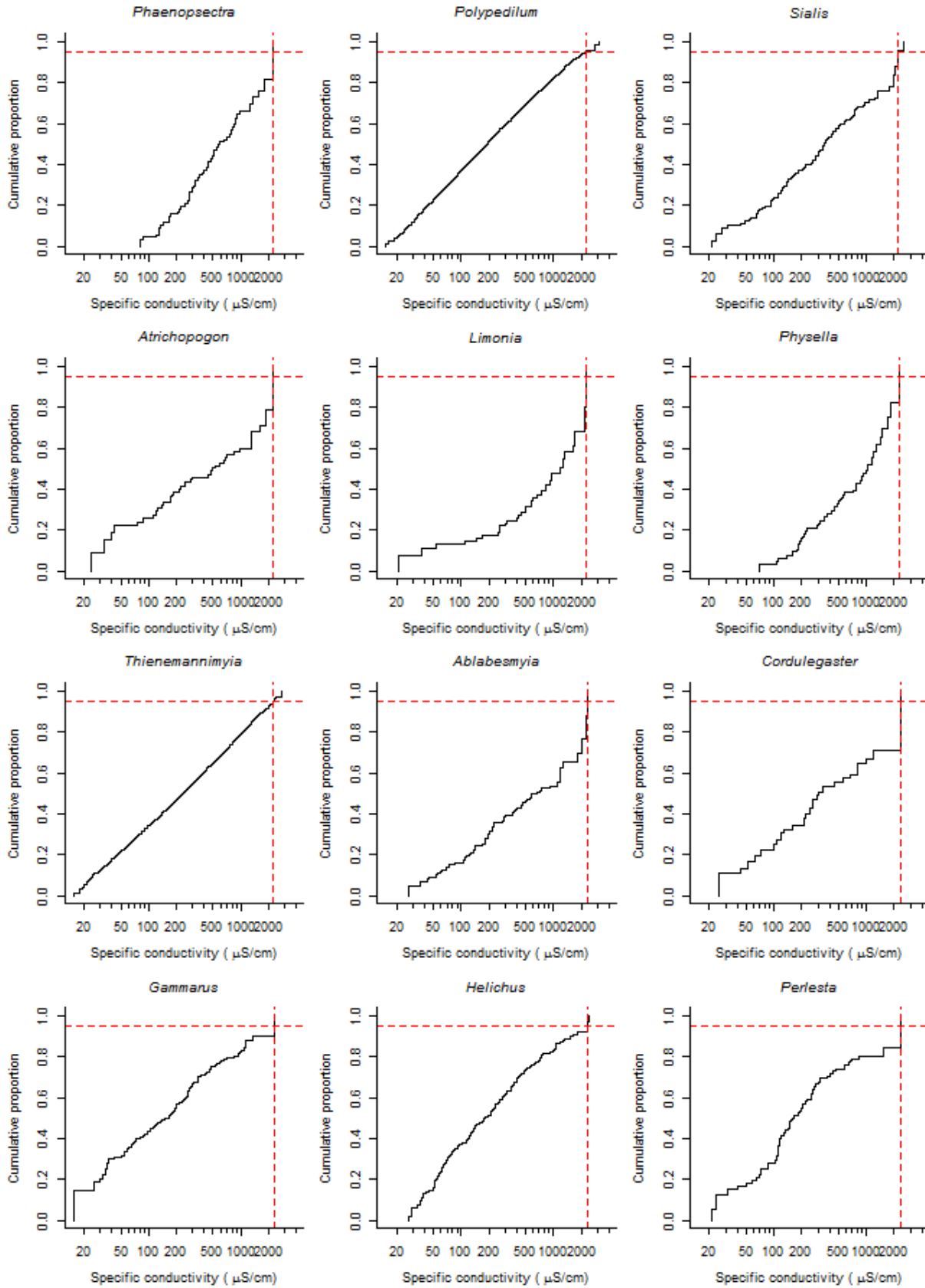


