APPENDIX F. CASE EXAMPLE USING AN ALTERNATIVE MEASURE OF EXPOSURE ([HCO_3^- + SO_4^{2-}])

This appendix describes an alternative measure of exposure for ionic strength for the purpose of field-based criteria development, the combination of two individual anions: bicarbonate plus sulfate, [HCO_3^-]+ [SO_4^{2-}] in mg/L. This method is provided as an alternative for adopters of the method who prefer to identify the ionic constituents as a measure of exposure. This alternative method is presented as an equivalent method; however, it has several logistical disadvantages compared with using specific conductivity (SC) including cost. Also, the option to use this alternative measure does not imply that the cause of benthic invertebrate decline is only due to these two anions. Only two ions are used because they are sufficient to model the mixture; however, in other situations more or other ions would be needed to estimate exposure to an ionic mixture. When feasible, measurement of all major ions in the mixture is preferred as the alternative measure of exposure.

F.1. SELECTION OF ALTERNATIVE MEASURE OF EXPOSURE

Several factors were considered in selecting an alternative measurement for developing example criteria for the mixture of ions: potential to reflect the exposure causing the loss of genera from streams, characterization of the ionic mixture, comparability to SC as a measure of exposure, size of data set, accuracy and reliability of the measurement method, and practicality.

The Science Advisory Board (SAB) noted in their review (U.S. EPA, 2011b, p. 20) of the U.S. Environmental Protection Agency (EPA) Benchmark Report (U.S. EPA, 2011a) that the mixture of ions is not necessarily represented by a single ion such as SO_4^{2-} The sum of all ions in the mixture is the most robust characterization of an ionic mixture and methods are well established for measuring individual ion concentrations. However, measurement of individual ions is generally more costly and time consuming than simply measuring SC, and many monitoring programs only measure SC, alkalinity, hardness, or a subset of ions in a given mixture. Additive, antagonistic, or synergistic modes of action preclude prediction of effects based on individual ion concentrations.

The dominant ions of the streams in the Case Study areas are Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}, and Cl^- (see Sections 4.1 and 5.1). On a mass basis, the sum of HCO_3^- plus SO_4^{2-} in mg/L was highly correlated with the dominant cations and SC (see Figure F-1c); however, when Cl^- was also included, the correlation was even stronger (Spearman correlation = 0.96; see Figure F-1d). However, a requirement to use HCO_3^-, SO_4^{2-} plus Cl^- would have greatly reduced the size of the data set and the number of genera that could be assessed with little gain in precision. Correlations between SC and either sulfate or bicarbonate alone are not as strong compared to SC and sulfate plus bicarbonate (see Figure F-1a, b, c). Ca^{2+} concentrations are also highly correlated with SC (r = 0.94; see Appendix A, Figure A-2); however, Ca^{2+} is not recommended as a measure of exposure because it suggests that Ca^{2+} alone is causing the effect. Furthermore, Na^+ was not recommended because it is less frequently measured than Cl^- in current monitoring data sets making it difficult to properly characterize the mixtures based on cations. However, Na^+ is important for pH and ionoregulation (Bradley, 2009; Griffith, 2016). If individual ion concentrations are used to develop water quality criteria for mixtures dominated by Ca^{2+} and Mg^{2+}, rather than a measure of total ionic concentration such as SC, then a combined concentration of anions, [HCO_3^- + SO_4^{2-}] or [HCO_3^- + SO_4^{2-} + Cl^-], is recommended as the measure of exposure. For simplicity sake, this appendix illustrates how the draft field-based method can be used to derive aquatic life criteria for the combined anions sulfate plus bicarbonate [HCO_3^- + SO_4^{2-}] (mg/L); however, in cases where chloride is a more significant contributor to overall ion toxicity, an appropriate data set would need to be developed. The statistical package R, Version 2.12.1 (December 2010), was used for all statistical analyses (R Development Core Team, 2011).

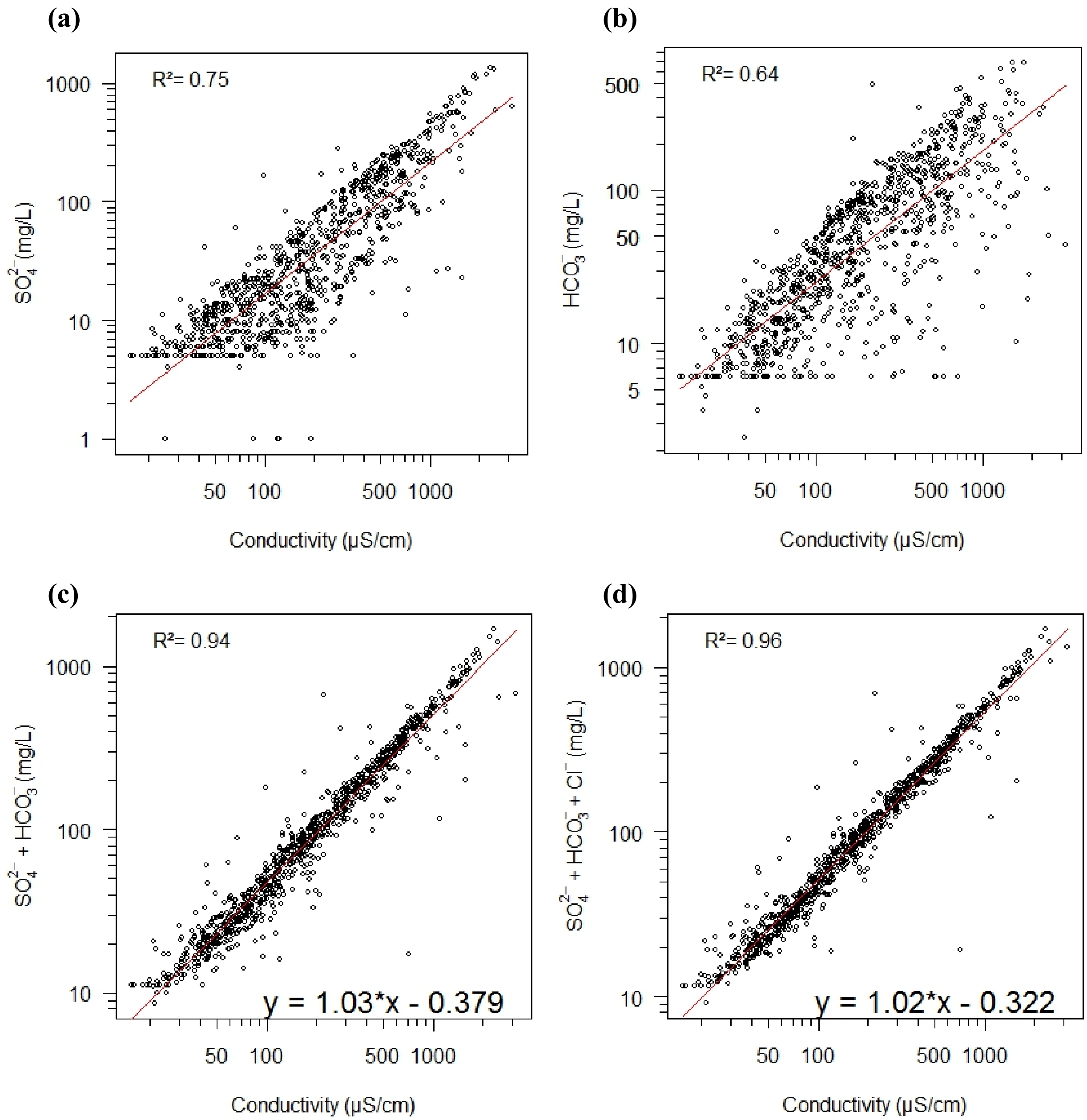


Figure F-1. **Four scatter plots and correlation values for specific conductivity and (a) [SO_4^{2-}], (b) [HCO_3^-], (c) [HCO_3^-] + [SO_4^{2-}] and (d) [HCO_3^-] + [SO_4^{2-}] + [Cl^-].** Conductivity reported as specific conductivity. Both *y* and *x* are log10 values.

F.2. DATA SET CHARACTERISTICS

The example presented here relies on the same data set used in Case Study I (see Section 4.1). The data set is a large field data set from the West Virginia Department of Environmental Protection (WVDEP)'s in-house Watershed Assessment Branch database (WABbase; see Section 4.1). Chemical and biological samples spanned from 1996−2011 and 1997−2010, respectively. The WABbase contains data from Level III Ecoregions 66, 67, 69, and 70 in West Virginia (U.S. EPA, 2010; Woods et al., 1996; Omernik, 1987). The WABbase data set provides consistent sampling and analytical methods, high quality, broad spatial coverage, and a large number of perennial streams (N = 2,299) from distinct locations in Ecoregion 69.

In addition to the several data filters described in Case Study I (see Section 4.1), sites lacking individual HCO_3^- or SO_4^{2-} measurements were removed from the data set. Out of the 1,661 samples from the Case Study I Criterion-data set (see Section 4.1), there are 925 paired samples with HCO_3^- and SO_4^{2-} measurements and biological samples which belong to 760 unique stations. Sampling points are depicted in Figure F-2.

Additional criteria were applied to identify macroinvertebrates for inclusion in the example extirpation concentration distribution (XCD): occurrence at reference sites and occurrence in 25 or more samples. Of the 219 macroinvertebrate genera identified in this ecoregion in the WABbase, 193 genera occurred at least once at one of the 64 identified reference sites where invertebrate samples were collected and identified to genus. A total of 117 genera occurred at 25 or more sampling locations. Of these sites, 94 (12.4%) were sampled more than once per year. Summary statistics for the data set used to derive the example criterion continuous concentration (CCC) is shown in Table F-1. The concentration of HCO_3^- + SO_4^{2-} in the data set ranged from 8.7−1,682 mg/L which allowed the response of organisms to be modeled for a wide range of HCO_3^- + SO_4^{2-} levels. Scatter plots of parameters and SC are depicted in Appendix Section A.1.

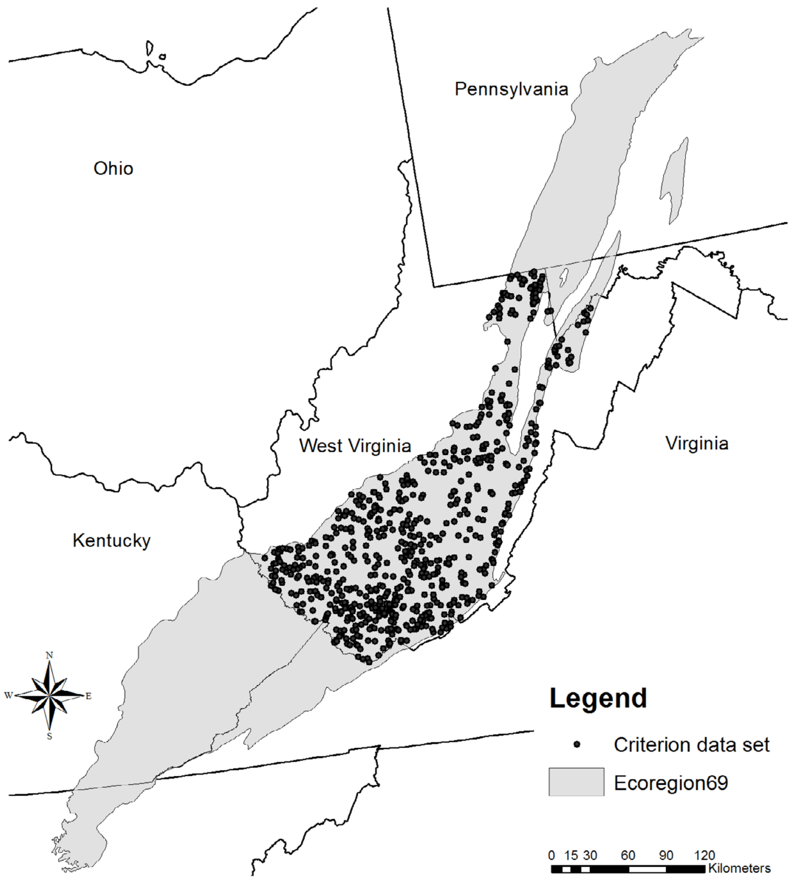


Figure F-2. **Ecoregion 69 extends from central Pennsylvania to northern Tennessee.** Sampling sites (N = 760) used to derive the case example criteria are indicated as points and represent those sites having biological measurements and both HCO_3^- and SO_4^{2-} measurements.

Table F-1. Summary statistics of the measured water-quality parameters used to derive the case example HCO_3^- + SO_4^{2-} criterion for Case Study I. The final data set has 925 samples belonging to 760 stations.

Parameter	Units	Min	25th	50th	75th	Max	Geomean	*N*
S. Conductivity	µS/cm	15.4	74	168	423	3,174	176.124	925
Hardness	mg/L	2.18	27.72	63.475	130.65	1,491.79	63.78	820
Total alkalinity	mg/L	2	12.2	34	78.9	560	33.355	925
SO_4	mg/L	1	10	22.2	91	1,336	31.13	925
Chloride	mg/L	0.5	2	3.4	8	650	4.126	925
SO_4 + HCO_3	mg/L	8.66	30.972	81.01	203.32	1,682.48	84.454	925
Ca, total	mg/L	0.686	6.8	16.7	33.1	430	15.605	825
Mg, total	mg/L	0.5	2.4	4.915	11.9	204	5.555	818
Na, total	mg/L	0.5	1.8	3.3	13.4	423	5.252	165
K, total	mg/L	0.5	0.7	1.1	2.45	16.3	1.39	163
TSS	mg/L	1	3	4	6	80	4.185	902
Fe, total	mg/L	0.02	0.09	0.18	0.39	2.72	0.184	924
Fe, dissolved	mg/L	0.01	0.02	0.03	0.06	1.1	0.041	785
Al, total	mg/L	0.01	0.06	0.1	0.2	3.32	0.115	923
Al, dissolved	mg/L	0.01	0.02	0.03	0.05	0.93	0.037	797
Mn, total	mg/L	0.003	0.013	0.025	0.059	4.44	0.03	923
Se, total	mg/L	0	0.001	0.001	0.003	1.26	0.002	578
DO	mg/L	2.21	8.495	9.34	10.23	17.14	9.411	919
Total phosphorus	mg/L	0.007	0.013	0.02	0.02	1.31	0.022	829
NO_x	mg/L	0.01	0.136	0.274	0.45	11	0.257	842
Fecal	counts/ 100 ml	0.5	10	45	214.5	250,000	50.704	811
pH	SU	6.03	6.89	7.41	7.88	9.26	7.392	925
Catchment area	km^2	0.34	3.395	15.21	82.345	17,985.73	19.44	783
Temperature	°C	−0.28	13.23	16.92	20.12	30.2	16.817	925
RBP_10Sc	RBP score	66	131	148	162	195	145.574	910

Table F-1. Summary statistics of the measured water quality parameters used to derive the case example $HCO_3^- + SO_4^{2-}$ criterion for Case Study I. The final data set has 925 samples belonging to 760 stations. (continued)

Parameter	Units	Min	25th	50th	75th	Max	Geomean	*N*
RBP_7Sc	RBP score	43	89	103	115.75	137	100.957	914
Embeddedness	RBP score	2	12	14	16	20	13.473	915
Fines (sand + silt)	%	0	5	10	20	100	11.506	900

All means are geometric means except pH, DO, Temperature, and Habitat Scores.

DO = dissolved oxygen; RBP = rapid bioassessment protocol (Barbour et al., 1999; RBP 10Sc has 10 parameters while RBP 7 does not include 3 flow-related parameters); S. Conductivity = specific conductivity; TSS = total suspended solids.

F.2.1. Background Ionic Concentration of $HCO_3^- + SO_4^{2-}$

Background of bicarbonate and sulfate concentration in mg/L [$HCO_3^- + SO_4^{2-}$] for the ecoregion was estimated at the 25th centile using the probability-based samples from the WABbase data set. The 25th centile was 34 mg/L [$HCO_3^- + SO_4^{2-}$] (see Figure F-3). Background was also estimated at 27 mg/L based on field data from reference sites from the WABbase data set (75th centile, 95 samples from 61 reference sites; see Figure F-4). By comparison, the 25th centile was 30 mg/L as for all samples in the example Criterion-data set (see Figure F-5). The monthly 25th centiles of probability-sampled sites (see Figure F-3), reference sites (see Figure F-4) and the full example Criterion data set (see Figure F-5) were relatively consistent and at or below 100 mg/L except in October (see Figure F-3). The large data set and the wide range in [$HCO_3^- + SO_4^{2-}$] levels in the example Criterion-data set allowed for characterization of the extirpation concentration affecting 95% of test organisms (XC_{95}).

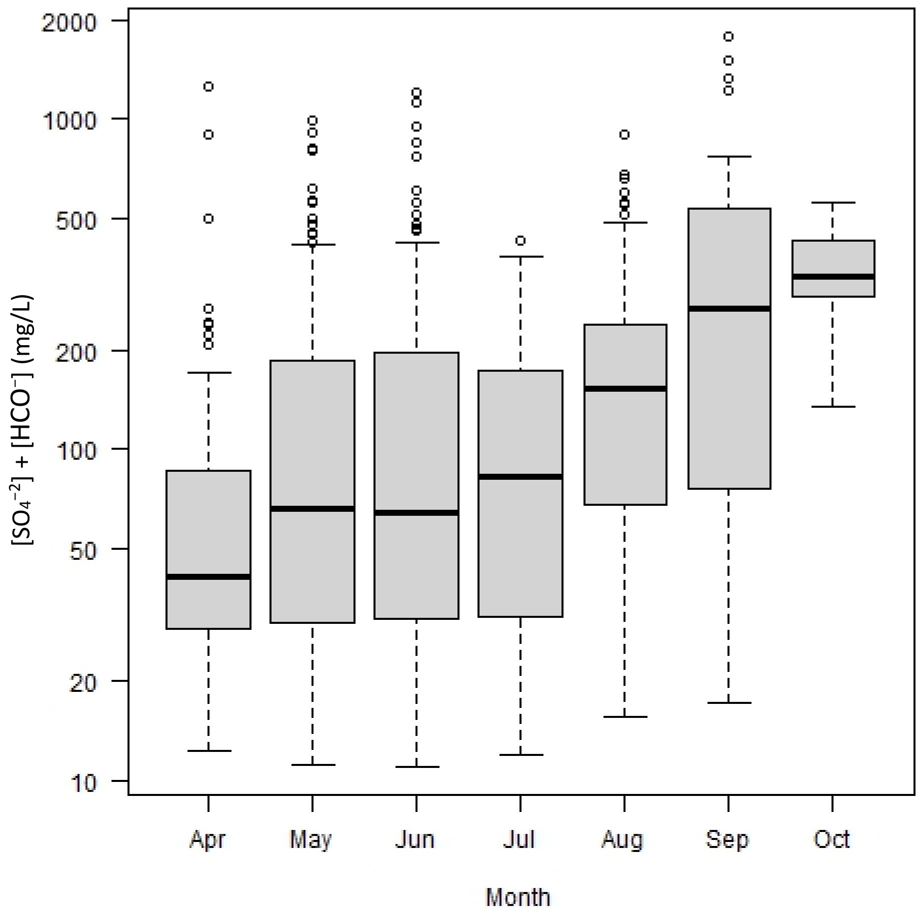


Figure F-3. Box plot showing seasonal variation of [HCO_3^- + SO_4^{2-}] (mg/L) from probability-sampled sites from Watershed Assessment Branch database (WABbase) 1997−2010. This represents a total of 539 sites with 592 samples from 1999−2011 from Ecoregion 69 in West Virginia with pH >6. Note the difference in scale along the y-axis between Figure F-3 (probability-sampled sites) and Figure F-4 (reference sites).

