APPENDIX G. CASE EXAMPLE USING AN ALTERNATIVE ASSESSMENT ENDPOINT (SPECIES OF FISH)

As with benthic macroinvertebrates, diverse assemblages of fish are important assessment endpoints for the protection of biotic integrity and aquatic life uses. This appendix assesses whether the example ecoregional criteria presented in the Case Studies, which were based on macroinvertebrate field data, are protective of fish. The extirpation concentrations (XC_{95}s) are derived for fish species and compared to the Case Study example criteria which were derived using benthic macroinvertebrate data (see Sections 4 and 5). The XC_{95} is the specific conductivity (SC) level above which 5% of observations of a species of fish were made in sampled streams. In this case study, a combined data set is used for fish in streams from four contiguous Level III ecoregions: Ecoregions 67 (Ridge and Valley), 68 (Southwestern Appalachians), 69 (Central Appalachians), and 70 (Western Allegheny Plateau). For illustrative purposes, the hazardous concentration of the 5th centile (HC_{05}) of extirpation concentration distributions (XCD) is also derived for species and genera of fish using the draft field-based method for SC criteria. Also, the effect of taxonomic resolution on the XC_{95} values is described.

G.1. INTRODUCTION

The draft field-based method for developing ecoregional criteria for SC is based on benthic macroinvertebrates for several reasons (see Section 2.6, *Assessment Endpoints and Measures of Effect*). Because macroinvertebrates are abundant, diverse, and easily collected, they are used more often than fish for water quality monitoring and bioassessment. This is partly due to there being fewer species of fish than macroinvertebrates, particularly in the western United States, and some streams by nature support no fish or very few species of fish. As a result, the fish data set is smaller and contains fewer genera than the macroinvertebrate data sets even though the sampled area included four ecoregions. Additionally, one practical advantage for using macroinvertebrate data is that fewer samples are required: sensitivity analyses indicate that the minimum samples size for the draft field-based method is 500−800 macroinvertebrate samples (e.g., see Figure 4-12) versus 800−1,000 or more fish samples (see Figure G-7).

Geology and water chemistry are broadly similar across the Case Study regions, so it is appropriate to compare effects on macroinvertebrates and fish in a general way. However, fish and macroinvertebrate data were not combined to estimate an effects endpoint (HC_{05}) for several reasons. First, salt-intolerant macroinvertebrates are observable primarily in the spring, when SC values tend to be close to the minimum or annual average. Fish are observable year-round, including during the summer when SC values are generally near an annual maximum. Therefore, effect levels for fish may reflect different exposure measurements.

Second, in Case Studies I and II, the macroinvertebrate data were analyzed within individual ecoregions and states for consistency, but to obtain sufficient data for this analysis, the fish data are aggregated across multiple sampling programs, states, and ecoregions including the addition of Ecoregion 67 (see Figure G-1). Hence, the spatial scopes of the fish and macroinvertebrate data are different.

Third, the mechanism of action may be different for fish and benthic invertebrates. Although both show an effect associated with increased SC and both are affected by ionic conditions outside their physiological range, they have somewhat different ionic regulatory mechanisms (Bradley, 2009; Evans, 2008a,b; Griffith 2016; Marshall, 2002; Wood and Shuttleworth, 2008). Furthermore, effects observed in the field may be an indirect effect associated with avoidance, food preferences, predation, diseases, or energetic demands, and those indirect effects are likely to differ between fish and invertebrates. Because the mechanisms of action for fish and invertebrates may differ, they may not follow a single unimodal XCD.

Fourth, fish are routinely identified to species; whereas, invertebrates are more difficult to identify to species. By calculating the XC_{95} to genus to be consistent with invertebrates, the XC_{95} values would represent the effect of the least salt-intolerant species in a genus (see Section G.4.3 and Figure G-11).