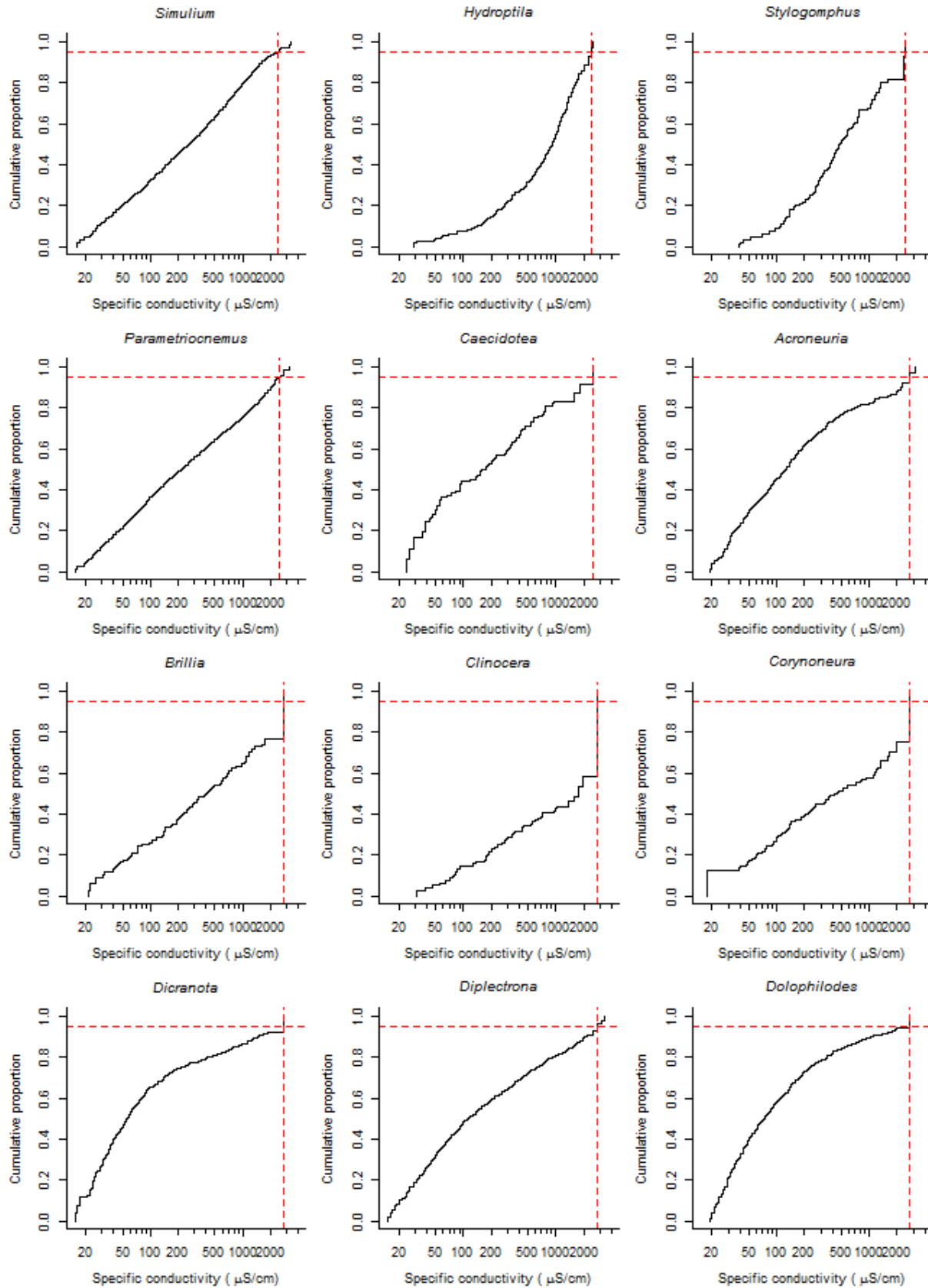


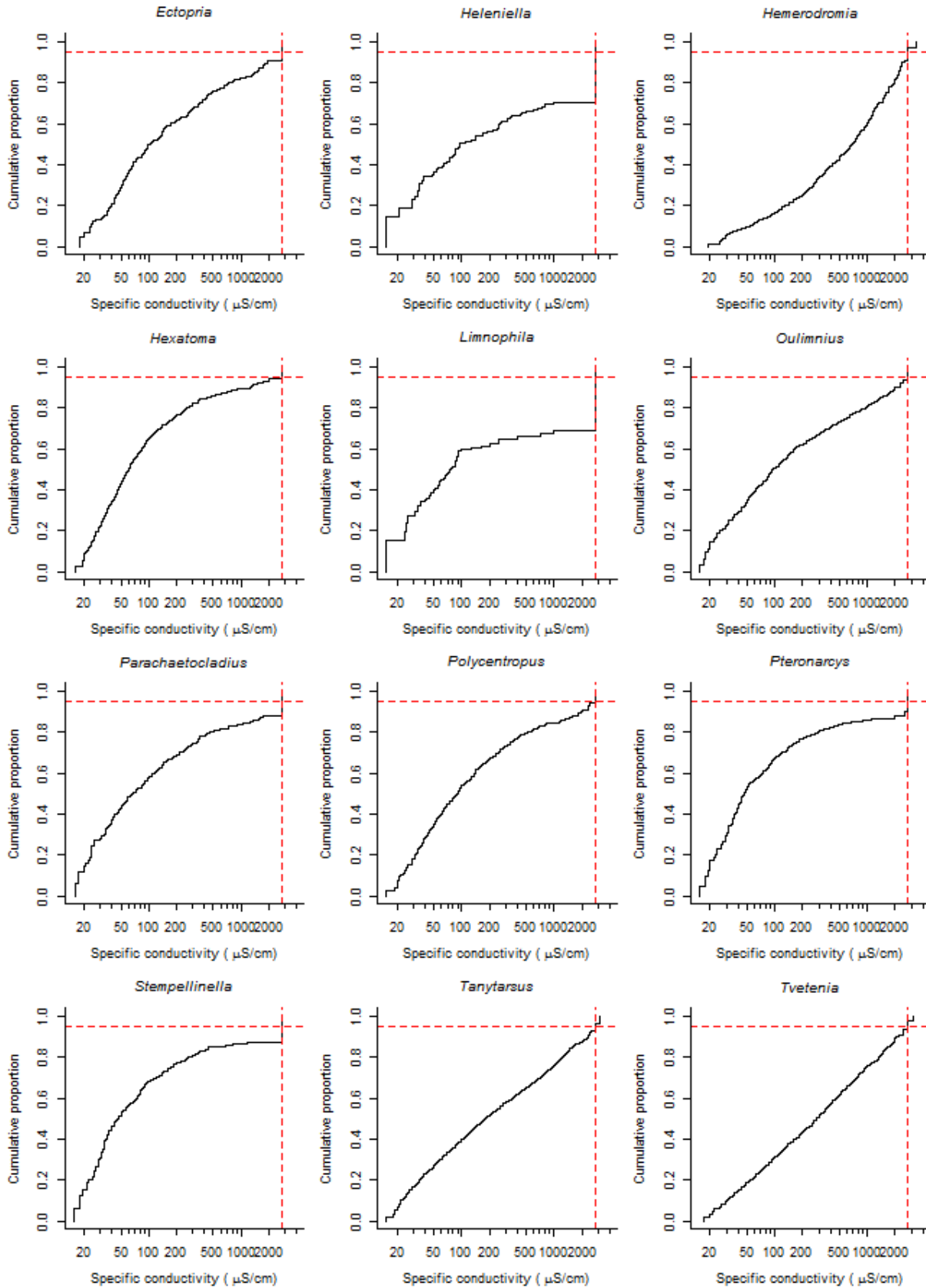