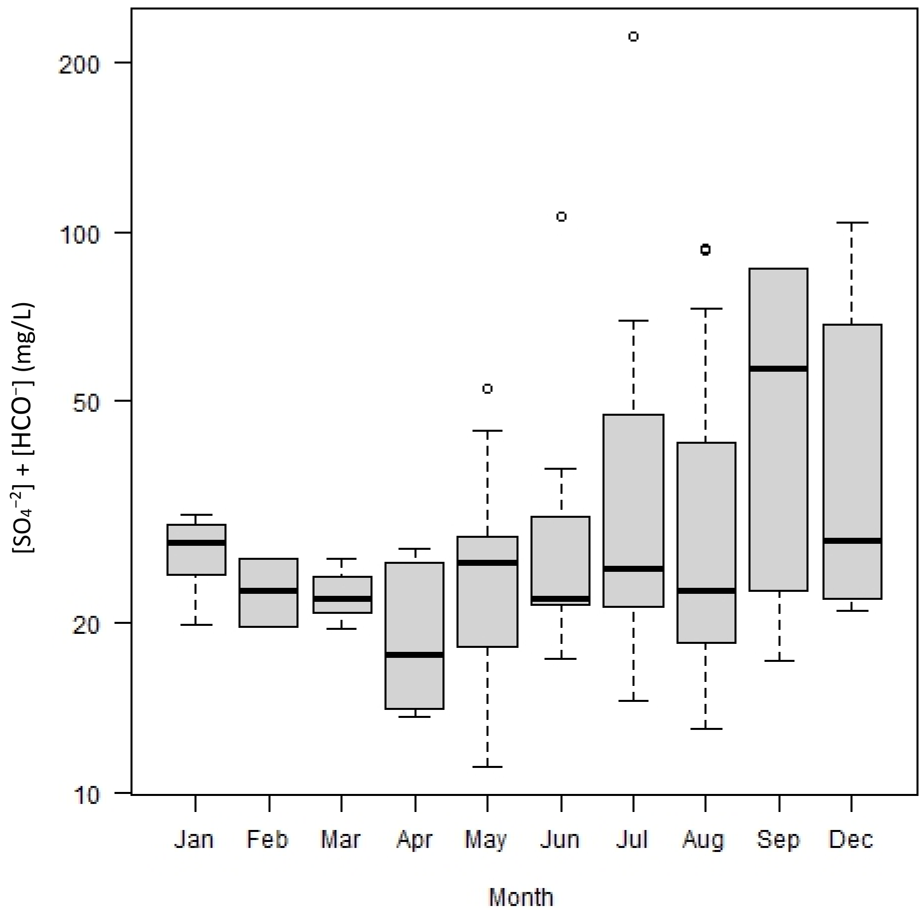


Figure F-4. **Box plot showing seasonal variation of [HCO_3^- + SO_4^{2-}] (mg/L) in the reference streams from Watershed Assessment Branch database (WABbase) 1997−2010.** A total of 95 samples from 61 reference stations were used for this analysis to estimate background.

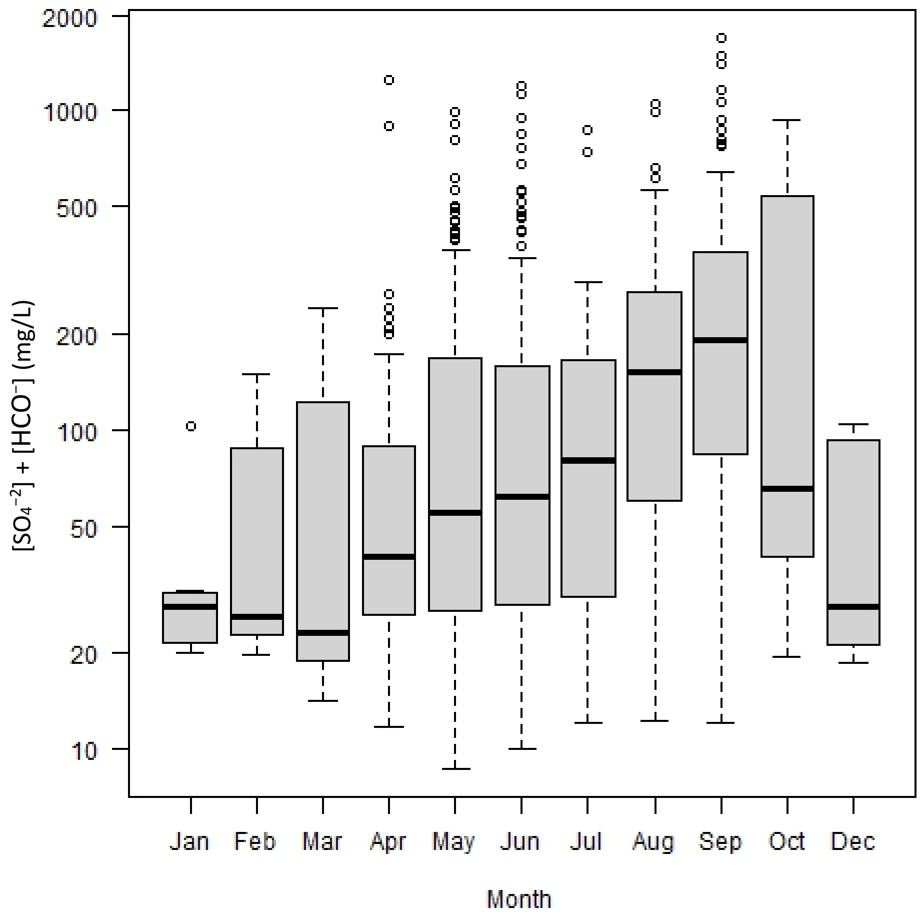


Figure F-5. Box plot showing seasonal variation of [HCO_3^- + SO_4^{2-}] (mg/L) from all Ecoregion 69 sites from Watershed Assessment Branch database (WABbase) 1997−2010 used to develop the example criteria. This represents a total of 925 samples from 760 sites from 1997−2010 in Ecoregion 69 in West Virginia. Note the difference in scale along the y-axis between Figure F-4 (reference sites) and Figure F-5 (all sites, reference and nonreference).

F.2.2. Ionic Composition

The ionic composition of the samples was assessed to ensure that the example criteria were derived for the dominant ions in the mixture, that is, sulfate and bicarbonate ions (see Section 4.1.2). Of the sites with ion measurements, 99% of sites were dominated by bicarbonate and sulfate anions ($[HCO_3^-] + [SO_4^{2-}] > [Cl^-]$). All sites in which $[HCO_3^-] + [SO_4^{2-}] \leq [Cl^-]$ were removed from the data set. Therefore, no known chloride dominated sites were used in the analysis.

F.2.3. Seasonal Specific Conductivity Regime

Chemical, physical, and/or biological samples were collected during the sampling years 1997−2010 (January−December). Most sites were sampled once during an annual sampling period, but some (e.g., sites being studied to improve stream condition within the Total Maximum Daily Load Program) were sampled monthly for water quality parameters (see Table F-2).

Table F-2. Number of samples with reported genera and [HCO_3^- + SO_4^{2-}] (mg/L) meeting acceptance criteria for the Ecoregion 69 analysis. Number of samples is presented for each month.

Samples	Month												Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Number	8	3	35	92	207	139	91	156	163	25	0	6	925
Percentage of total	0.9	0.3	3.8	9.9	22.4	15.0	9.8	16.9	17.6	2.7	0.0	0.6	100

Samples collected from the WVDEP-identified reference sites indicate that [HCO_3^- + SO_4^{2-}] concentrations are generally low and similar throughout the year, although slightly higher in summer/fall months of August, September, and October (see Figures F-3−F-5). These data show that [HCO_3^- + SO_4^{2-}] concentrations in flowing waters in the study area can vary somewhat by season, likely depending on stream discharge, rainfall, snowmelt, and other hydrological factors (Kaushal et al., 2013). As described in Section 3.1.4 (and in greater detail in U.S. EPA, 2011a), the effects of seasonal differences in SC levels and aquatic insect life history were evaluated by comparing hazardous concentration of the 5th centile (HC_{05}) values of the genus XCDs partitioned for season. In the final assessment, due to the similarity at the low end of the genus XCD between the spring HC_{05} and the HC_{05} based on the full data set, the case example criteria was derived using all available data, regardless of the time of year that they were collected (see Section 4.1.3).

F.3. RESULTS

F.3.1. Extirpation Concentration (XC_{95}) and Hazardous Concentration (HC_{05}) Values (Example Criterion Continuous Concentration [CCC])

The Case Study I Criterion-data set (see Table F-1) was used to develop XC_{95} values from weighted cumulative distribution functions (CDFs). The histogram used to develop weights is depicted in Figure F-6. The XC_{95} values that were used in the XCD model are listed in the order of least to most tolerant in Appendix Section F.7. The Generalized Additive Model (GAM) plots used to designate ~ and > values for those XC_{95} values are depicted in Appendix Section F.8 and the CDFs used to derive the XC_{95} values are shown in Appendix Section F.9. The HC_{05} was calculated at 161 mg/L (see Figures F-7 and F-8); the two-tailed 95% confidence bounds were 113−175 mg/L (see methods Section 3.1.3.1; U.S. EPA, 2011a). Those bounds, derived by bootstrap resampling, indicate that different data sets could be expected to yield HC_{05} values within that interval. Rounding to two significant figures, the case example CCC is 160 mg/L.

F.3.2. Example Criterion Maximum Exposure Concentration

The example criterion maximum exposure concentration (CMEC) is estimated at the 90th centile of observations of the concentration of the ionic mixture that contribute to the annual CCC. (see Section 3.2). The example CMEC was derived using the full Case Study I data set (1997−2010) for which there were ionic measurements. [HCO_3^- + SO_4^{2-}] measurements were included in 6,620 samples within Ecoregions 69 (see Table F-3). Of the 6,620 samples in Ecoregion 69, there are 4,321 samples in a July-to-June rotating year representing 387 unique stations, 406 rotation years, 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. To calculate the example field-based CMEC, 4,321 samples were included in the analysis. Note that inclusion of samples is not contingent on biological data. Reference and nonreference sites were included to ensure a range of [HCO_3^- + SO_4^{2-}] concentrations.

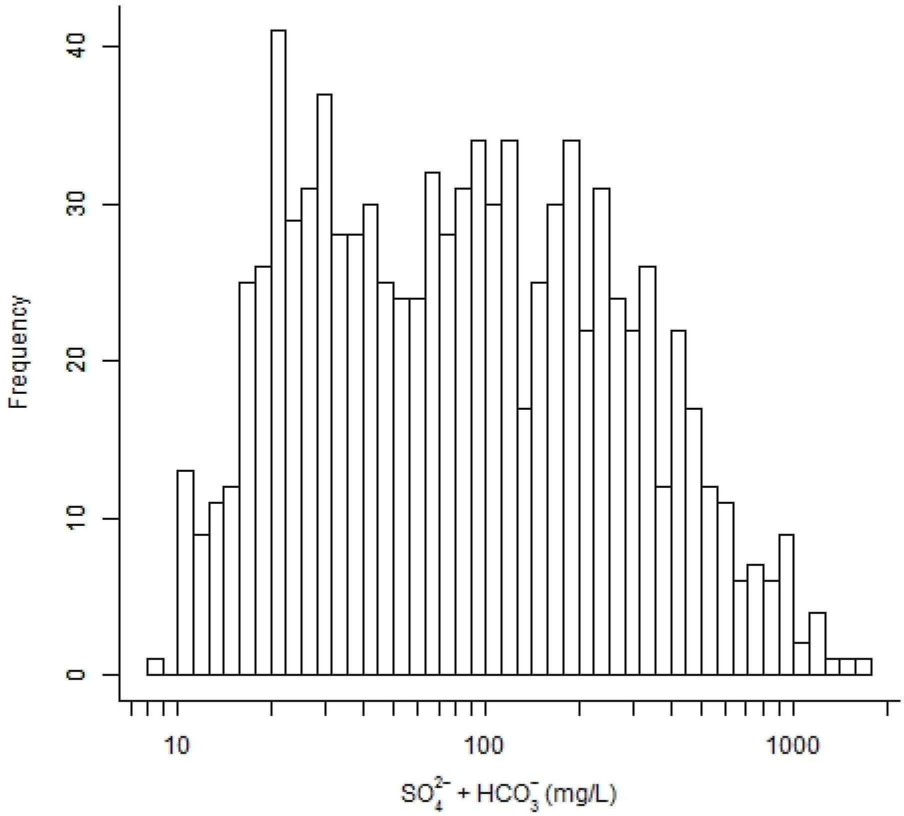


Figure F-6. Histograms of the frequencies of observed sulfate plus bicarbonate values in samples from Ecoregion 69 sampled 1997−2010. Because the distribution of the exposure data spans three orders of magnitude a log scale base 10 was used. Bins are each 0.017 units wide, 1/60 of the range of $\log_{10}([HCO_3^-] + [SO_4^{2-}])$ in mg/L.

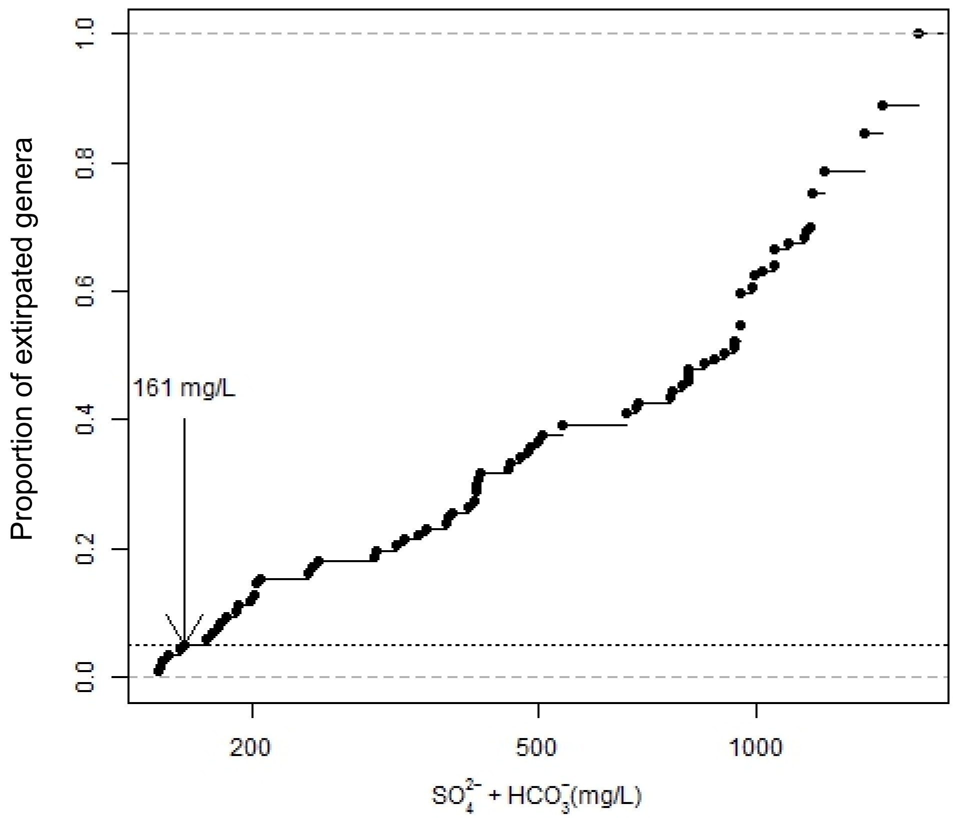


Figure F-7. Extirpation concentration distribution (XCD) for Ecoregion 69. Each point is an extirpation concentration (XC_{95}) value for a genus. There are 117 genera. The hazardous concentration (HC_{05}) is 161 mg/L (95% confidence interval is 113−175 mg/L) and is the [HCO_3^- + SO_4^{2-}] concentrations at the intersection of the XCD with the horizontal line at the 5th centile.

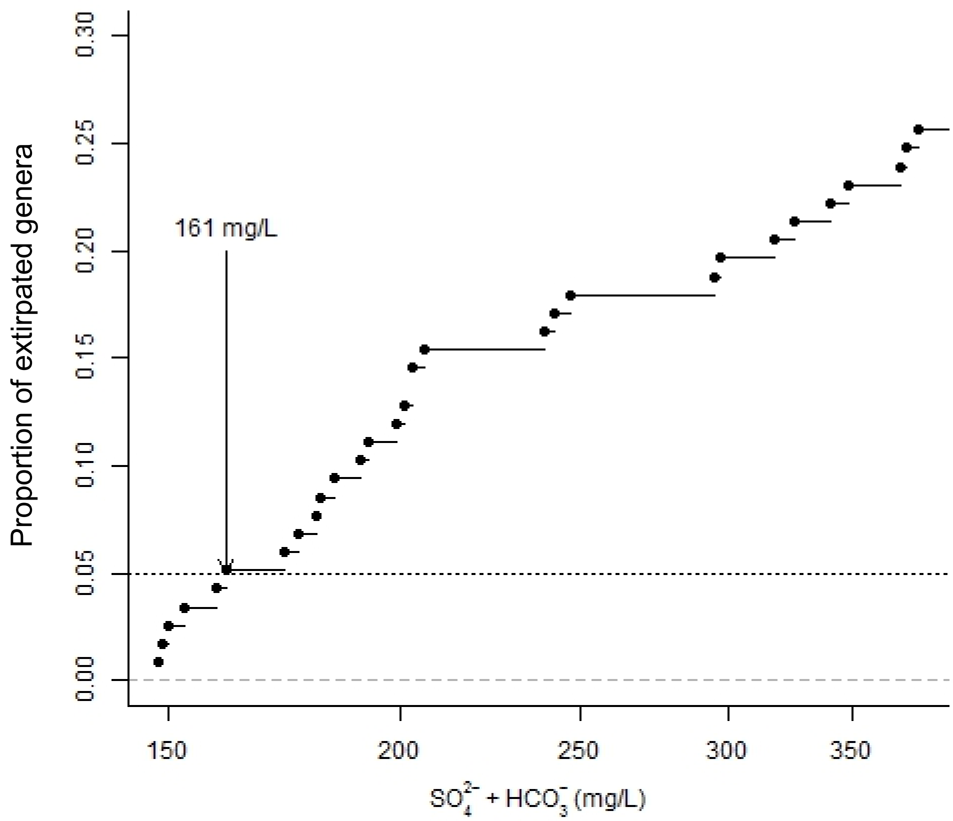


Figure F-8. The lower end of the genus sensitivity distribution for Ecoregion 69. The dotted horizontal line is the 5th centile. The vertical arrow indicates the hazardous concentration (HC_{05}) of 161 mg/L (95% confidence interval is 113−175 µS/cm). Only the 30 most intolerant genera are shown to better discriminate the points on the left side of the distribution. The six most intolerant genera (i.e., extirpation concentration [XC_{95}] ≤ 161 mg/L) are *Pycnopsyche, Eurylophella, Stempellina, Paraleptophlebia, Leptophlebia,* and *Ameletus*.

Table F-3. **Summary data related to the calculation of the example criterion maximum exposure concentration (CMEC) for Ecoregion 69**

# samples July to June prior to biological sampling	4,321
# unique stations	406 rotation years, 387 stations
CCC used in the calculation of CMEC	160 mg/L
CMEC	304 mg/L
CMEC rounded to two significant figures	300 mg/L

CCC = criterion continuous concentration.