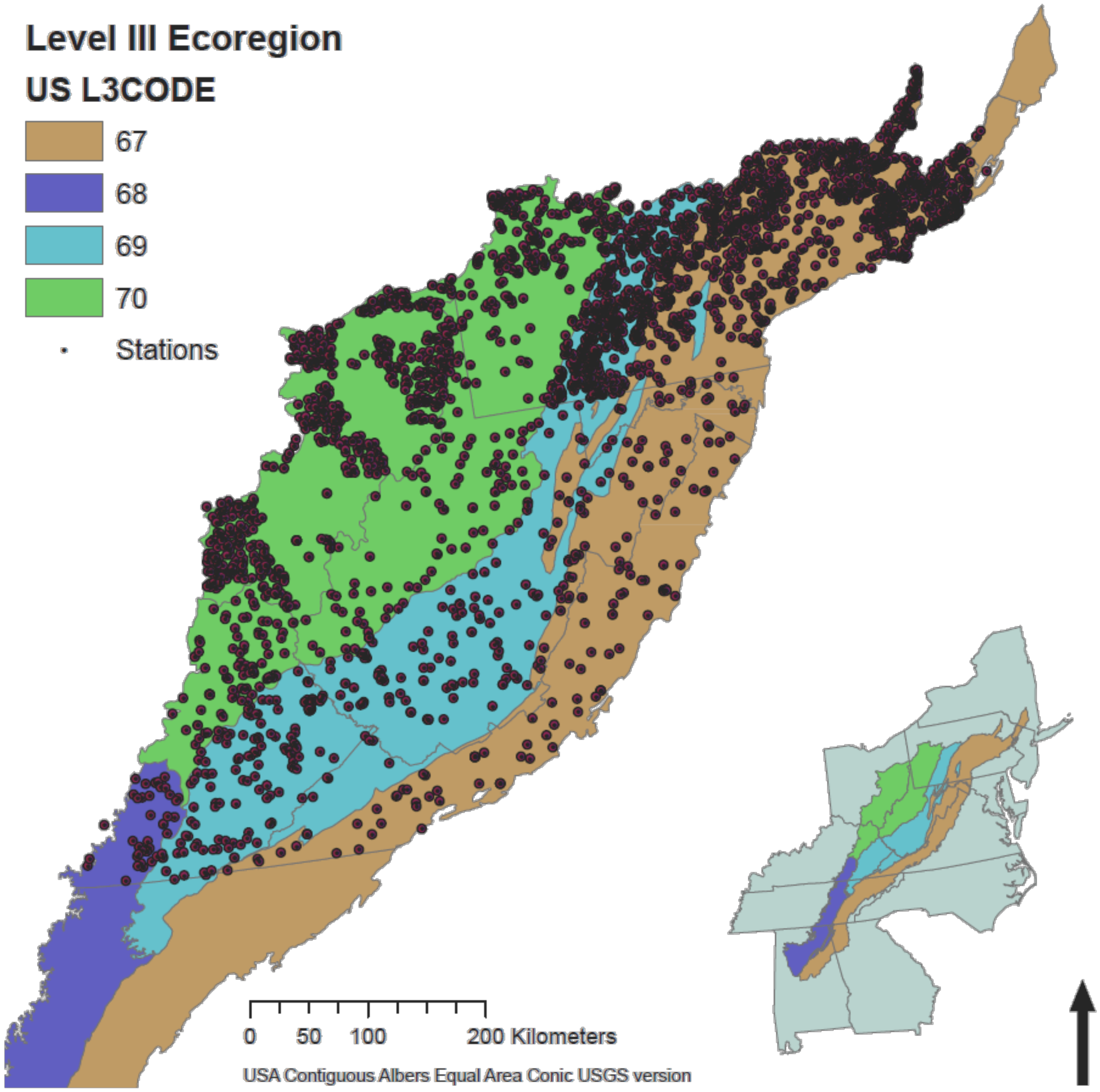


Figure G-1. The fish sampling locations (*N* = 3,465) are from Level III Ecoregions 67, 68, 69, and 70 spanning the states of Kentucky, West Virginia, Virginia, Ohio, Maryland, Pennsylvania, and New Jersey.

Data source: State outlines from U.S. Environmental Protection Agency (EPA) Base Map Shapefile. Omernik Level III Ecoregions from National Atlas Projection NAD1982UTM17N.