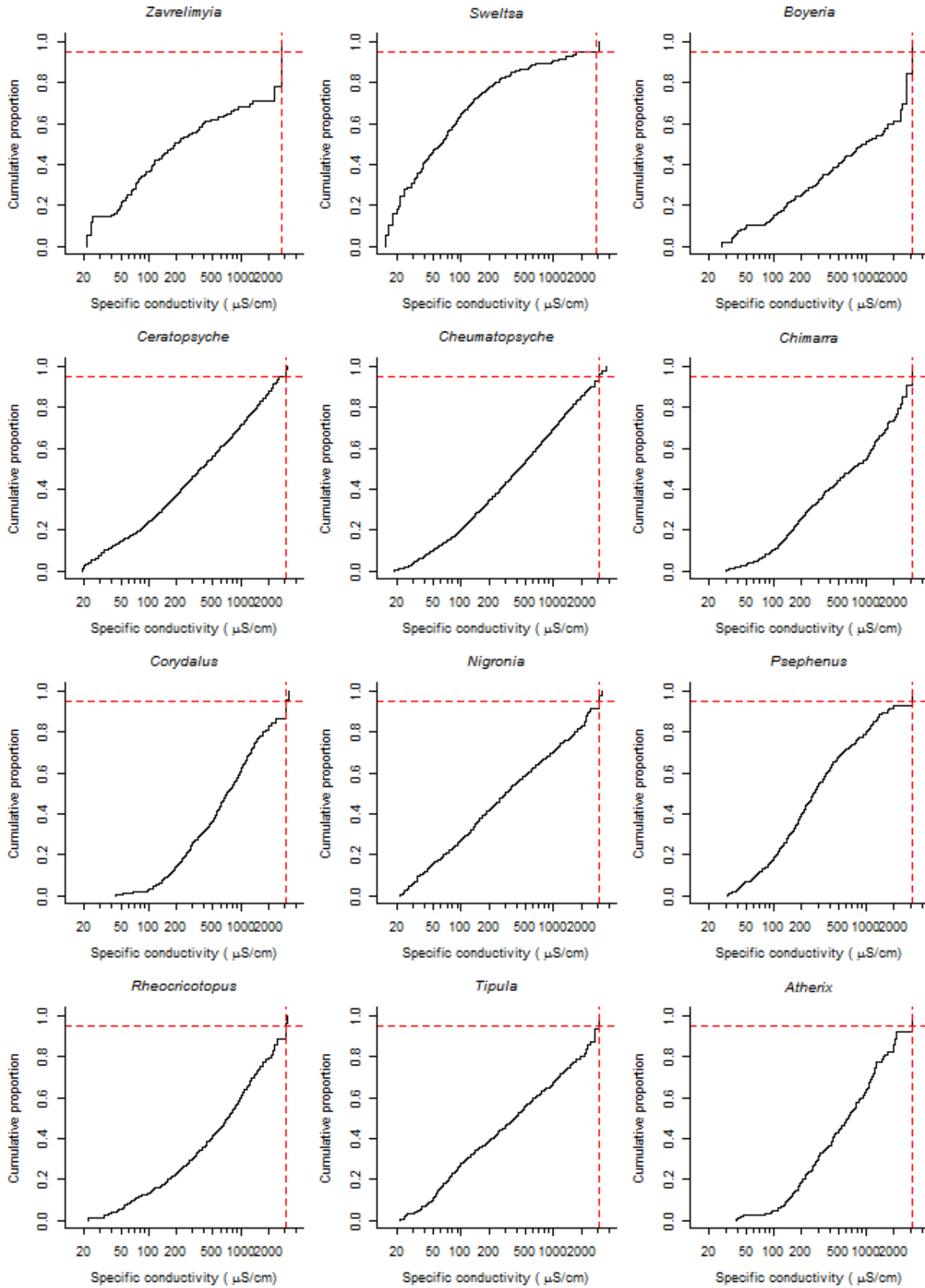