Of the 406 rotation years in Ecoregion 69 with multiple [HCO_3^- + SO_4^{2-}] measurements, the variability of within station [HCO_3^- + SO_4^{2-}] (mg/L) was found to be only slightly different for streams with different mean [HCO_3^- + SO_4^{2-}] mg/L (see Figure F-9). A locally weighted smoothing spline (LOWESS) estimates a line for a scatter plot by combining nonparametric multiple regression models using local approximation from neighboring points. The LOWESS-fitted line indicated that the average variability (residual standard deviation for a station) in the middle of the [HCO_3^- + SO_4^{2-}] gradient and around the CCC values in Ecoregion 69 is fairly similar (approximately between 100−250 mg/L in Ecoregion 69). Therefore, these stations with a mean [HCO_3^- + SO_4^{2-}] between 100 to 250 mg/L were selected to estimate the variance of annual mean [HCO_3^- + SO_4^{2-}] (at 160 mg/L, the CCC). The selected data sets with mean [HCO_3^- + SO_4^{2-}] values between 100 and 250 mg/L in respective data sets have a sample size of 124 rotation years and 1,269 observations for Ecoregion 69. The grand mean and standard deviation of this data set was determined and the CMEC. The example CMEC calculation is shown below:

$$\text{Ecoregion 69: } 10^{\log 10(160) + 1.28 * \sqrt{0.0475}} = 304 \text{ mg/L} \qquad \text{(F-1)}$$

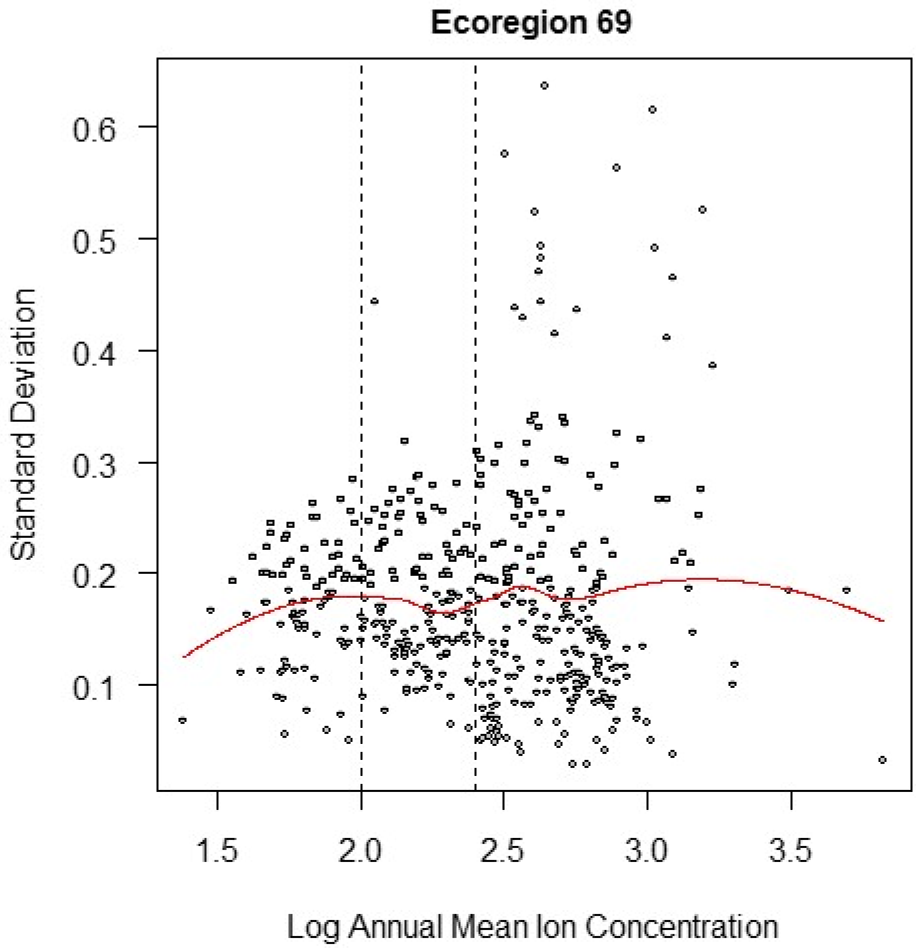


Figure F-9. **Illustration of within site variability (residual standard deviation for each station) along the [HCO_3^- + SO_4^{2-}] gradient (station mean) in Ecoregions 69**. Each dot represents a station. The fitted lines is a locally weighted smoothing spline (LOWESS; span = 0.75), while the two vertical dashed lines represent logarithm mean [HCO_3^- + SO_4^{2-}] of 100 and 250 mg/L, respectively. Within those bounds the standard deviation is fairly constant.

The field-based CMEC is 304 mg/L for Ecoregion 69 (see Table F-3). Rounding to two significant figures yields an example CMEC of 300 mg/L for Ecoregion 69. If this maximum is not exceeded, the annual average [HCO_3^- + SO_4^{2-}] is expected to be <160 mg/L 90% of the time. At this level, where the annual average ionic concentration of bicarbonate plus sulfate (mg/L) is <160 mg/L, 90% of the ionic measurement observations are expected to be less than the CMEC.

F.4. GEOGRAPHIC APPLICABILITY

The geographical applicability was assessed using the background-matching approach (see Section 3.7.1). The background [HCO_3^- + SO_4^{2-}] (mg/L) of the new area (i.e., Ecoregion 69 beyond the original Criterion-data set) was estimated at the 25th centile (see Section 3.7.1.2; Suter and Cormier, 2013) and compared with the background estimates for the original area (Case Study I Criterion-data set).

Because the example [HCO_3^- + SO_4^{2-}] (mg/L) criteria presented here have been developed for ionic mixtures in which ([HCO_3^-] + [SO_4^{2-}]) > [Cl^-] in mg/L, all samples used to estimate background in the new area with ([HCO_3^-] + [SO_4^{2-}]) < [Cl^-] in mg/L were removed from the data set before estimating background [HCO_3^- + SO_4^{2-}]. Thereby, the background is estimated for the same mixture as the example criteria.

F.4.1. Data Sources

Available data were used for this assessment. Two data sets were used: the original data set used to derive the HC_{05} described in Section 4.1 and an EPA-survey data set described in Section 4.3.1 with the exception that sites without [HCO_3^- + SO_4^{2-}] (mg/L) were removed (see Figure F-10).

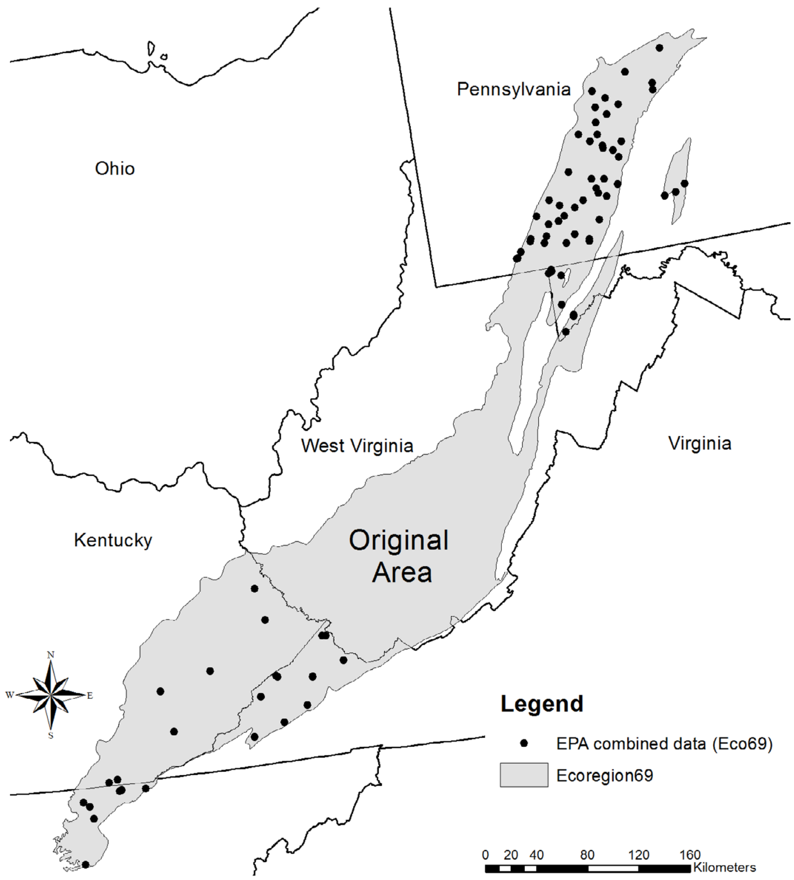


Figure F-10. **Ecoregion 69 extends from central Pennsylvania to northern Tennessee.** Sampling sites (85) in the EPA-survey data set that was used to estimate background for Ecoregion 69 are indicated as points (*N* = 9 [Kentucky], 8 [Maryland], 48 [Pennsylvania], 9 [Tennessee], 11 [Virginia]).

F.4.2. Geographic Applicability Results

A summary of water quality for the EPA-survey data set of Ecoregion 69, including major ionic constituents, is provided in Table F-4. The natural background of $[HCO_3^-] + [SO_4^{2-}]$ (mg/L) was estimated without exclusions from EPA-survey data set because no sample was dominated by Cl^-.

Table F-4. Summary of water quality parameters for Ecoregion 69 from the EPA-survey data set

Ecoregion	Ion	Min	Centile				Max	Relevant *N*
			10th	25th	50th	75th		
Ecoregion 69	HCO_3^- (mg/L)	1.01	3.68	8.12	20.95	51.98	312.9	209
	SO_4^{2-} (mg/L)	3.19	6.48	8.92	15.79	68.81	896.5	211
	Cl^- (mg/L)	0.25	0.58	1.10	1.93	4.15	59.1	211
	Ca^{2+} (mg/L)	1.27	2.97	5.46	11.68	27.23	177.1	211
	Mg^{2+} (mg/L)	0.41	1.15	1.64	2.99	9.77	152.1	211
	Na^+ (mg/L)	0.23	0.60	1.05	2.22	5.88	93.4	211
	K^+ (mg/L)	0.19	0.65	0.80	1.21	1.93	8.0	211
	pH (SU)	6.06	6.55	6.91	7.33	7.80	8.6	220
	$(HCO_3^- + SO_4^{2-})/Cl^-$ [a]	1.38	4.56	12.21	23.82	50.57	351.1	209
	Specific conductivity	16.8	37.9	63.7	112.7	300.0	2,060	220

[a]Value within category calculated from individual sample ion concentrations.

Background $[HCO_3^- + SO_4^{2-}]$ (mg/L), estimated as the 25th centile of the EPA-survey data set in Ecoregion 69 outside the area used to develop the example criteria, is 23 mg/L (95% confidence interval [CI] 19−32 mg/L; see Table F-5). The 25th centile for the probability based samples is 34 mg/L; (95% CI 30−38) from the WABbase data set (see Table F-5). The confidence intervals from the EPA-survey data set outside the area of the example criterion data set overlap with the interval for the WABbase data set reference subsample (27 mg/L; 95% CI 26−29 mg/L), and the probability and full Criterion-derivation data sets (30 mg/L; 95% CI 29−34). Therefore, the case example criterion developed for Ecoregion 69 in West Virginia (160 mg/L) is applicable throughout the ecoregion wherever $[HCO_3^- + SO_4^{2-}]$ is elevated due to salts dominated by $HCO_3^- + SO_4^{2-}$ (mg/L).

Table F-5. Background $[HCO_3^- + SO_4^{2-}]$ estimates for Ecoregion 69 expressed as mg/L

Data set	Centile used to estimate background	Background	Confidence interval	Relevant *N* (sites/samples)
EPA—survey data set from geographic area not represented in the example criterion derivation data set	25th	23	19−32	85/85
WABbase data set, probability sample subset	25th	34	30−38	539/592
WABbase data set, reference sample subset	75th	27	26−29	61/95
Example criterion derivation data set, full data set	25th	30	29−34	780/925

WABbase = Watershed Assessment Branch database.

The 95% CI of other estimates of background for the original area in Ecoregion 69 also overlap with estimates for Ecoregion 69 outside the original area used for the example criterion. The 75th centile from the reference samples is 27 mg/L (95% CI 26−29 mg/L) and the 25th centile from the example Criterion-data set is 30 mg/L (95% CI 29−34; see Table F-5).

The analysis presented in this section demonstrates that the $[HCO_3^- + SO_4^{2-}]$ (mg/L) case example criterion developed using the Case Study I data set is applicable to the entire ecoregion. The example geographical applicability was assessed using the background-matching approach (see Section 3.7.1). The background and ionic mixture $[([HCO_3^-] + [SO_4^{2-}]) > [Cl^-]$ in mg/L] is similar throughout the ecoregion.

F.5. SUMMARY OF EXAMPLE ECOREGIONAL CRITERIA

The case example for Ecoregion 69 includes an annual geometric mean (i.e., CCC) and a 1-day mean (i.e., CMEC), which are not to be exceeded more than once in 3 years on average (see Table F-6). It would be necessary to meet both of these distinct expressions of the example $[HCO_3^- + SO_4^{2-}]$ (mg/L) criteria in order to adequately protect aquatic life. Freshwater animals would have an appropriate level of protection if the annual geometric mean concentration of

HCO_3^- + SO_4^{2-} (mg/L) does not exceed 160 mg/L and the 1-day mean does not exceed 300 mg/L, more than once every 3 years on average (see Table 5-7).

Table F-6. Summary [HCO_3^- + SO_4^{2-}] (mg/L) example criteria for Ecoregion 69

	CCC	CMEC
Magnitude	160 mg/L	300 mg/L
Duration	Annual geometric mean (i.e., long-term average)	1-day mean (i.e., maximum daily limit)
Frequency	No more than once every 3 years on average	
Geographic region	Ecoregion 69 inclusive of Kentucky, West Virginia, Maryland, Virginia, Tennessee, and Pennsylvania	
Waterbody type	All flowing fresh waters (ephemeral, intermittent, and perennial streams)	
Considerations for site-specific implementation	On a site by site basis, these example ecoregional criteria apply if the ionic mixture is dominated by anions of bicarbonate plus sulfate. For streams crossing into Ecoregion 69, professional judgment may be needed to assess the potential effect of different ionic composition or concentration. Professional judgment is recommended when applying to sites with a catchment area greater than 1,000 km^2 (386 mi^2) owing to lesser representation in the example data set by this class of stream. On a site by site basis, alternative ionic criteria may be more appropriate if the natural background of a site is shown to be substantially lower or higher than its regional background ionic concentration.	

CCC = criterion continuous concentration; CMEC = criterion maximum exposure concentration.

F.6. EXAMPLE CRITERIA CHARACTERIZATION

F.6.1. Factors Potentially Affecting the Extirpation Concentration Distribution (XCD) Model

F.6.1.1. *Ecoregion 69: Sensitivity Analyses*

As the minimum number of occurrences of a genus for inclusion in the data set increases, fewer genera are included in the XCD (see Figure F-11). The HC_{05} increases greatly when a taxon in the lower 5th centile is removed because it does not meet the minimum number of samples and then more slowly alternates between increasing and decreasing as genera either above or below the 5th centile are removed because they do not meet the minimum number of samples. The pattern repeats until all genera above and below the lower 5th centile have the same XC_{95} value (not shown). A minimum of 25 occurrences was used, which is just above the inflection point in the HC_{05} curve (see Figure F-11).

The number of samples in the data set affected the number of genera included in the XCD model and the resulting HC_{05}. The effect of data set size on the HC_{05} estimates and their confidence bounds was estimated using a bootstrapping technique. The mean of all bootstrapped HC_{05} values, the numbers of genera used for the Ecoregion 69 data set HC_{05} calculation, and their 95% confidence intervals were plotted to show the effect of sample sizes. As shown in Figure F-12, for this data set the HC_{05} declines and then stabilizes reaching an asymptote at approximately 500 sites sampled and 90 genera evaluated. Therefore, the original data set was adequate for estimating the example CCC.

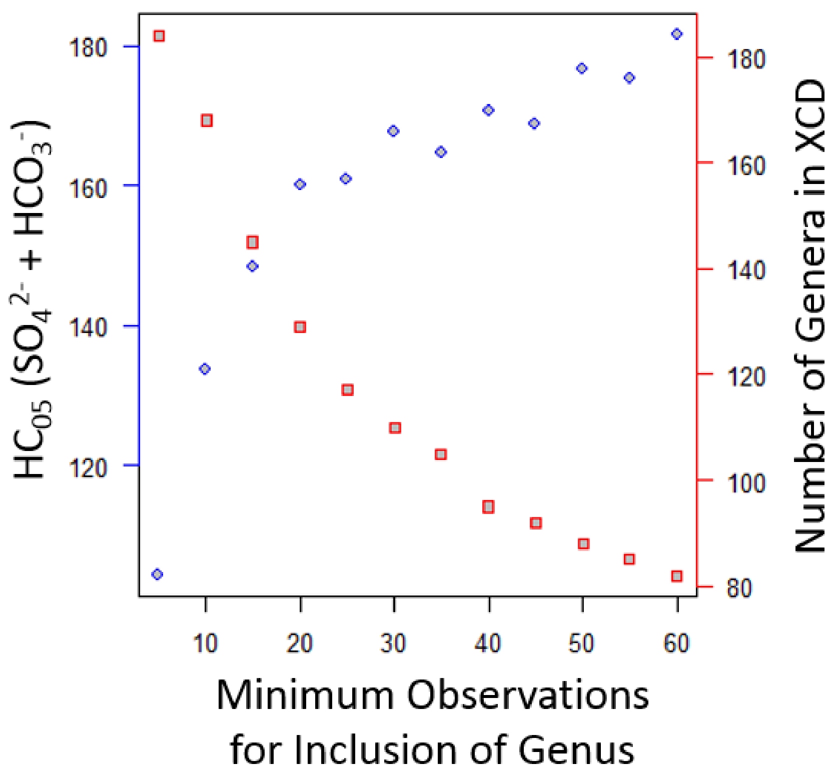


Figure F-11. Relationship of number of occurrences of a genus and the hazardous concentration (HC_{05}) based on Ecoregion 69 example Criterion data set. The HC_{05} (diamonds) increases rapidly and then changes more slowly with ≥25 observations of a genus and as fewer genera (squares) are included in the extirpation concentration distribution (XCD).

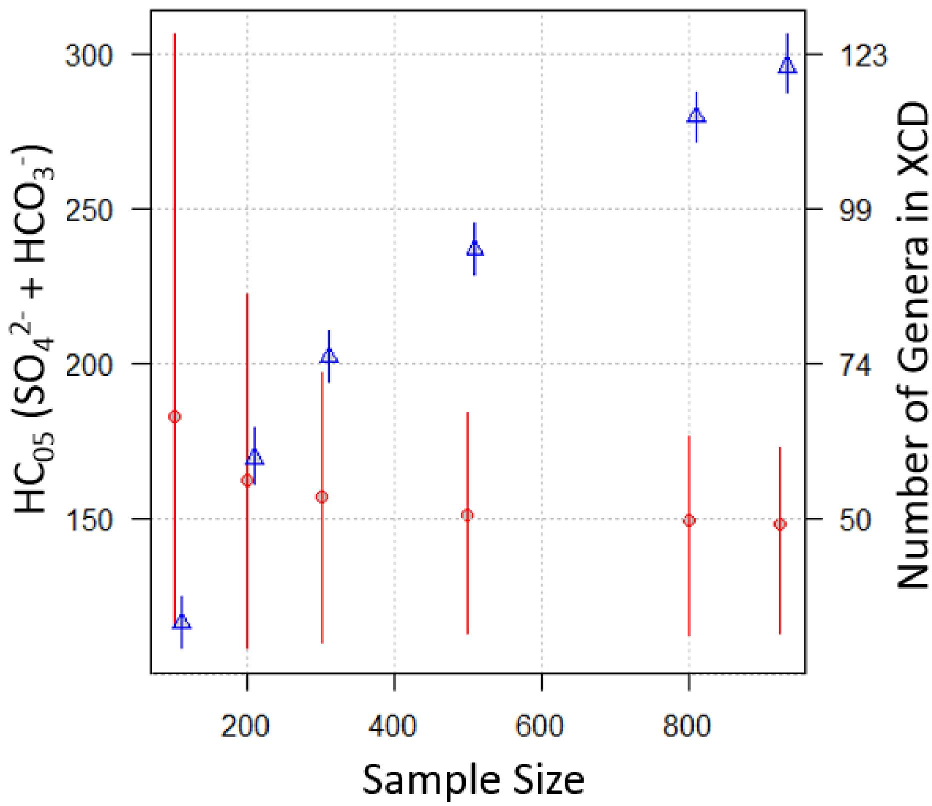


Figure F-12. **The effect of the number of samples used to model the hazardous concentration (HC_{05}) based on the Case Study I data set**. As sample size increases the number of genera included in the extirpation concentration distribution (XCD) increases (triangles). The HC_{05} (circles) declines and stabilizes, reaching an asymptote at approximately 500 sites sampled and 90 genera evaluated. Note that the mean of all bootstrapped HC_{05} values shown in the figure is lower than the HC_{05} value for Ecoregion 69 at a similar sample size because the Monte Carlo results are asymmetrical (i.e., there are more ways that the sample variance can result in lower values than higher values).