Finally, the fish data provide a weaker exposure-response relationships than the macroinvertebrate data. This appears to be due in part to biological factors (fewer species, lower cross-basin mobility and potentially lower sensitivities of fish to SC) and in part to statistical factors (a smaller fish data set with greater extraneous variance and a narrower range of exposures). The greater mobility in a stream network and less mobility across basins and physical structures is particularly important. The greater mobility of fish within a system compared to invertebrates allows them to enter upstream systems that may support adults but may not support salt-intolerant early life stages. Therefore, the presence of a species may not be a sustainable one. Fish may be absent because of limited interbasin dispersal in contrast to the winged stages of most aquatic insects which permit them to disperse among disconnected basins. As a result of this combination of biological and statistical factors, the estimates of the relationships of fish observations and SC may not be directly comparable to those for macroinvertebrates.

For these reasons, fish and macroinvertebrate data were not combined in a single genus level XCD to derive an HC_{05}. However, the fish data still allow assessment of whether a criterion derived using the draft macroinvertebrate-based field method is protective of fish.

G.2. PROBLEM FORMULATION

The problem formulation for this assessment of fish is largely the same as for the benthic macroinvertebrate cases (see Section 2). The stressor of concern is the same as is the conceptual model for its sources, transport, exposure, and effects (see Section 2.2.2). The routes of exposure are the same for direct exposure (see Section 2.3), but fish may be stressed indirectly through reduced food resources.

The nature of the effect and mechanism of action are largely the same but have some differences. The direct effects on fish, as with macroinvertebrates, are caused by internal ionic concentrations that affect homeostasis, which can result in reduced survival and fecundity (see Section 2.4). However, indirect effects are also possible, because a principle food of stream fish is benthic macroinvertebrates (Allan, 1981; Cada et al., 1987; Richardson, 1993) which are affected by high SC (U.S. EPA, 2011a). Hitt and Chambers (2014) suggest that reduced fish diversity and abundance in high SC streams may be due to decreased food availability.

The assessment endpoint is equivalent to that for macroinvertebrates (see Section 2.6). The entities of concern are fish. The attribute is local extirpation of species from streams in their natural range. Fish are ecologically and socioeconomically important. In addition, they have been shown to be affected by elevated SC. In a study of the South Fork of Tenmile Creek in southwestern Pennsylvania, Kimmel and Argent (2010) assessed the fish assemblage along a SC gradient. At two sites where SC levels exceeded 1,200 µS/cm, the fish assemblage included only

Ambloplites rupestris, *Hypentelium nigricans*, *Lepomis cyanellus, Micropterus dolomieu*, *Moxostoma erythrurum*, and *Notropis volucellus*, all of which are freshwater fish that are tolerant of elevated SC with XC_{95} values of >2,122 to >3,594 μS/cm based on analyses in this assessment.

The same field-based method was applied to fish as to benthic macroinvertebrates (see main document Section 3). As with macroinvertebrates, the field data represent realistic exposures of actual fish communities to the actual mixture of ions found in the regions (see Section 2.3). In addition, because the purpose of this assessment is to determine the sensitivity of fish relative to macroinvertebrates, it is appropriate to use the same methods for deriving effect levels (i.e., XC_{95} values). Because fish are reliably reported as species, species-level XC_{95} values are calculated as well as genus-level values.

These supplementary fish analyses use a combined data set for fish from four contiguous ecoregions. This was necessary in order to be able to reasonably derive XC_{95} values for more fish species, a total of 101. Because the values are for species, rather than for genera, the species-level XC_{95} is not affected by variance among species within a genus. The regions are Level III Ecoregions 67 (Ridge and Valley), 68 (Southwestern Appalachians), 69 (Central Appalachians), and 70 (Western Allegheny Plateau; see Figure G-1; [U.S. EPA, 2007; Omernik, 1987; Woods et al., 1996, 2002, 2007]). Portions of these ecoregions are located in seven states: Kentucky, West Virginia, Virginia, Ohio, Maryland, Pennsylvania, and New Jersey. They are characterized by mountain ridges and valleys underlain by sedimentary rock formations and by extensive areas of forest and agriculture with few large metropolitan areas (i.e., Pittsburgh, PA, and Charleston-Huntington, WV). At the Level II ecoregion, these four ecoregions are placed in the Ozark, Ouachita-Appalachian Forests ecoregion (Wilken et al., 2011), while physiographically these ecoregions are placed in the Ridge and Valley and the Appalachian Plateau provinces of the Appalachian Highlands (Fenneman, 1938). Larger-scale land disturbance is the result of forestry, some agriculture, and resource extraction, primarily coal mining.