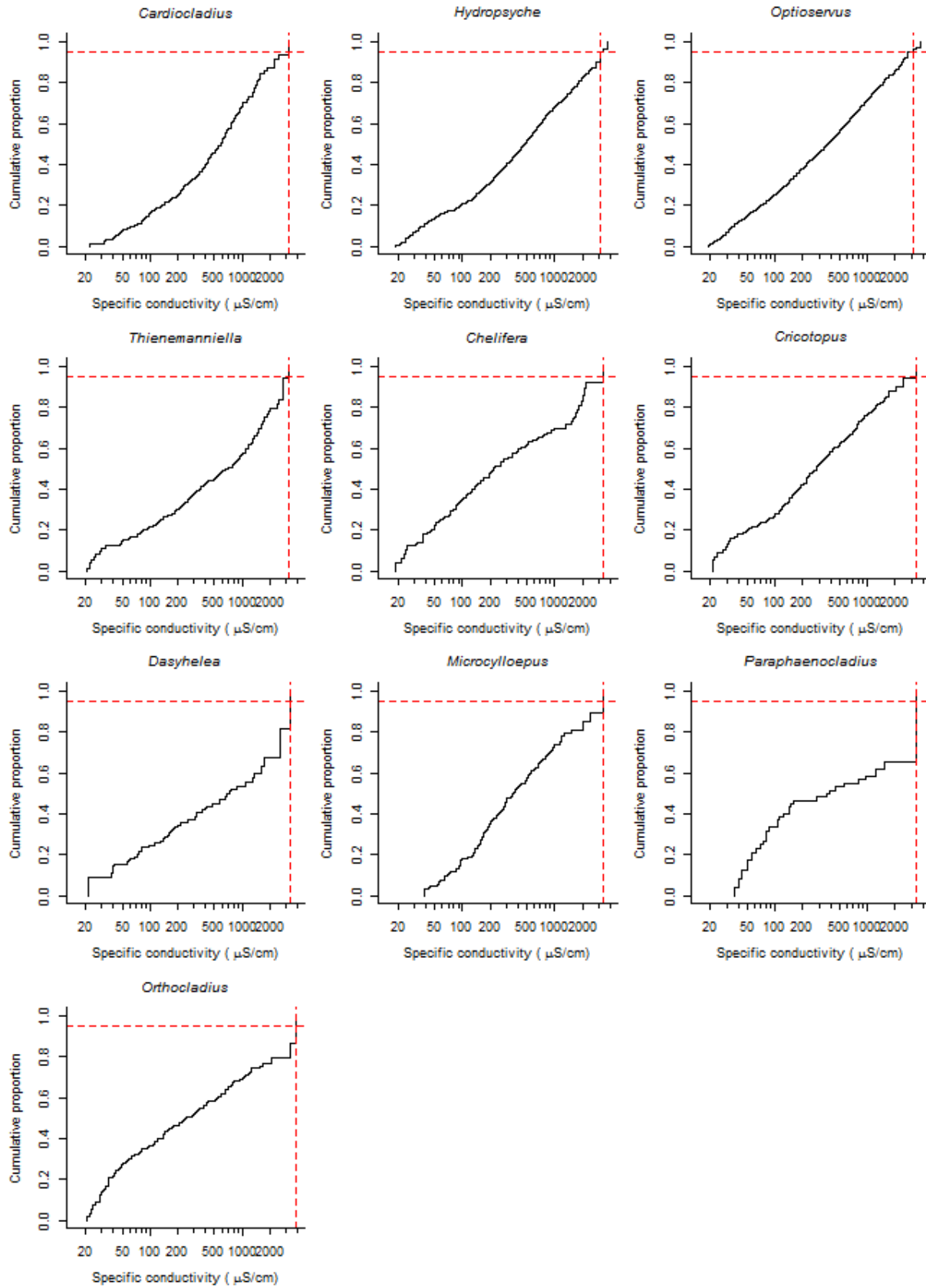














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