F.6.2. Validation of the Model

As recommended by the SAB (U.S. EPA, 2011b), the XCD model was validated and uncertainty around the HC_{05} values was estimated using bootstrapping, a statistical technique of repeated random sampling from the data set that is commonly used in environmental studies to estimate confidence limits of a parameter (Newman et al., 2000, 2001). This is akin to having different data sets to compare results and fidelity of the model. The results show that the median bootstrapped XCD is similar to the calculated XCD. This suggests that the model would not change greatly if generated using an independent data set (see Figure F-13).

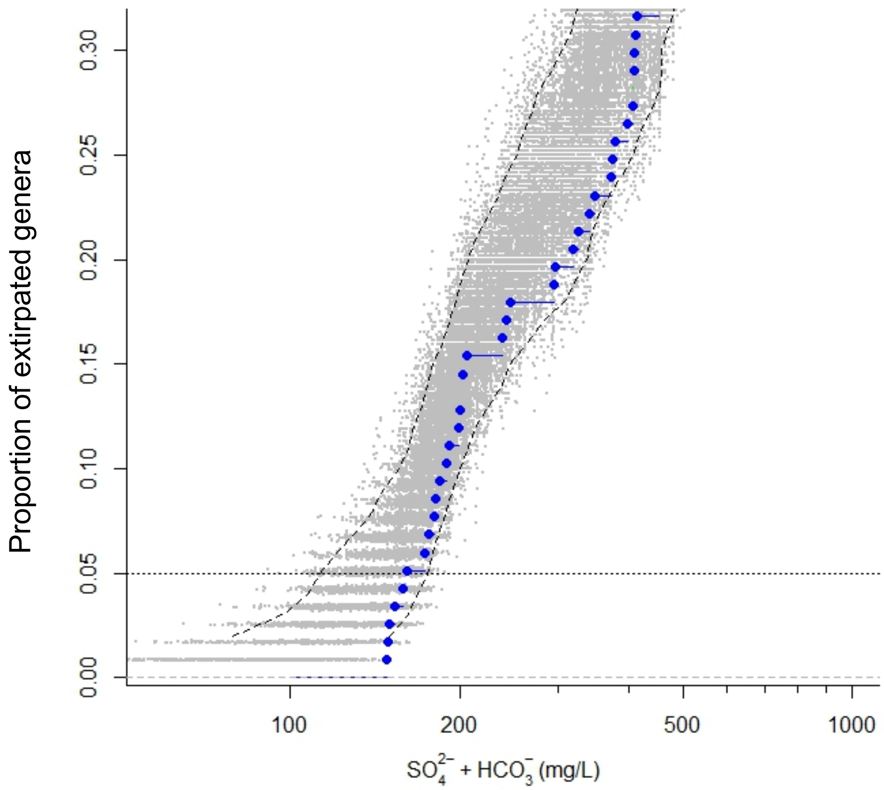


Figure F-13. **Cumulative distribution of the extirpation concentration (XC_{95}) values for 30% of genera (blue circles) and 95% confidence intervals (dotted lines) based on 1,000 bootstrapping results.** Each small gray dot represents an XC_{95} value for a bootstrapping iteration (note that the genus in each percentage varies with each extirpation concentration distribution [XCD] iteration). Each larger dark dot represents the original calculated XC_{95} of the XCD for the example criterion continuous concentration (CCC). The median bootstrapped hazardous concentration (HC_{05}) is 149 mg/L.

F.6.3. Assessment of Potential Confounders

Previous assessments of the factors potentially influencing the model of the causal relationship between ionic strength and extirpation of benthic invertebrates indicated that the following factors did not substantially confound the causal relationship between SC and benthic macroinvertebrate assemblages: rapid bioassessment protocol 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. However, low pH could possibly affect the model (Suter and Cormier, 2013; U.S. EPA, 2011a

Appendix B). As a result, sampling sites with acidic waters (pH <6) were excluded from the analysis in order to minimize any effects.

Analyses performed with the Ecoregion 69 data sets (see Appendix Section A.2) confirmed the results reported in U.S. EPA (2011a) that the SC relationship is not appreciably confounded. No additional confounding analyses were performed for this case, because the alternative measure of exposure of [HCO_3^- + SO_4^{2-}] (mg/L) is highly correlated with SC and the same data set was used in both assessments (see Figure F-1c).

F.6.4. Duration and Frequency

Numeric criteria include magnitude (i.e., how much), duration (i.e., how long), and frequency (i.e., how often) components. Appropriate duration and frequency components of criteria are determined based on consideration of available data and understanding the exposure-response relationship in the context of protecting the designated use of a water body. The significant consideration used in setting the duration component of aquatic life criteria is how long the exposure concentration can be above the criteria without affecting the endpoint on which the criteria are based (U.S. EPA, 1985, 1991). Based on the temporal resolution of the available field data set and an analysis of within-site variability of [HCO_3^- + SO_4^{2-}] levels, EPA developed two different expressions for the example [HCO_3^- + SO_4^{2-}] (mg/L) criteria in order to provide adequate protection for aquatic life.

In this case, the majority (>87%) of sites used to derive the example CCC were sampled once during an annual sampling period and thus represent the average stream chemistry [HCO_3^- + SO_4^{2-}] (mg/L) and macroinvertebrate assemblage information over the course of 1 year. As a result, the appropriate duration for the CCC is 1 year. The duration for the CMEC, a level of protection from acutely toxic exposures, is 1 day based on a review of the literature on the onset of macroinvertebrate drift in response to elevated [HCO_3^- + SO_4^{2-}] concentration, and because the exposures used to derive a CMEC represent grab samples from the field on a single day (see Section 3.3). At sites meeting the CCC, 90% of the observations of the bicarbonate plus sulfate concentrations are estimated to occur below the CMEC.

EPA has determined that an appropriate allowable frequency of exceedance for these example criteria is no more than once in 3 years, based on recovery rates from literature reviews and consideration of the life history of insects that are able to recolonize a site via drift or aerial dispersal (see Section 3.4). Recovery is expected to occur in 3 years if the following conditions are met: (1) the [HCO_3^- + SO_4^{2-}] regime returns to a yearly average below the CCC, (2) there are nearby streams with low [HCO_3^- + SO_4^{2-}] concentrations supporting a diverse community, and (3) there is an upstream source of colonizers or the flight or recolonizing distance is within the dispersal range of genetically diverse, reproducing adult colonizers. If any of these conditions are not met, the time required for ecosystem recovery (and thus, the allowable frequency of exceedance) would likely be longer than 3 years.

F.6.5. Protection of Federally-Listed Species

Although the derivation of the example criteria was limited to the macroinvertebrate taxa represented in the data sets, the available evidence indicates that other taxa in the streams are likely to be protected as well (see Section 2.6 and Appendix G). Hence, no adjustment was made for unanalyzed taxa. However, on a site-specific basis, the example criterion may be adjusted or recalculated to protect important species, highly valued aquatic communities, or specially protected waters.

F.7. EXAMPLE ECOREGION 69 EXTIRPATION CONCENTRATION (XC_{95}) VALUES FOR [HCO_3^- + SO_4^{2-}].

The XC_{95} values (HCO_3^- + SO_4^{2-} in mg/L) that were used in the XCD model are listed in the order of most to least intolerant below.

Order	Family	Genus	Symbol	XC_{95}	*N*	95% CI
Trichoptera	Limnephilidae	*Pycnopsyche*		148	45	72−176
Ephemeroptera	Ephemerellidae	*Eurylophella*		149	67	62−199
Diptera	Chironomidae	*Stempellina*		150	28	89−178
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*		153	193	104−222
Ephemeroptera	Leptophlebiidae	*Leptophlebia*		159	38	86−293
Ephemeroptera	Ameletidae	*Ameletus*		161	113	114−319
Ephemeroptera	Heptageniidae	*Heptagenia*		173	55	105−215
Diptera	Chironomidae	*Heleniella*	>	176	36	124−233
Plecoptera	Chloroperlidae	*Alloperla*	~	180	49	89−226
Ephemeroptera	Heptageniidae	*Epeorus*		181	334	154−254
Ephemeroptera	Baetidae	*Diphetor*		184	79	131−303
Diptera	Chironomidae	*Conchapelopia*	~	190	25	148−228
Ephemeroptera	Heptageniidae	*Leucrocuta*		192	116	170−222
Ephemeroptera	Heptageniidae	*Cinygmula*	~	199	76	127−265
Diptera	Chironomidae	*Stempellinella*	>	201	97	137−343
Diptera	Chironomidae	*Demicryptochironomus*	~	203	39	96−517
Coleoptera	Elmidae	*Promoresia*	>	203	65	146−368
Ephemeroptera	Heptageniidae	*Stenacron*	~	206	158	172−224
Ephemeroptera	Ephemerellidae	*Ephemerella*	~	239	268	192−338
Plecoptera	Perlodidae	*Diploperla*	=	242	28	81−344
Plecoptera	Pteronarcyidae	*Pteronarcys*	~	247	149	163−399
Ephemeroptera	Baetidae	*Procloeon*	~	295	34	201−339
Plecoptera	Chloroperlidae	*Haploperla*	>	297	178	242−517
Plecoptera	Perlodidae	*Yugus*	>	318	92	125−531
Ephemeroptera	Heptageniidae	*Stenonema*	>	326	258	268−513

Order	Family	Genus	Symbol	XC_{95}	*N*	95% CI
Trichoptera	Philopotamidae	*Wormaldia*	>	341	43	199−483
Plecoptera	Perlodidae	*Remenus*		348	40	68−457
Ephemeroptera	Ephemerellidae	*Serratella*	~	372	104	176−663
Diptera	Tipulidae	*Limnophila*		374	35	48−457
Diptera	Chironomidae	*Microtendipes*	>	380	220	294−873
Diptera	Simuliidae	*Prosimulium*	>	399	93	170−594
Plecoptera	Perlidae	*Eccoptura*	>	407	29	141−457
Diptera	Chironomidae	*Cladotanytarsus*	>	409	32	200−466
Diptera	Tipulidae	*Pseudolimnophila*	>	409	55	278−438
Plecoptera	Perlodidae	*Isoperla*	>	410	224	215−517
Diptera	Tipulidae	*Dicranota*	>	412	183	221−590
Diptera	Chironomidae	*Parachaetocladius*	>	415	105	247−935
Diptera	Chironomidae	*Cryptochironomus*	>	453	37	296−559
Plecoptera	Perlidae	*Acroneuria*	>	457	328	294−760
Diptera	Tipulidae	*Hexatoma*	>	470	291	236−848
Ephemeroptera	Ephemeridae	*Ephemera*	~	483	80	145−642
Ephemeroptera	Baetidae	*Plauditus*	>	488	215	427−848
Plecoptera	Peltoperlidae	*Tallaperla*	>	498	80	225−897
Diptera	Chironomidae	*Brillia*	>	506	68	245−607
Ephemeroptera	Baetidae	*Acentrella*	>	539	407	416−791
Ephemeroptera	Heptageniidae	*Maccaffertium*	>	539	257	378−740
Ephemeroptera	Baetidae	*Acerpenna*	>	663	33	194−663
Diptera	Chironomidae	*Dicrotendipes*	>	663	30	475−663
Diptera	Chironomidae	*Orthocladius*	>	681	76	373−902
Diptera	Chironomidae	*Micropsectra*	>	686	145	192−996
Amphipoda	Crangonyctidae	*Crangonyx*	>	760	31	176−760
Ephemeroptera	Isonychiidae	*Isonychia*	>	767	338	615−937
Diptera	Chironomidae	*Cricotopus*	>	793	132	566−913
Coleoptera	Elmidae	*Stenelmis*	>	804	285	634−1,167
Coleoptera	Psephenidae	*Psephenus*	>	805	291	607−1,063

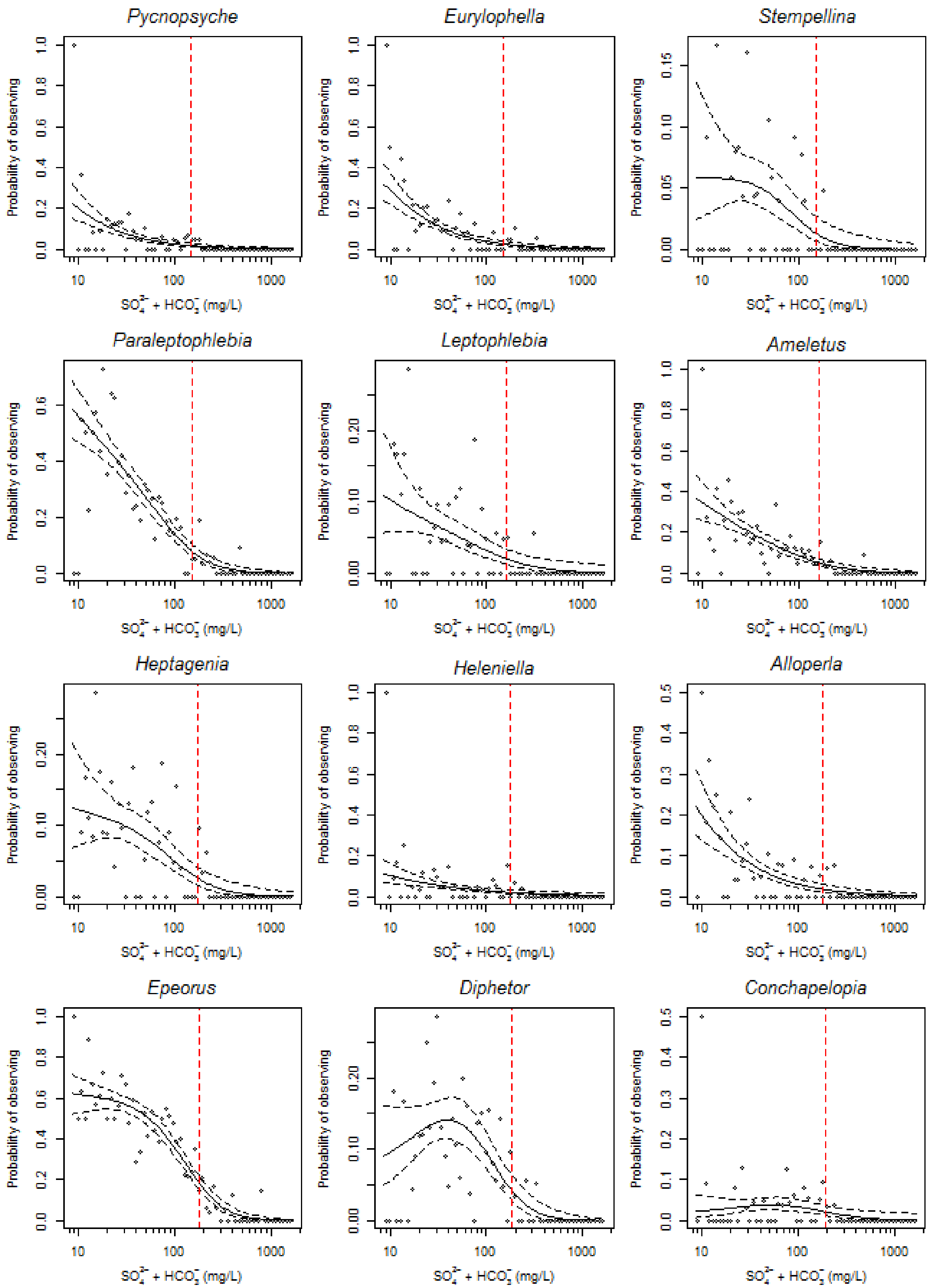
Order	Family	Genus	Symbol	XC_{95}	*N*	95% CI
Diptera	Chironomidae	*Paratanytarsus*	>	806	28	348−806
Trichoptera	Philopotamidae	*Dolophilodes*	>	848	312	438−1,127
Ephemeroptera	Caenidae	*Caenis*	>	875	140	417−996
Diptera	Chironomidae	*Potthastia*	>	902	28	270−902
Trichoptera	Glossosomatidae	*Glossosoma*	>	935	105	369−954
Ephemeroptera	Baetidae	*Baetis*	>	936	736	780−991
Trichoptera	Hydropsychidae	*Diplectrona*	>	951	409	660−1,127
Coleoptera	Psephenidae	*Ectopria*	>	951	170	378−1,063
Plecoptera	Leuctridae	*Leuctra*	>	951	560	748−1,167
Diptera	Tipulidae	*Antocha*	>	954	231	772−1,058
Decapoda	Cambaridae	*Cambarus*	>	954	207	459−1,498
Diptera	Chironomidae	*Chaetocladius*	>	954	60	285−954
Diptera	Chironomidae	*Corynoneura*	>	954	35	251−954
Plecoptera	Perlidae	*Paragnetina*		954	50	97−954
Trichoptera	Rhyacophilidae	*Rhyacophila*	>	954	354	414−1,199
Diptera	Chironomidae	*Cardiocladius*	>	987	156	792−1,248
Diptera	Athericidae	*Atherix*	>	995	92	739−996
Megaloptera	Corydalidae	*Corydalus*	>	995	170	792−1,412
Plecoptera	Nemouridae	*Amphinemura*	>	1,020	335	342−1,248
Diptera	Chironomidae	*Rheotanytarsus*	>	1,058	323	848−1,243
Isopoda	Asellidae	*Caecidotea*	>	1,063	40	201−1,063
Diptera	Chironomidae	*Polypedilum*	>	1,063	619	748−1,412
Diptera	Simuliidae	*Simulium*	>	1,063	494	848−1,248
Plecoptera	Chloroperlidae	*Sweltsa*	>	1,109	146	268−1,167
Plecoptera	Peltoperlidae	*Peltoperla*	>	1,167	106	565−1,167
Trichoptera	Hydropsychidae	*Ceratopsyche*	>	1,173	477	873−1,412
Trichoptera	Hydropsychidae	*Cheumatopsyche*	>	1,189	639	902−1,498
Ephemeroptera	Ephemerellidae	*Drunella*	~	1,199	169	193−1,199
Coleoptera	Elmidae	*Dubiraphia*	>	1,199	38	238−1,199
Trichoptera	Lepidostomatidae	*Lepidostoma*	>	1,199	121	154−1,199