These ecoregions are broadly similar in terms of water chemistry and quality, resident fish assemblages, and sources of SC owing to the type of underlying sedimentary rock formations and the unglaciated geological history of the regions. Therefore, like the macroinvertebrate case examples, these fish analyses are relevant to flowing waters with increased loadings of ionic mixtures dominated by salts of calcium (Ca^{2+}) plus magnesium (Mg^{2+}), and sulfate (SO_4^{2-}) plus bicarbonate (HCO_3^-).

G.2.1. Data Sources

The data set for fish was assembled from several sources because no single data set provided sufficient data for the analysis. Data available for this analysis included results from studies conducted by the U.S. Environmental Protection Agency (EPA), either as part of the Environmental Monitoring and Assessment Program (EMAP) or for the Environmental Impact Statement (EIS) for mountaintop mining and valley fills; the West Virginia Department of Environmental Protection (WVDEP), as part of a pilot bioassessment program; the Kentucky Department for Environmental Protection, Division of Water (KDEP-DOW); Ohio Environmental Protection Agency, Division of Surface Water (OEPA-DSW), as part of their bioassessment programs; and the Pennsylvania Fish and Boat Commission (PFBC) as part of their stream fisheries assessment program.

The eight data sets used in this study to calculate the XC_{95}s for fish are:

1. The Mid-Atlantic Highlands Assessment conducted by the EPA's EMAP from 1993 to 1996 (*n* = 172 sites),

2. The Mid-Atlantic Integrated Assessment conducted by the EPA's EMAP in 1997 and 1998 (*n* = 119 sites),

3. Fish bioassessment data collected by the KDEP-DOW as part of their stream bioassessment program from 1991 to 2004 (*n* = 285 sites),

4. Fish and chemistry data collected by Stauffer and Ferreri (2002) and Bryant et al. (2002) from 1999 to 2001 as part of the Programmatic EIS for mountaintop mining and valley fills (*n* = 34 sites),

5. Fish and chemistry data collected by EPA's Regional Applied Research Effort (RARE) program in cooperation with the West Virginia Department of Natural Resources (WVDNR) in 2001 and 2002 (Detenbeck et al., 2005; *n* = 118 sites),

6. Fish bioassessment data collected by the WVDEP from 2007 to 2009 (*n* = 43 sites),

7. Fish bioassessment data collected by the OEPA-DSW as part of their stream bioassessment program from 1999 to 2013 (*n* = 593 sites), and

8. Fish survey data collected by PFBC as part of their stream fisheries assessments from 1990 to 2014 (n = 2,101) sites.

Fish survey data, along with chemical and physical data, were collected from a total of 3,465 distinct sites during the sampling years 1990−2014. The EMAP (i.e., 1st and 2nd data sets), RARE (i.e., 5th data set), and WVDEP (i.e., 6th data set) sites were probability sites selected as part of regional surveys (Herlihy et al., 2000; Detenbeck et al., 2005; Smithson, 2007), and those sampled by Stauffer and Ferreri (2002), KDEP-DOW, OEPA-DSW, and PFBC (i.e., 4th, 5th, 7th, and 8th data sets) included targeted-sampling sites (e.g., above and below permitted outfalls such as wastewater treatment plant, or as general surveys of fish occurrences) that were part of bioassessment studies. All sites were not dry at the time of sampling but may be intermittent at other times.