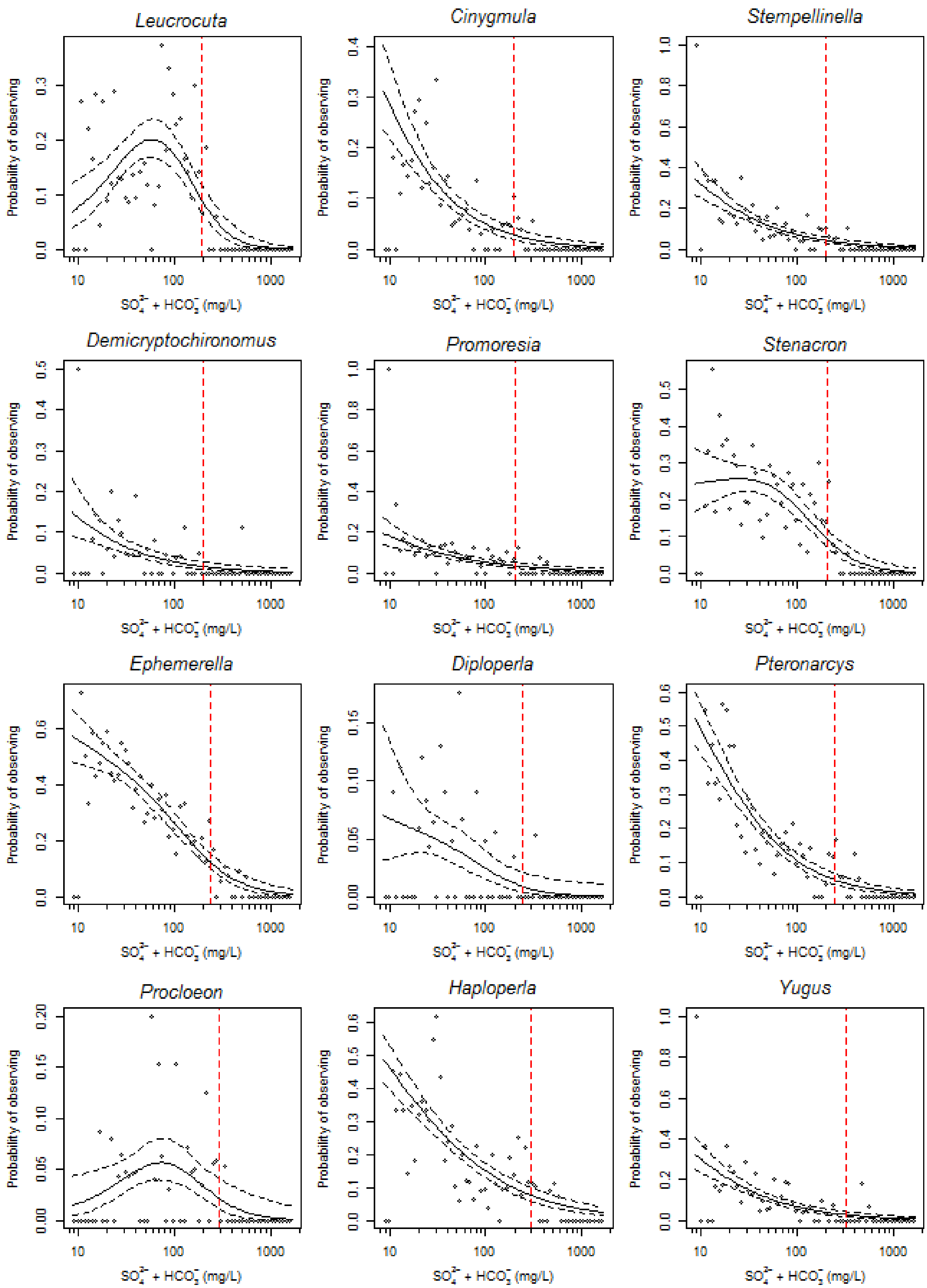
Order	Family	Genus	Symbol	XC_{95}	*N*	95% CI
Diptera	Chironomidae	*Limnophyes*	>	1,199	36	471−1,199
Coleoptera	Elmidae	*Oulimnius*	>	1,199	226	526−1,412
Diptera	Chironomidae	*Tvetenia*	>	1,199	391	909−1,248
Diptera	Empididae	*Chelifera*	>	1,248	94	951−1,248
Diptera	Dixidae	*Dixa*	>	1,248	48	189−1,248
Diptera	Chironomidae	*Eukiefferiella*	>	1,248	265	906−1,412
Trichoptera	Uenoidae	*Neophylax*	=	1,248	109	122−1,248
Trichoptera	Hydropsychidae	*Hydropsyche*	>	1,412	412	937−1,412
Coleoptera	Elmidae	*Microcylloepus*	>	1,412	88	498−1,412
Coleoptera	Elmidae	*Optioservus*	>	1,412	586	873−1,498
Diptera	Chironomidae	*Parametriocnemus*	>	1,412	562	954−1,498
Diptera	Chironomidae	*Sublettea*	>	1,412	78	565−1,412
Diptera	Chironomidae	*Thienemanniella*	>	1,412	106	878−1,412
Diptera	Chironomidae	*Thienemannimyia*	>	1,412	526	873−1,498
Trichoptera	Hydroptilidae	*Hydroptila*	>	1,498	110	910−1,498
Megaloptera	Corydalidae	*Nigronia*	>	1,498	331	873−1,682
Decapoda	Cambaridae	*Orconectes*	>	1,498	29	512−1,498
Megaloptera	Sialidae	*Sialis*	>	1,498	51	227−1,498
Diptera	Chironomidae	*Tanytarsus*	>	1,498	414	806−1,498
Diptera	Chironomidae	*Ablabesmyia*	>	1,682	38	663−1,682
Odonata	Aeshnidae	*Boyeria*	>	1,682	57	600−1,682
Trichoptera	Philopotamidae	*Chimarra*	>	1,682	220	897−1,682
Diptera	Chironomidae	*Diamesa*	>	1,682	173	951−1,682
Amphipoda	Gammaridae	*Gammarus*	>	1,682	50	359−1,682
Coleoptera	Dryopidae	*Helichus*	>	1,682	118	470−1,682
Diptera	Empididae	*Hemerodromia*	>	1,682	170	951−1,682
Plecoptera	Perlidae	*Perlesta*	>	1,682	45	339−1,682
Trichoptera	Polycentropodidae	*Polycentropus*	>	1,682	300	517−1,682
Diptera	Chironomidae	*Rheocricotopus*	>	1,682	144	897−1,682
Odonata	Gomphidae	*Stylogomphus*	>	1,682	39	405−1,682

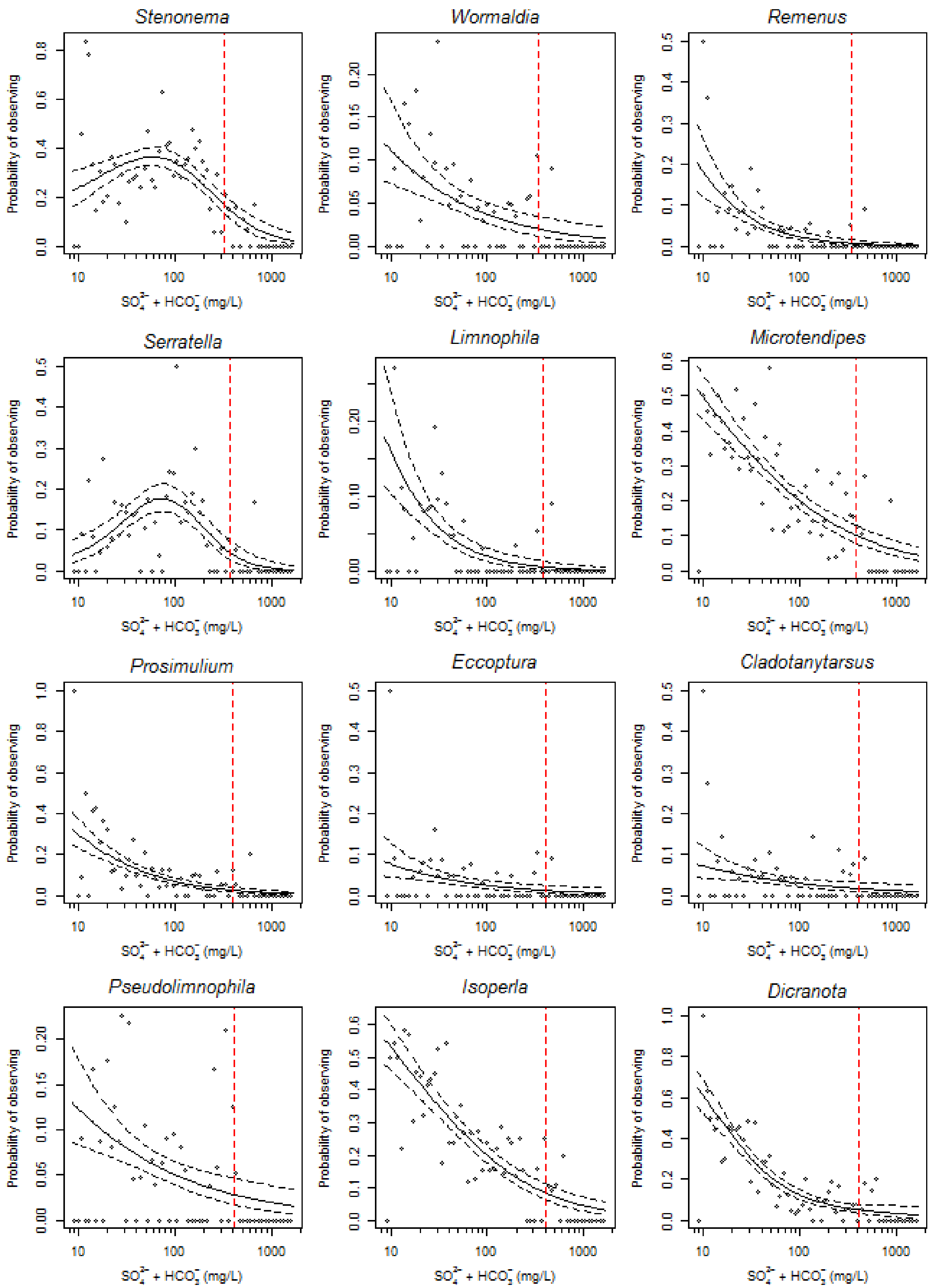
Order	Family	Genus	Symbol	XC_{95}	*N*	95% CI
Diptera	Tipulidae	*Tipula*	>	1,682	221	848−1,682
Diptera	Chironomidae	*Zavrelimyia*	>	1,682	67	255−1,682

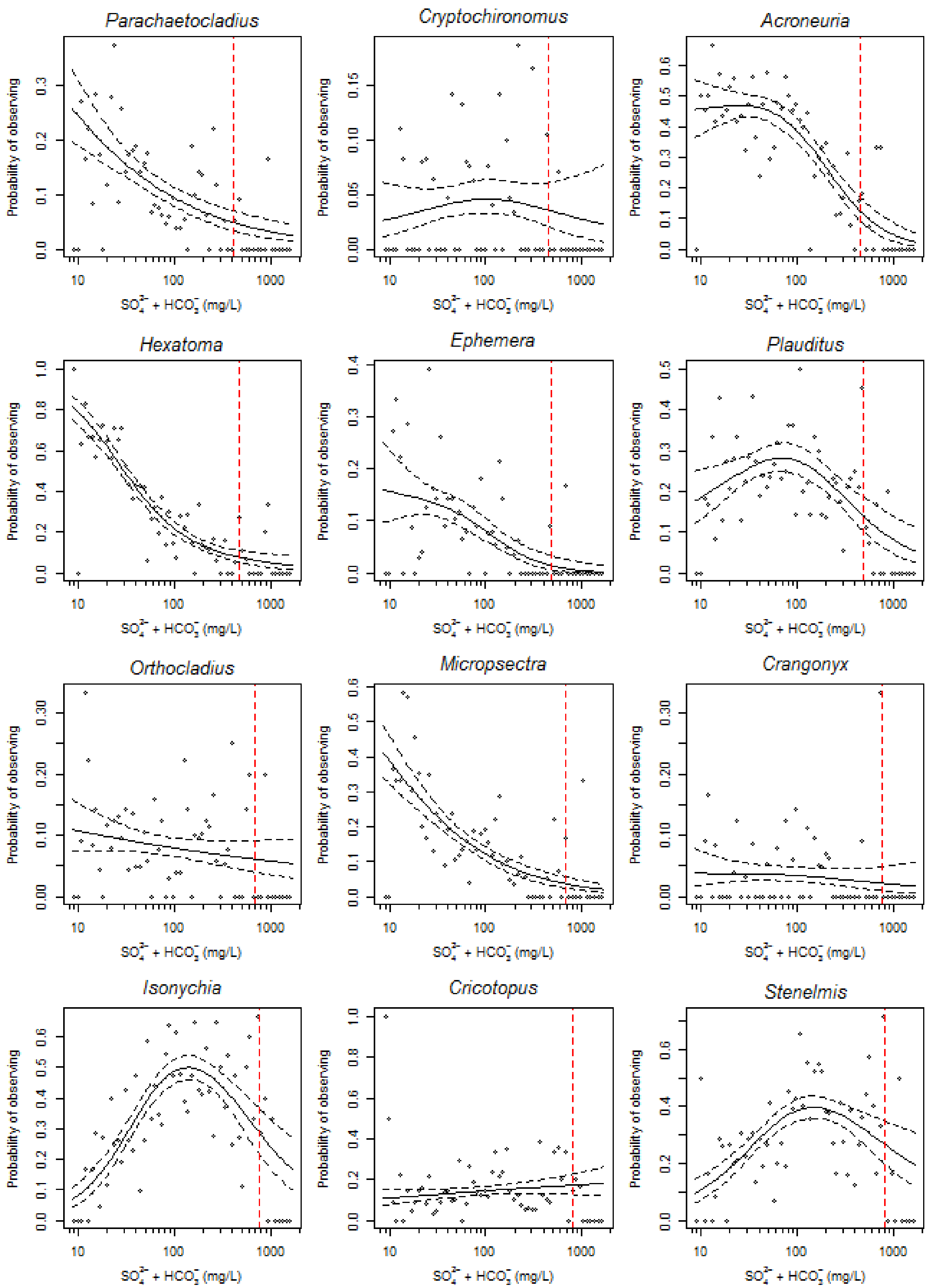
F.8. EXAMPLE ECOREGION 69 GENERALIZED ADDITIVE MODEL (GAM) PLOTS FOR [HCO_3^- + SO_4^{2-}].

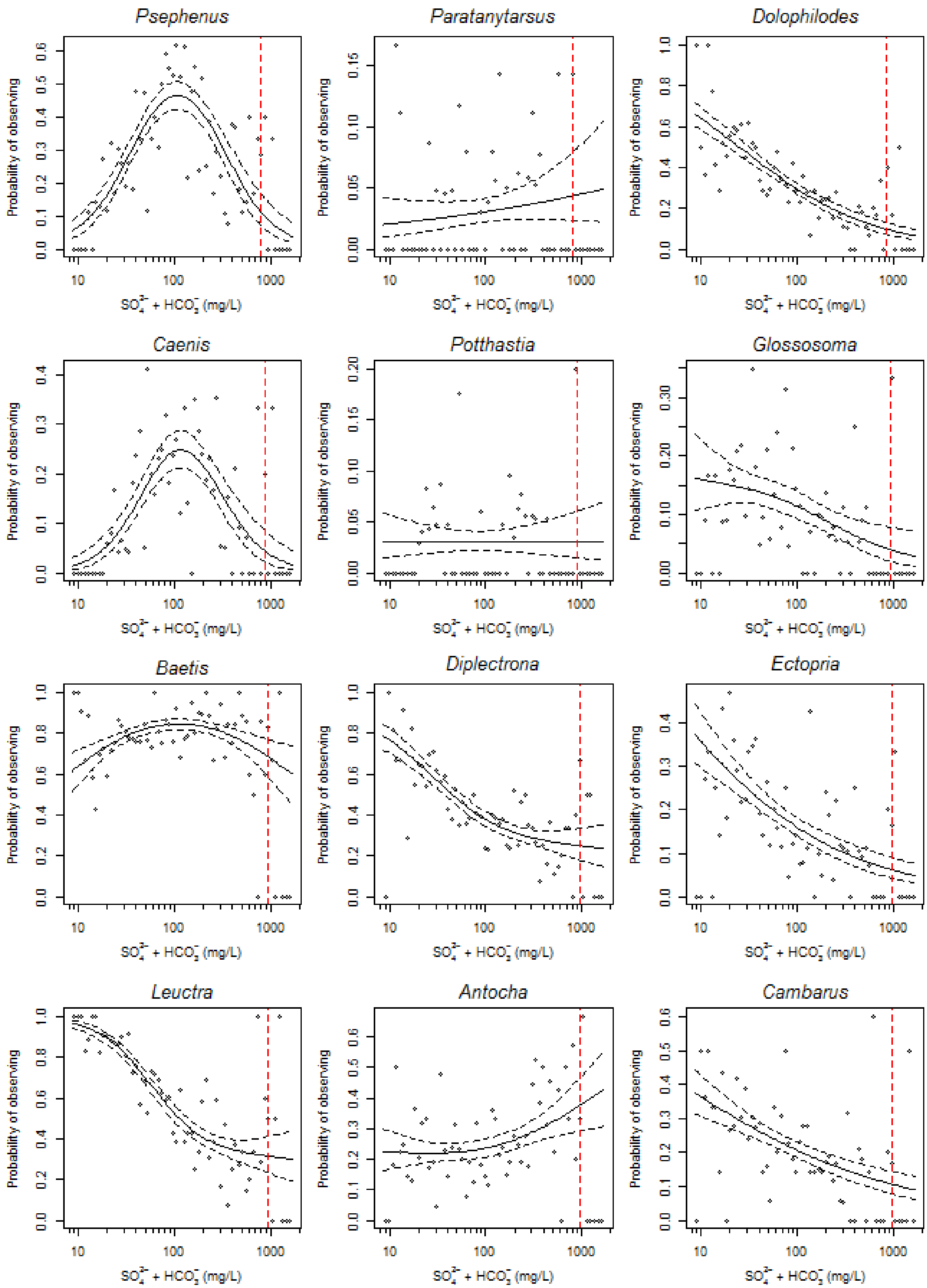
The GAM plots are depicted below that were used to designate ~ and > values for those XC_{95} values, ([HCO_3^-] + [SO_4^{2-}]) in mg/L. In this example, the capture probability is the proportion of sampled stations in an ionic concentration bin with the genus present based on taxonomic identification of 200 individuals per sample. The red, dashed vertical line is the XC_{95} value for the genus obtained from the plots of the CDFs in Appendix Section F.9. Plots are arranged from the lowest to the highest XC_{95} value.

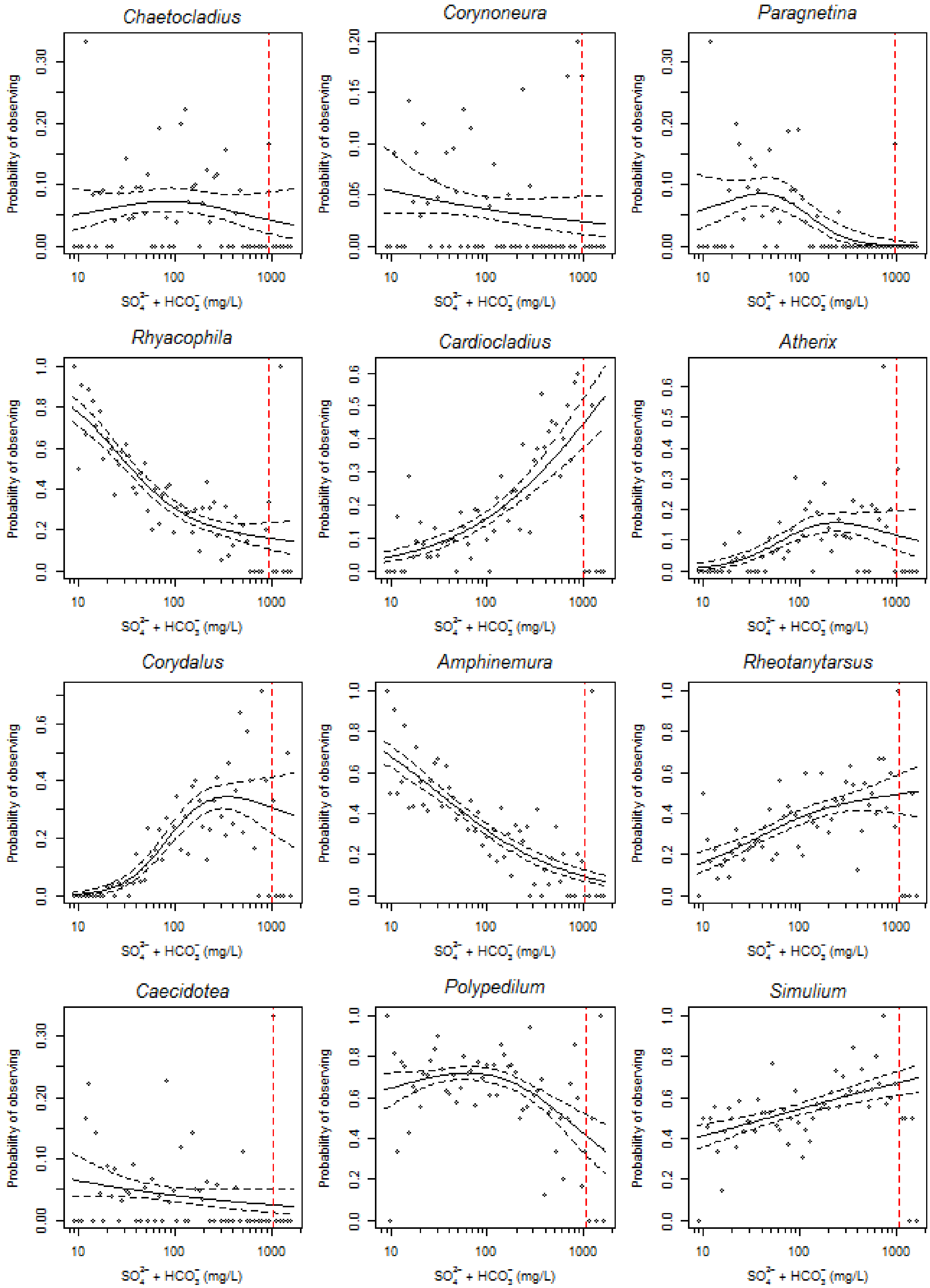

Pycnopsyche
Eurylophella
Stempellina
Paraleptophlebia
Leptophlebia
Ameletus
Heptagenia
Heleniella
Alloperla
Epeorus
Diphetor
Conchapelopia
Probability of observing
$SO_4^{2-} + HCO_3^-$ (mg/L)
10
100
1000

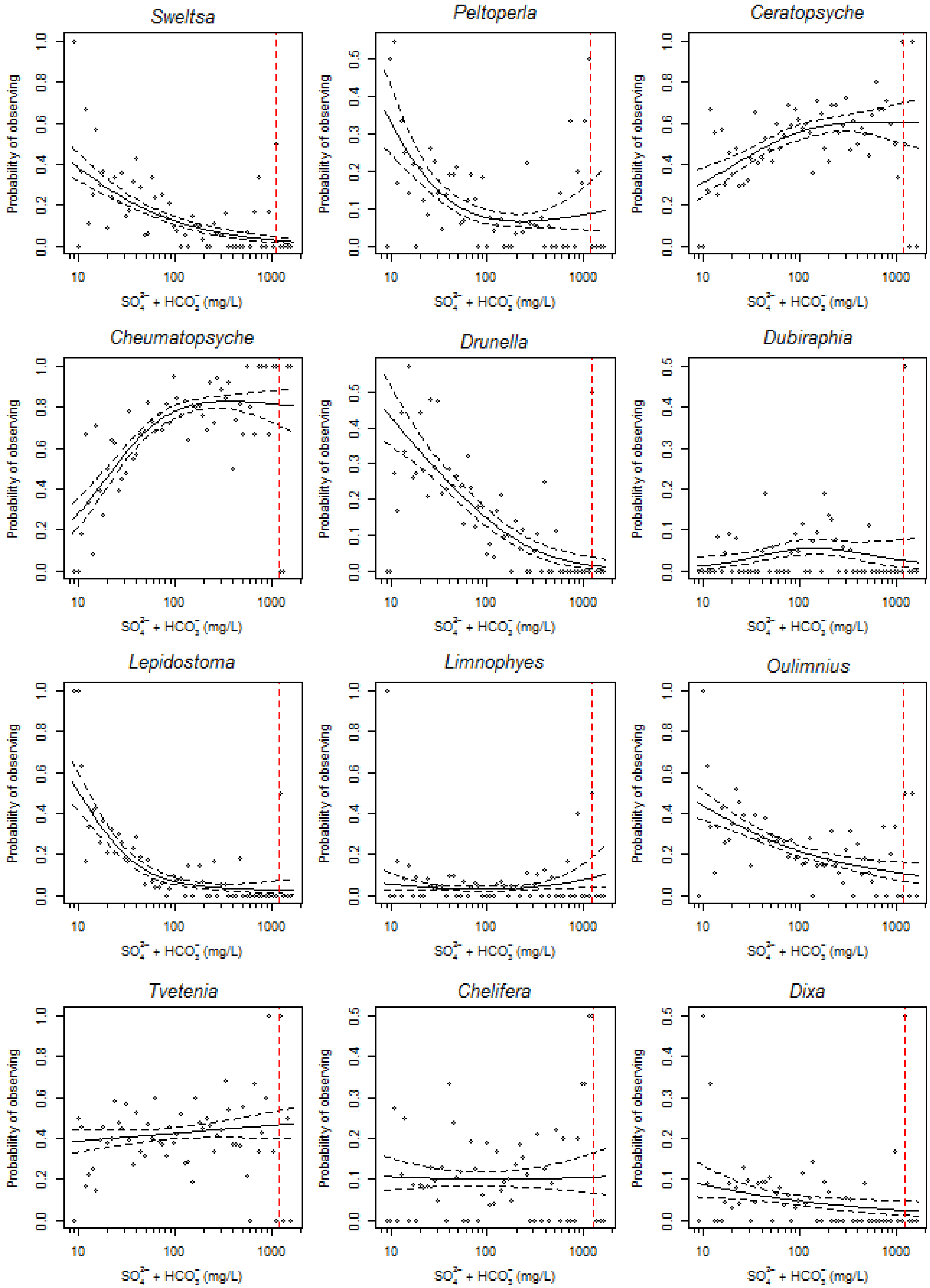

Leucrocuta
Cinygmula
Stempellinella
Demicryptochironomus
Promoresia
Stenacron
Ephemerella
Diploperla
Pteronarcys
Procloeon
Haploperla
Yugus
Probability of observing
$SO_4^{2-} + HCO_3^-$ (mg/L)

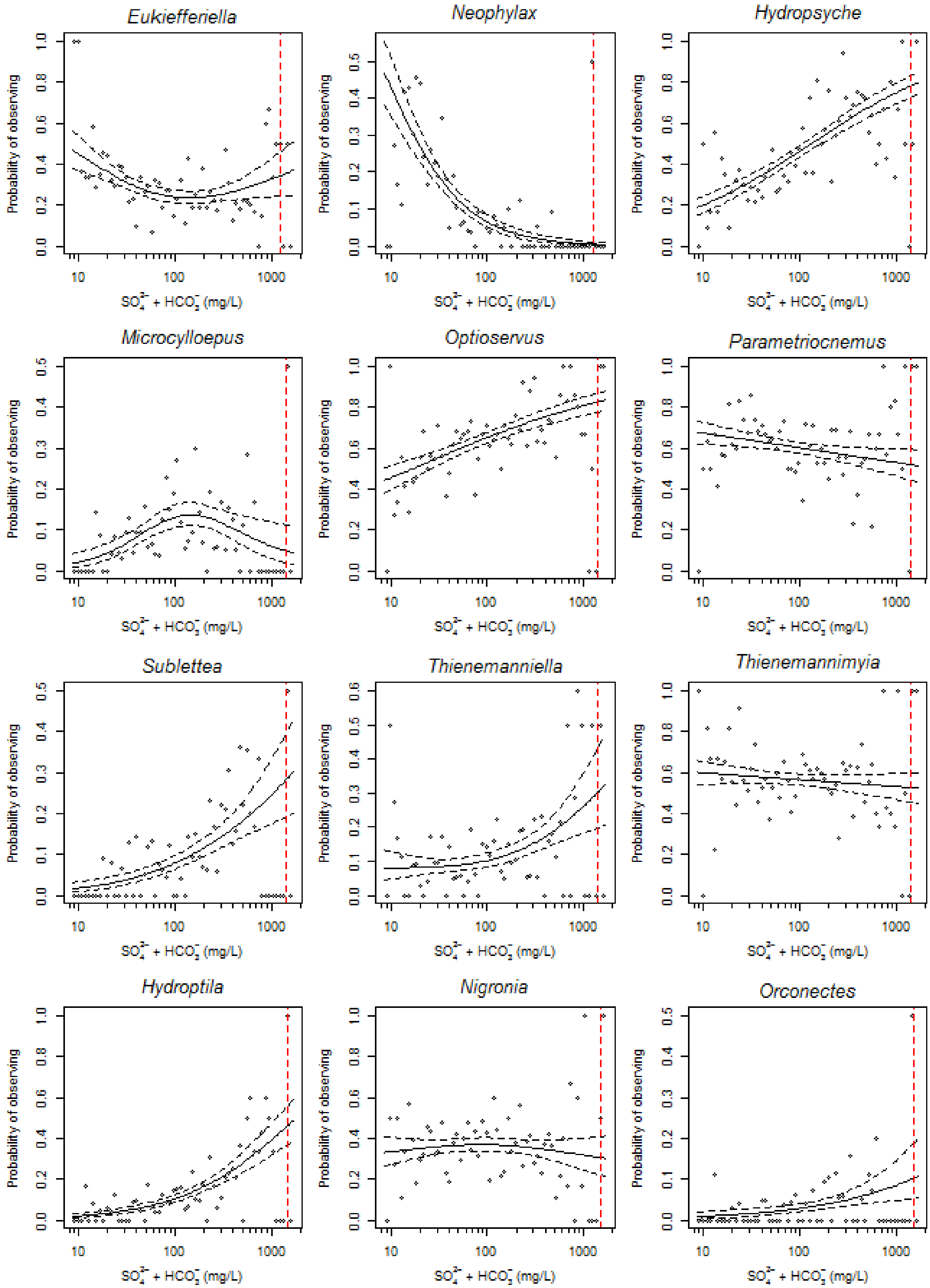

Stenonema
Wormaldia
Remenus
Serratella
Limnophila
Microtendipes
Prosimulium
Eccoptura
Cladotanytarsus
Pseudolimnophila
Isoperla
Dicranota
Probability of observing
$SO_4^{2-} + HCO_3^-$ (mg/L)

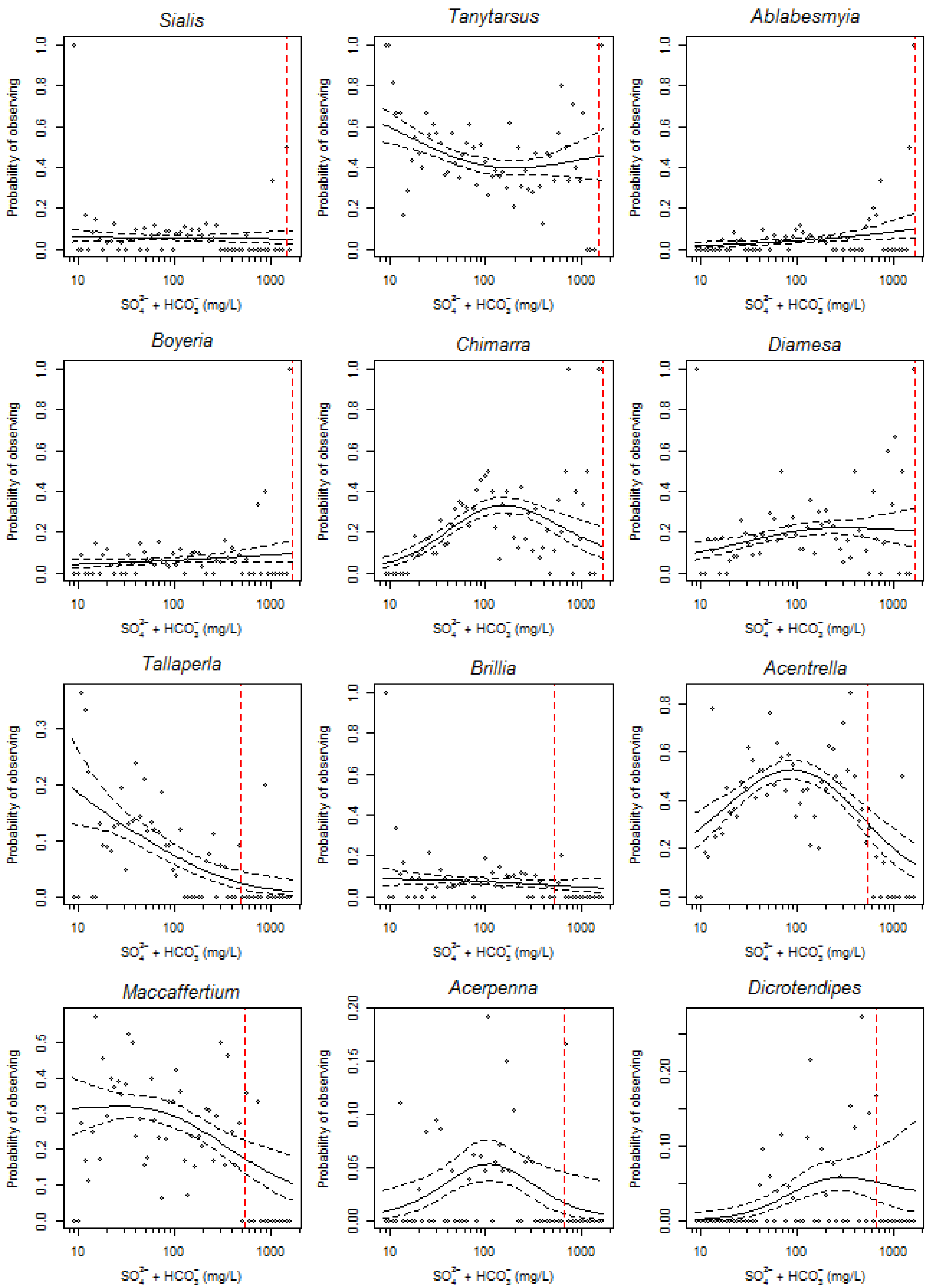

Parachaetocladius
Cryptochironomus
Acroneuria
Hexatoma
Ephemera
Plauditus
Orthocladius
Micropsectra
Crangonyx
Isonychia
Cricotopus
Stenelmis
Probability of observing
$SO_4^{2-} + HCO_3^-$ (mg/L)

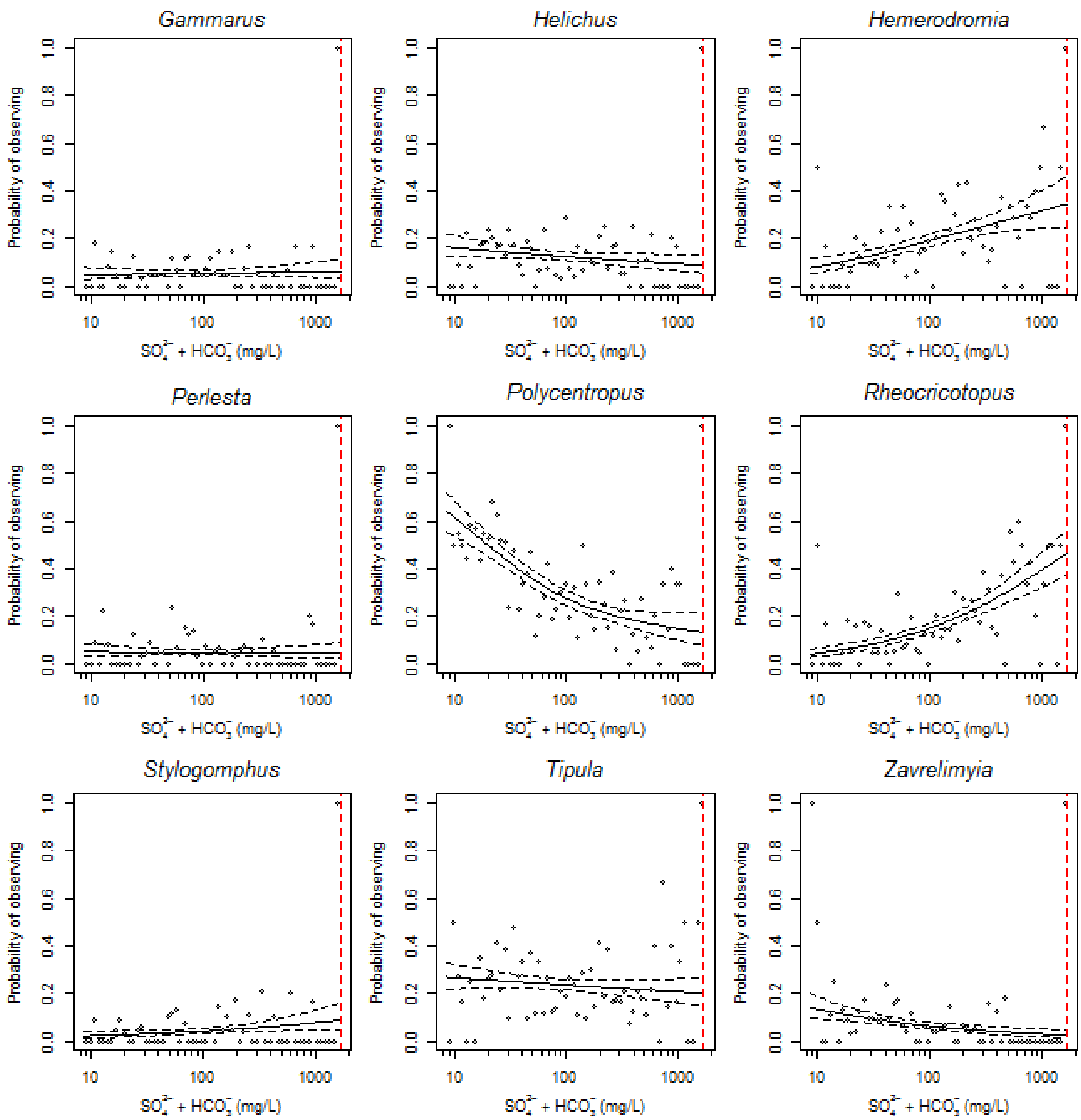

Psephenus
Paratanytarsus
Dolophilodes
Caenis
Potthastia
Glossosoma
Baetis
Diplectrona
Ectopria
Leuctra
Antocha
Cambarus
Probability of observing
SO$_4^{2-}$ + HCO$_3^-$ (mg/L)
10
100
1000


Chaetocladius
Corynoneura
Paragnetina
Rhyacophila
Cardiocladius
Atherix
Corydalus
Amphinemura
Rheotanytarsus
Caecidotea
Polypedilum
Simulium
Probability of observing
$SO_4^{2-} + HCO_3^-$ (mg/L)
10
100
1000


Sweltsa
Peltoperla
Ceratopsyche
Cheumatopsyche
Drunella
Dubiraphia
Lepidostoma
Limnophyes
Oulimnius
Tvetenia
Chelifera
Dixa
Probability of observing
$SO_4^{2-} + HCO_3^-$ (mg/L)
10
100
1000

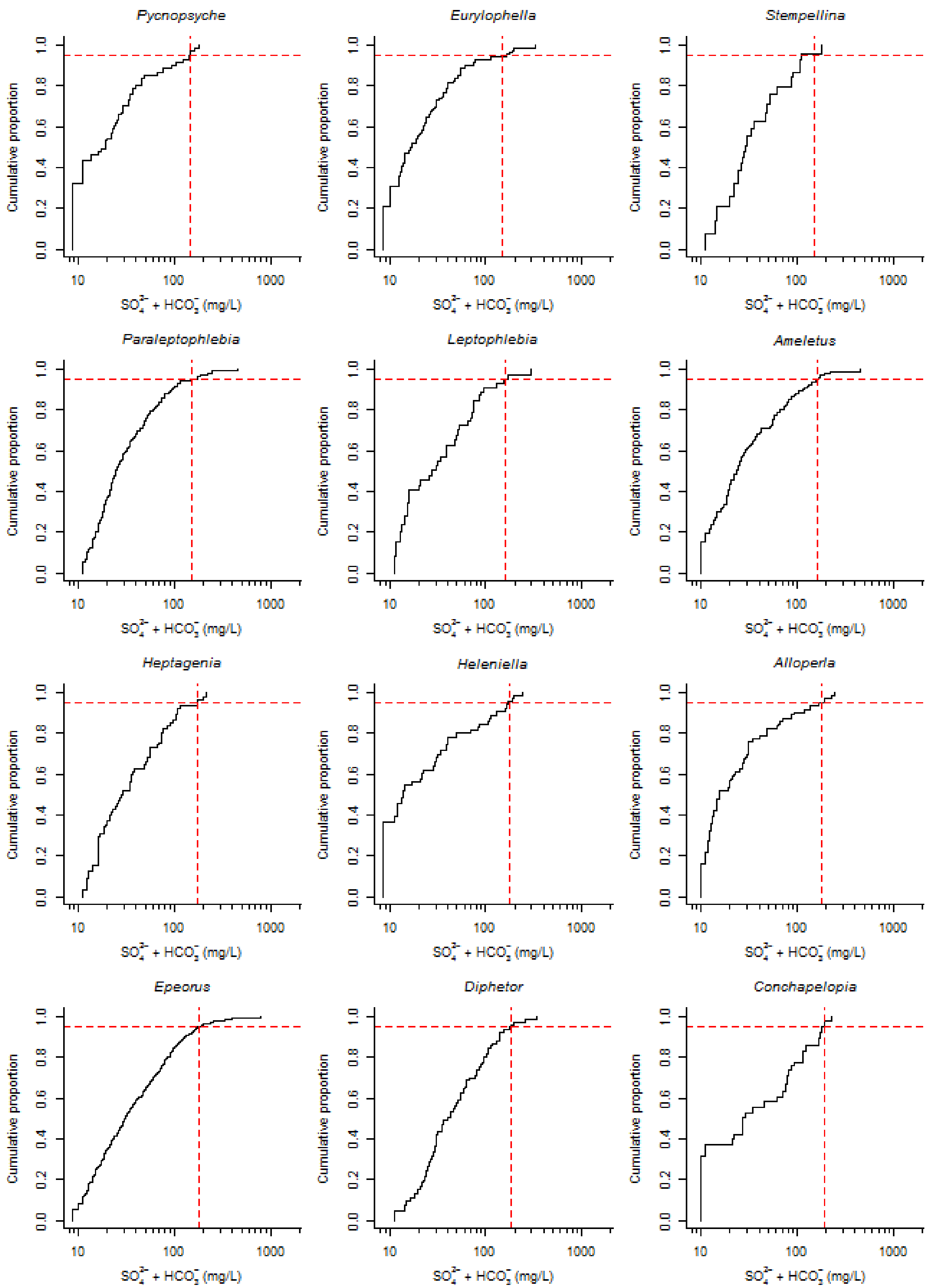

Eukiefferiella
Neophylax
Hydropsyche
Microcylloepus
Optioservus
Parametriocnemus
Sublettea
Thienemanniella
Thienemannimyia
Hydroptila
Nigronia
Orconectes
Probability of observing
SO$_4^{2-}$ + HCO$_3^-$ (mg/L)