Most sites in the parent data sets were sampled once, but some sites were revisited and sampled one or more times. Data from only the most recent visit to a site was used in these analyses. Sites were not identified as “least disturbed” or reference sites. However, at least 134 sites were in catchments described as >90% forested, one characteristic often used to identify reference site. Water quality, habitat, and fish data (both raw data and calculated metrics) were collected as part of these regional bioassessment surveys.

Quality assurance and standard procedures are described by Lazorchak et al. (1998), U.S. EPA (1987), KDEP-DOW (2009a, b, c, 2010), Stauffer and Ferreri (2002), Bryant et al. (2002), WVDEP (2009), OEPA-DSW (1989a, b, 2013a, b), and Pennsyvania Department of Environmental Protection (2013).

G.2.2. Data Set Characteristics

Biological sampling usually occurred once from March through November with fish sampling protocols designed to collect all except very rare species. Table G-1 provides summary statistics for ion concentrations and other parameters for the 3,277 observations in the combined data set used in the analyses. The results of this analysis are relevant to waters with a similar composition.

Data from 3,277 sites out of 3,465 total sites from Ecoregions 67, 68, 69, and 70 (see Figure G-1) were used in the calculation of the XC_{95} values for fish (see Table G-2). Data from a sampling event at a site were *excluded* from the analysis if they lacked a SC measurement (*n* = 62; see Table G-3). Observations from 26 sites were excluded where no fish were collected in order to minimize bias from sites that were too small to support fish. To prevent potential confounding by the effects of acid mine drainage or acid deposition, 102 sites with a pH <6 were excluded from the analysis (see Table G-3). All analyses represent waters having a pH between 6.0 and 9.5. These circumneutral waters are within the range of low or high pH conditions tolerated by most fish. Because many of the observations lacked data about ionic concentration, we did not exclude sites where $[Cl^-] \geq ([SO_4^{2-}] + [HCO_3^-])$ in mg/L. Inspection of the few sites that were dominated by chloride indicated that these sites generally had very low total ionic concentrations and SC and therefore were not chloride dominated due to salt inputs.

Table G-1. Summary statistics of the water quality parameters from the eight combined data sets described in Section G.2.1

Parameter	Units	Minimum	25th centile	Median	75th centile	Maximum	Mean	Valid *n*
Specific conductivity	µS/cm	9.4	84.0	217	430	4,000	328	3,277
Hardness	mg/L	0.00	20.0	42.0	118	772	83.4	1,488
Alkalinity	µeq/L	6.28	983	1,960	3,160	7,670	2,120	995
Bicarbonate (HCO_3^-)	µeq/L	0.00	887	1,910	3,120	7,680	2,060	1,014
Sulfate (SO_4^{2-})	µeq/L	44.4	365	1,000	3,160	52,900	3,240	1,014
Calcium (Ca^{2+})	µeq/L	29.9	1,100	2,150	3,660	18,300	2,900	1,029
Magnesium (Mg^{2+})	µeq/L	28.8	637	1,150	1,970	21,600	1,810	917
Sodium (Na^+)	µeq/L	4.35	223	478	1,070	27,900	1,160	877
Potassium (K^+)	µeq/L	6.39	51.2	76.7	102	1,240	87.0	872
Chloride (Cl^-)	µeq/L	0.726	139	310	673	8,610	587	1,035
Iron (Fe), total	µg/L	1.00	10.0	36.3	110	2,690	143	369
Nitrate (NO_3^-)	µg/L	6.00	125	298	794	875,000	2,270	1,099
Nitrogen (N), total	µg/L	45.0	210	436	860	875,000	2,400	956
Aluminum (Al), total	µg/L	1.00	6.00	16.0	31.0	1,060	52.8	360
Manganese (Mn), total	µg/L	1.10	10.0	20.0	82.0	2,090	82.6	367
Phosphorus (P), total	µg/L	1.0	6.0	13.0	24.0	971	28.1	532
Selenium (Se), total	µg/L	1.0	1.0	2.0	3.0	1,300	98.9	85

Table G-1. Summary statistics of the water quality parameters from the eight combined data sets described in Section G.2.1 (continued)

Variable	Units	Minimum	25th centile	Median	75th centile	Maximum	Mean	Valid *n*
Dissolved oxygen (O_2)	mg/L	1.2	7.3	8.6	9.6	18.6	8.5	822
pH	Standard units	6.00	6.90	7.31	7.80	9.50	7.36	3,190
Water temperature	ºC	0.4	14.0	17.0	19.7	31.0	16.7	2,601
[a]RBP habitat score (rbp score)	Unitless	38	75	114	139	191	111	801
Catchment area	km^2	0.111	11.47	28.79	88.70	18,640	272	1,280

[a]RBP (Rapid Bioassessment Protocol, Barbour et al., 1999).