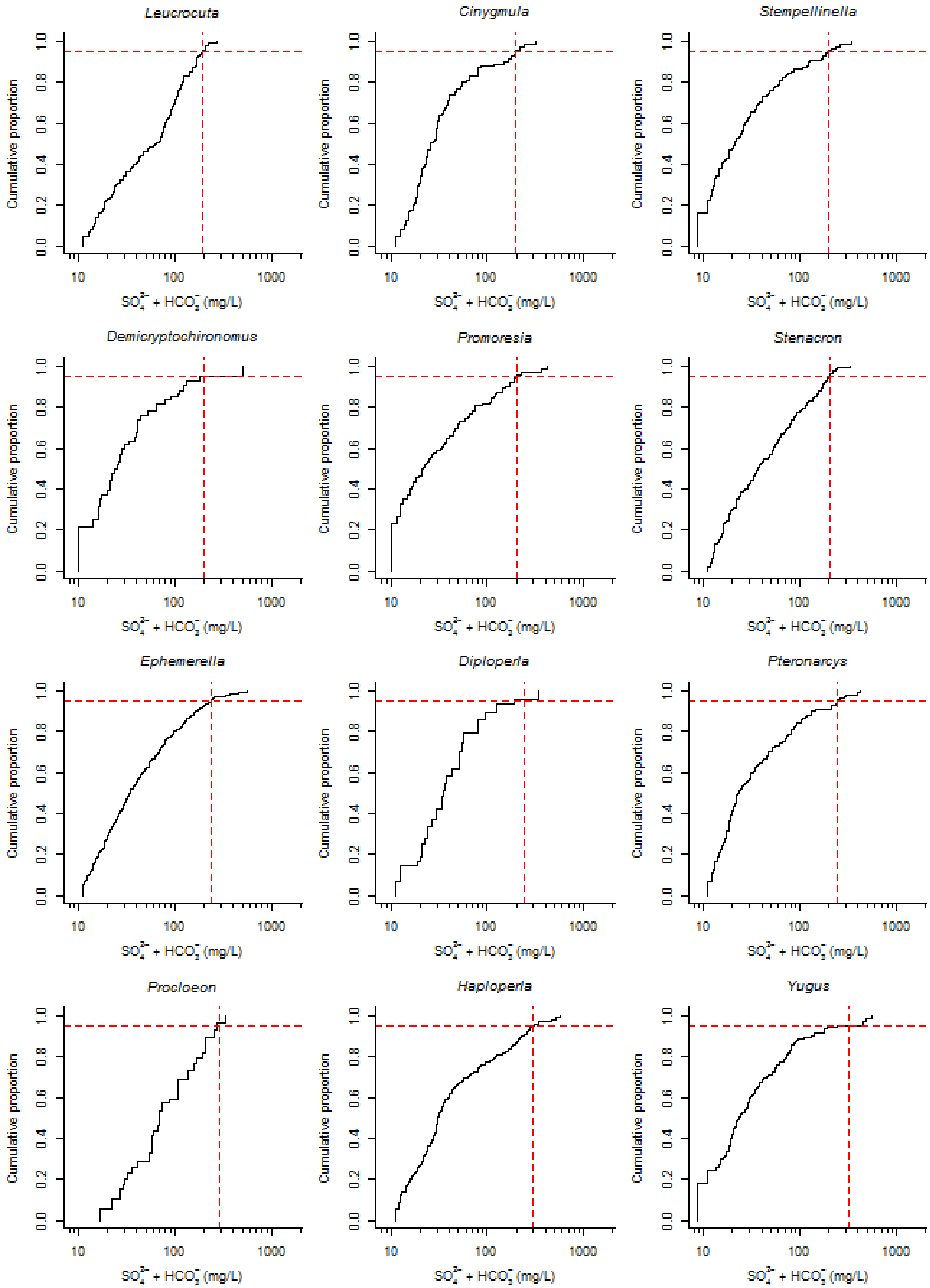

Sialis
Tanytarsus
Ablabesmyia
Boyeria
Chimarra
Diamesa
Tallaperla
Brillia
Acentrella
Maccaffertium
Acerpenna
Dicrotendipes
Probability of observing
SO$_4^{2-}$ + HCO$_3^-$ (mg/L)

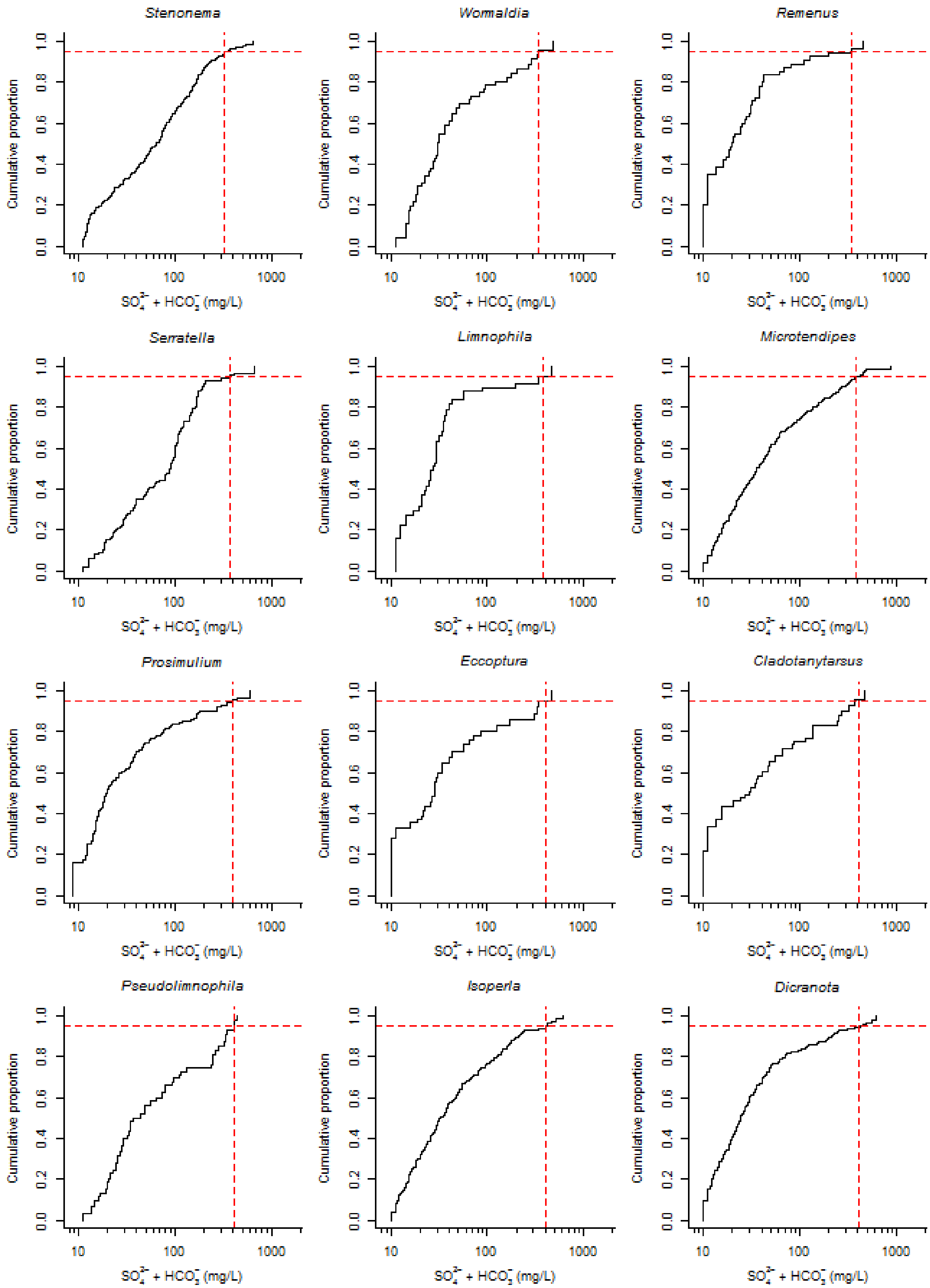

Gammarus
Helichus
Hemerodromia
Perlesta
Polycentropus
Rheocricotopus
Stylogomphus
Tipula
Zavrelimyia
Probability of observing
$SO_4^{2-} + HCO_3^-$ (mg/L)

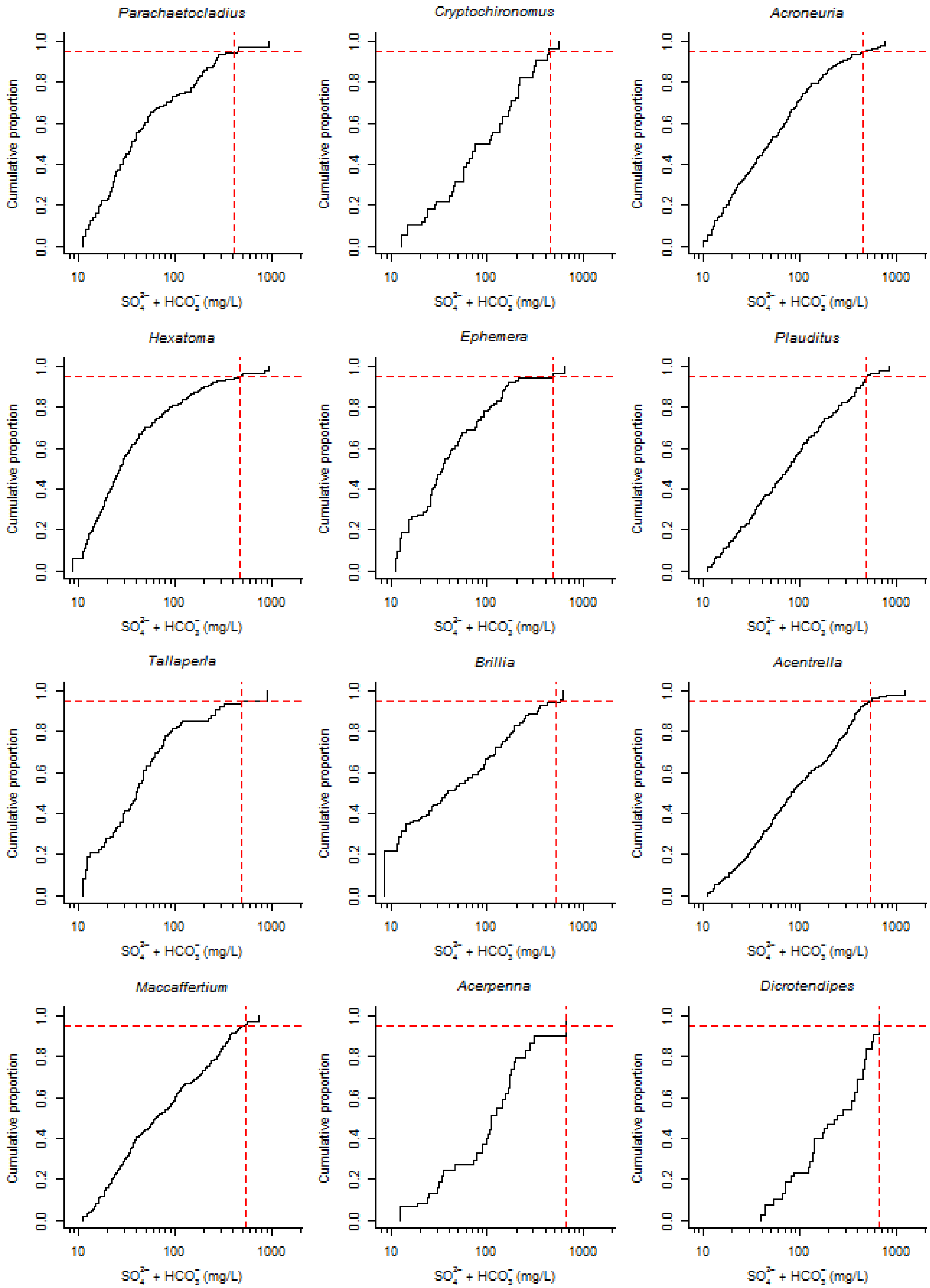
F.9. EXAMPLE ECOREGION 69 CUMULATIVE DISTRIBUTION FUNCTIONS (CDFS) PLOTS FOR [HCO_3^- + SO_4^{2-}].

The CDFs used to derive the XC_{95} values for ([HCO_3^-] + [SO_4^{2-}]) in mg/L are shown in this Appendix (see Section F.9). The red, dashed vertical line is the XC_{95} value for the genus obtained from each plotted CDF in this Appendix (see Section F.9) and the values are listed in this Appendix (see Section F.7). Plots are arranged from the lowest to the highest XC_{95} value.

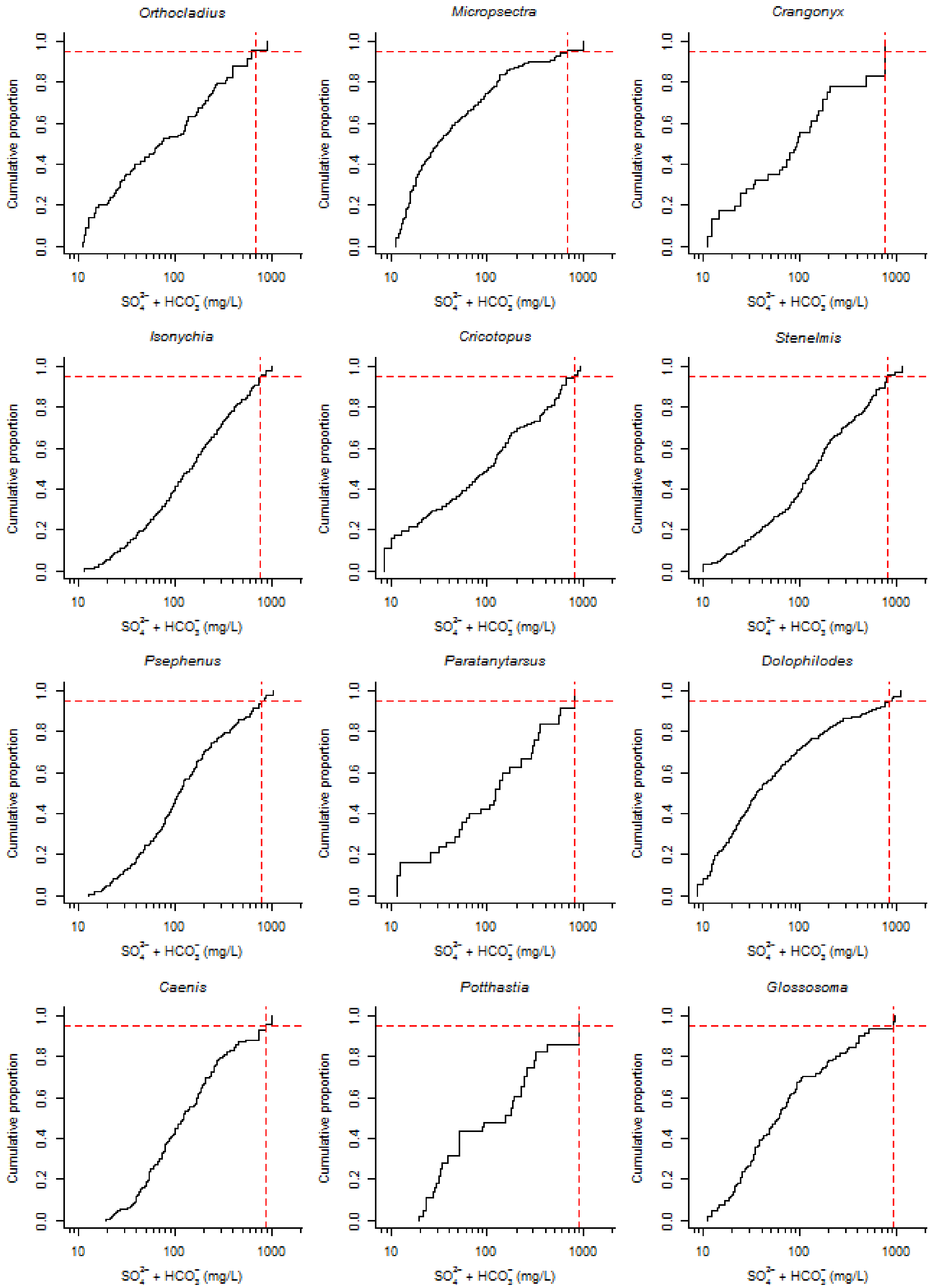

Pycnopsyche
Eurylophella
Stempellina
Paraleptophlebia
Leptophlebia
Ameletus
Heptagenia
Heleniella
Alloperla
Epeorus
Diphetor
Conchapelopia
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
$SO_4^{2-} + HCO_3^-$ (mg/L)

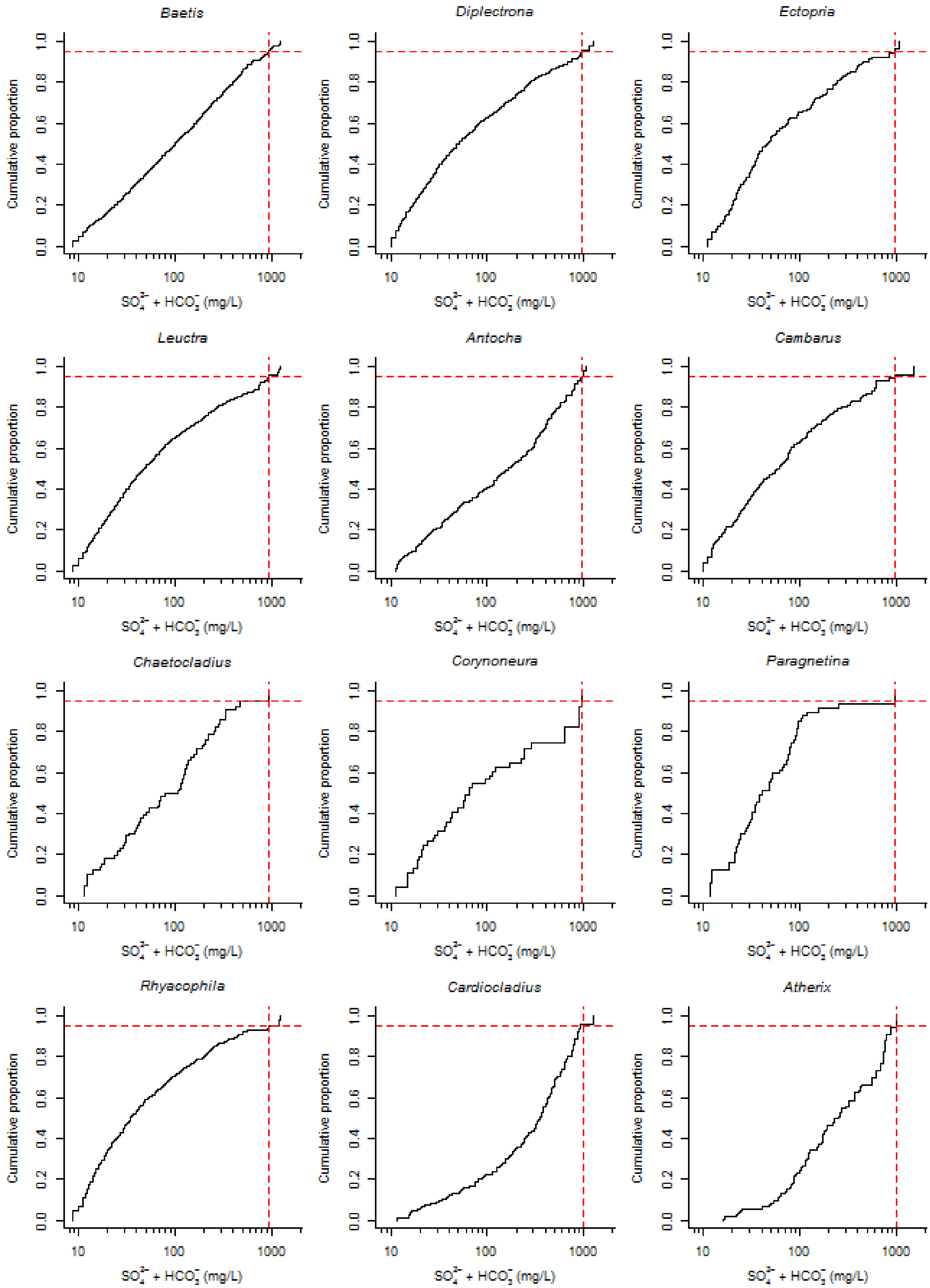

Leucrocuta
Cinygmula
Stempellinella
Demicryptochironomus
Promoresia
Stenacron
Ephemerella
Diploperla
Pteronarcys
Procloeon
Haploperla
Yugus
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
SO$_4^{2-}$ + HCO$_3^-$ (mg/L)

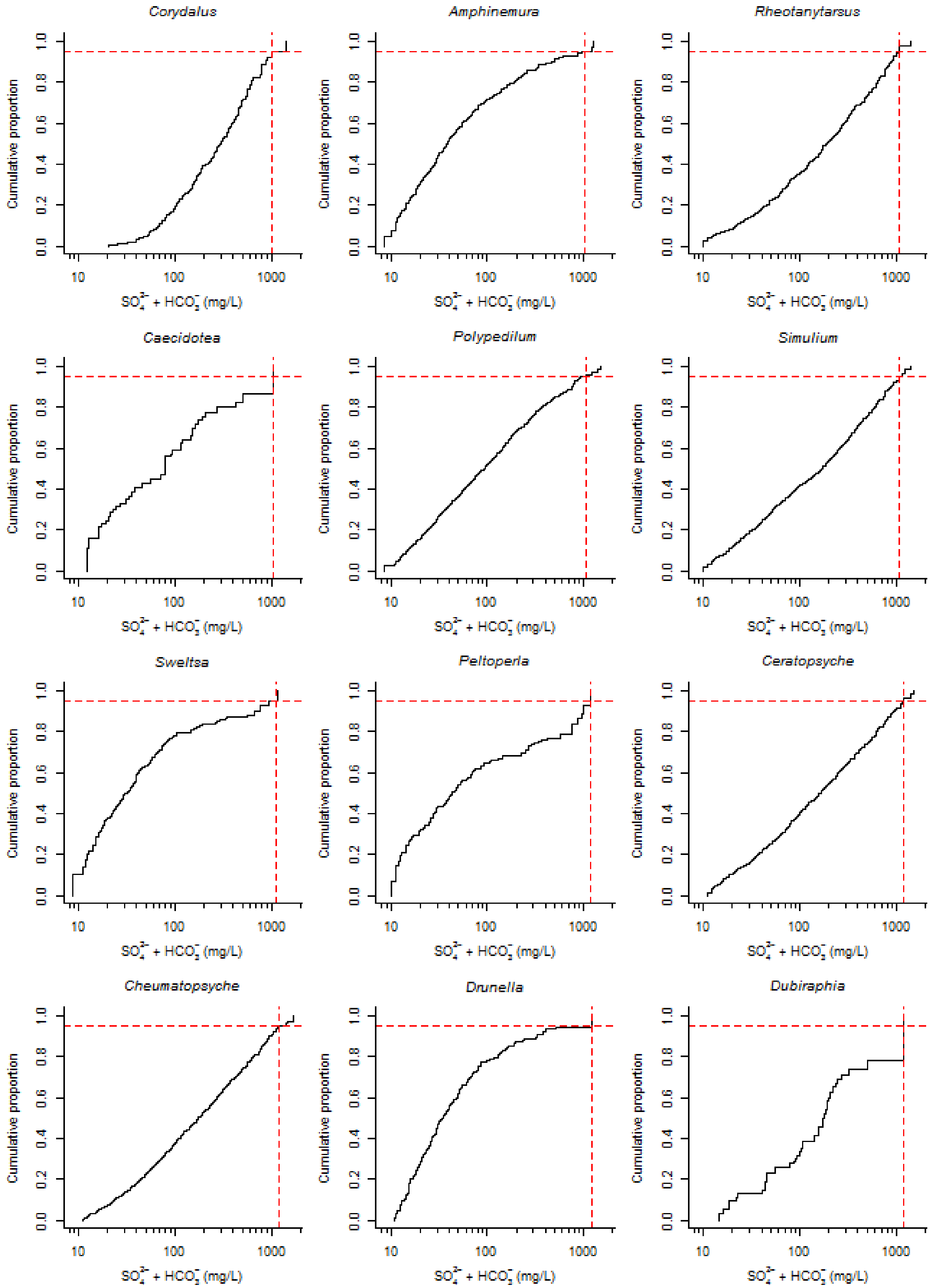

Stenonema
Wormaldia
Remenus
Serratella
Limnophila
Microtendipes
Prosimulium
Eccoptura
Cladotanytarsus
Pseudolimnophila
Isoperla
Dicranota
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
SO_4^{2-} + HCO_3^- (mg/L)

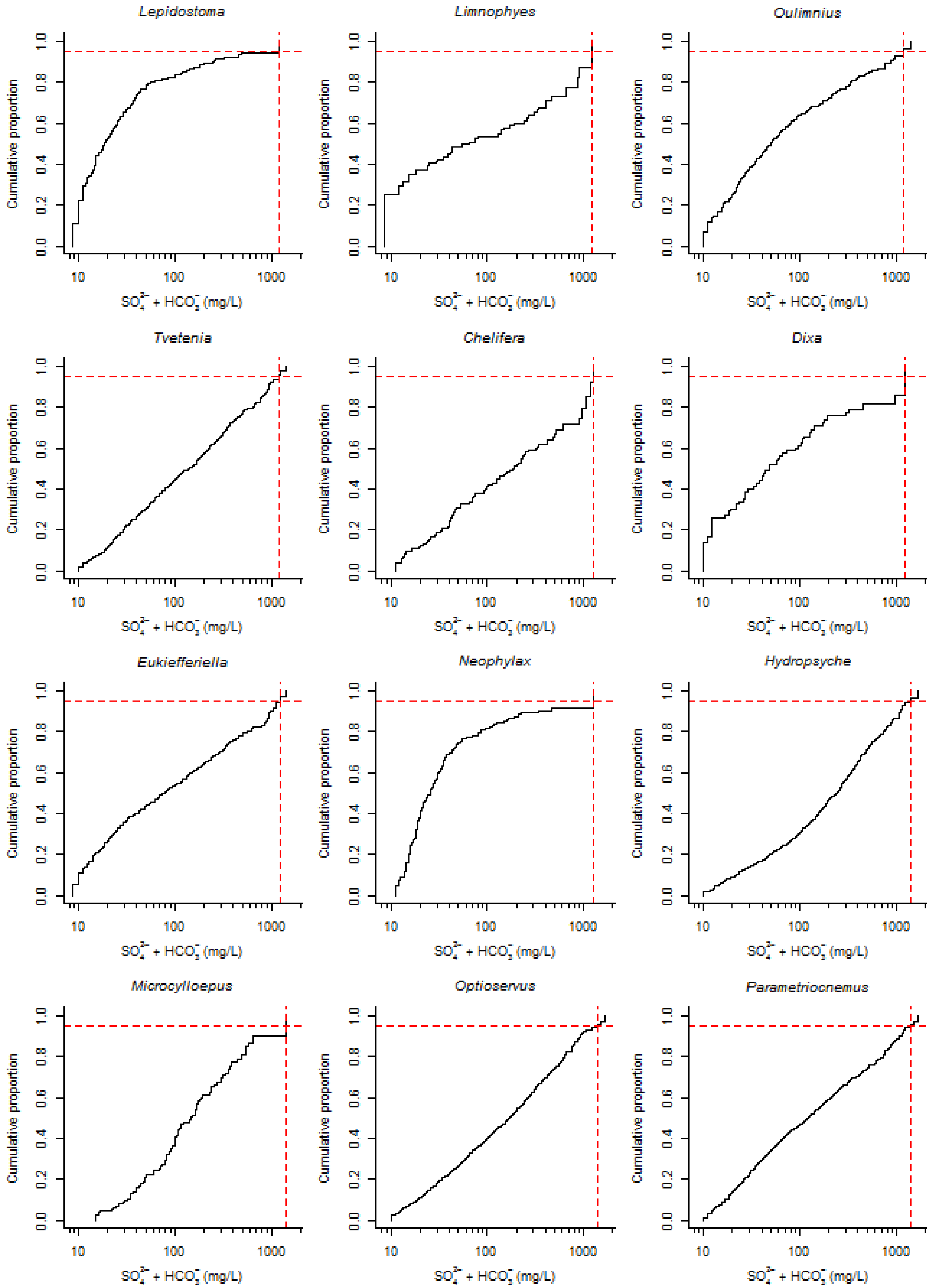


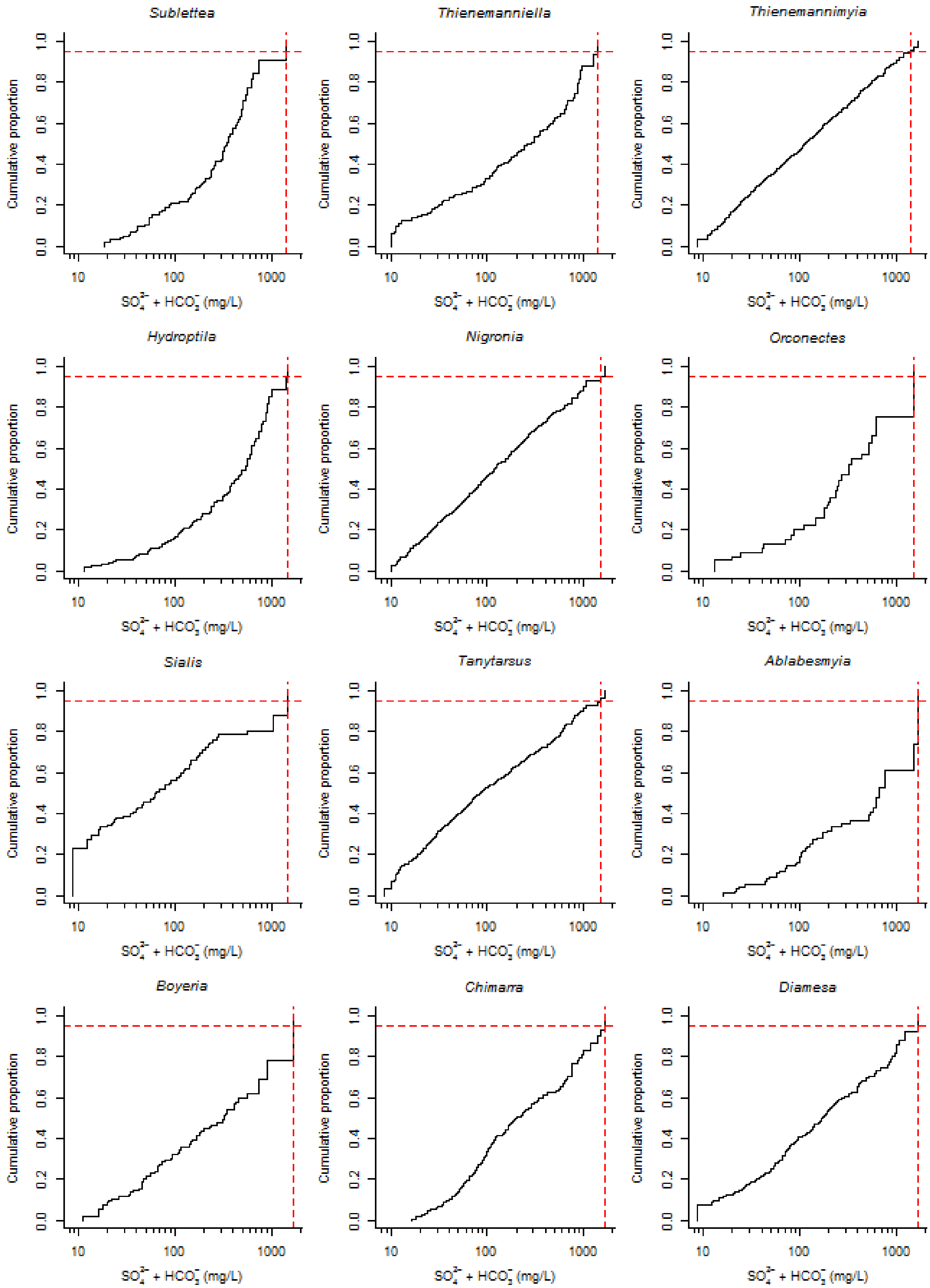
Parachaetocladius
Cryptochironomus
Acroneuria
Hexatoma
Ephemera
Plauditus
Tallaperla
Brillia
Acentrella
Maccaffertium
Acerpenna
Dicrotendipes
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
$SO_4^{2-} + HCO_3^-$ (mg/L)

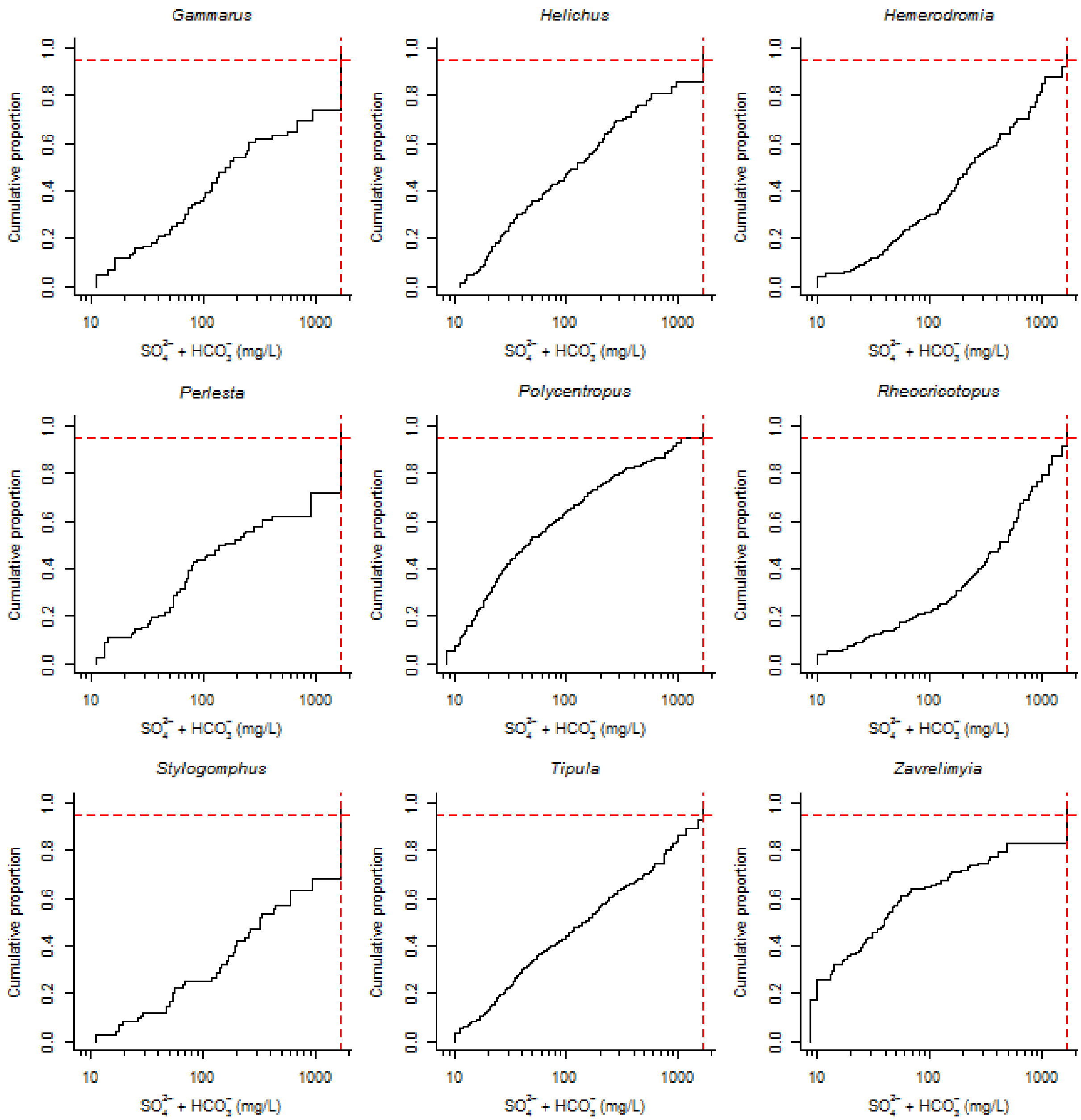

Orthocladius
Micropsectra
Crangonyx
Isonychia
Cricotopus
Stenelmis
Psephenus
Paratanytarsus
Dolophilodes
Caenis
Potthastia
Glossosoma
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
$SO_4^{2-} + HCO_3^-$ (mg/L)


Baetis
Diplectrona
Ectopria
Leuctra
Antocha
Cambarus
Chaetocladius
Corynoneura
Paragnetina
Rhyacophila
Cardiocladius
Atherix
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
$SO_4^{2-} + HCO_3^-$ (mg/L)


Corydalus
Amphinemura
Rheotanytarsus
Caecidotea
Polypedilum
Simulium
Sweltsa
Peltoperla
Ceratopsyche
Cheumatopsyche
Drunella
Dubiraphia
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
$SO_4^{2-} + HCO_3^-$ (mg/L)


Lepidostoma
Limnophyes
Oulimnius
Tvetenia
Chelifera
Dixa
Eukiefferiella
Neophylax
Hydropsyche
Microcylloepus
Optioservus
Parametriocnemus
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
$SO_4^{2-} + HCO_3^-$ (mg/L)


Sublettea
Thienemanniella
Thienemannimyia
Hydroptila
Nigronia
Orconectes
Sialis
Tanytarsus
Ablabesmyia
Boyeria
Chimarra
Diamesa
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
$SO_4^{2-} + HCO_3^-$ (mg/L)



Gammarus
Helichus
Hemerodromia
Perlesta
Polycentropus
Rheocricotopus
Stylogomphus
Tipula
Zavrelimyia
Cumulative proportion
0.0
0.2
0.4
0.6
0.8
1.0
10
100
1000
$SO_4^{2-} + HCO_3^-$ (mg/L)

F.10. REFERENCES

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Suter, GW, II; Cormier, SM. (2013) A method for assessing the potential for confounding applied to ionic strength in Central Appalachian streams. Environ Toxicol Chem 32(2):288−295.

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U.S. EPA (Environmental Protection Agency). (1991) Technical support document for water quality-based toxics control. Washington, DC: Office of Science and Technology, Office of Water. EPA/505/2-90/001. Available online at https://www3.epa.gov/npdes/pubs/owm0264.pdf.

U.S. EPA (Environmental Protection Agency). (2010) Primary distinguishing characteristics of Level III Ecoregions of the Continental United States. Corvallis, OR: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory Available online at: ftp://ftp.epa.gov/wed/ecoregions/us/Eco_Level_III_descriptions.doc.

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