Table G-2. Number of samples with reported fish species and specific conductivity meeting the acceptance criteria for calculating the hazardous concentration (HC_{05}). The number of sites is presented for each month and ecoregion.

Level III ecoregion	Number of Samples per Month												Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
67	0	2	89	75	85	383	325	325	97	23	12	3	1,419
68	0	0	0	3	0	4	9	15	6	2	0	0	39
69	1	0	12	51	33	175	231	170	70	60	5	0	808
70	1	0	7	9	29	237	332	250	93	52	0	1	1,011
Total	2	2	108	138	147	799	897	760	266	137	17	4	3,277

Table G-3. Observations excluded from the original data sets before analysis

Characteristic	Exclusion level	*n* of observations excluded
Specific conductivity	No measurement	58
No fish were collected	0	26
pH	<6	102

Observations were also *excluded* from calculations if the fish were not identified to the species level. Such fish were generally immature specimens, and identifiable mature specimens of the species were generally present in the same sample. No fish were observed that were not considered to be freshwater species. Species observed at fewer than 25 sampling locations in the aggregated ecoregions were excluded to ensure reasonable confidence in the evaluation of the relationship between SC and the observation of a species. Although stocking could raise the XC_{95} estimates, the native salmonid species, brook trout (*Salvelinus fontinalis*), was included even though the effect of stocking is not known. Two nonnative salmonids, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) were included because some trout populations are established in the region and they are recreationally important; however, it is uncertain how stocking may have affected the estimation of their XC_{95} values. Although common carp (*Cyprinus carpio*) is an invasive species, it was included because it has become irreversibly established in the region.

In the combined database, 210 fish species were identified of which 101 species were observed in at least 25 sites. The four ecoregions had 36 of these 101 species in common, with 76 species in Ecoregion 67, 47 species in Ecoregion 68, 97 species in Ecoregion 69, and 86 species in Ecoregion 70.

The calculation of XC_{95} values uses weighted observations of a species to adjust for uneven sampling along the SC exposure gradient (see Figure G-2). Because the distribution and therefore the observation of fish species are affected by biogeography (Hocutt and Wiley, 1986; Stauffer et al., 1995) and stream size (McCormick et al., 2001), the number of sites used to weight the observations of a species to estimate the XC_{95} values was restricted to the number of sampled sites in river systems with catchment areas in which a species is likely to occur.

Freshwater fish have a limited ability to disperse among river systems, particularly among larger river systems that drain separately to the ocean (Stauffer et al., 1995). The case study region includes several river basins that each drain separately to the Chesapeake Bay or Atlantic Ocean (i.e., Delaware, Susquehanna, Potomac, James, and Roanoke River basins) or to the Tennessee River and Ohio River basins, which are major tributaries of the Mississippi River, and the distributions of some fish species are limited to one or more but not all of these river basins (Stauffer et al., 1995). To prospectively account for these factors, the range of stream sizes (based on the $\log_{10}$-transformed catchment area [km^2]) and river basins (based on 4-digit hydrological unit codes [HUCs] from the data set) were identified where fish species collected from at least 25 sites were observed. Prior to calculating weights and XC_{95} for each fish species or genus, the data set was subsetted by excluding any stream sites where that fish species was unlikely to occur because the stream was too small or too large or because the stream was in a river basin outside the distribution of that species. Specifically, the data set was subsetted for each species to include sites in 4-digit HUCs where the species was observed in the data set and to exclude sites in catchments greater than the maximum and less than the minimum size where the fish species were observed (see Table G-4).

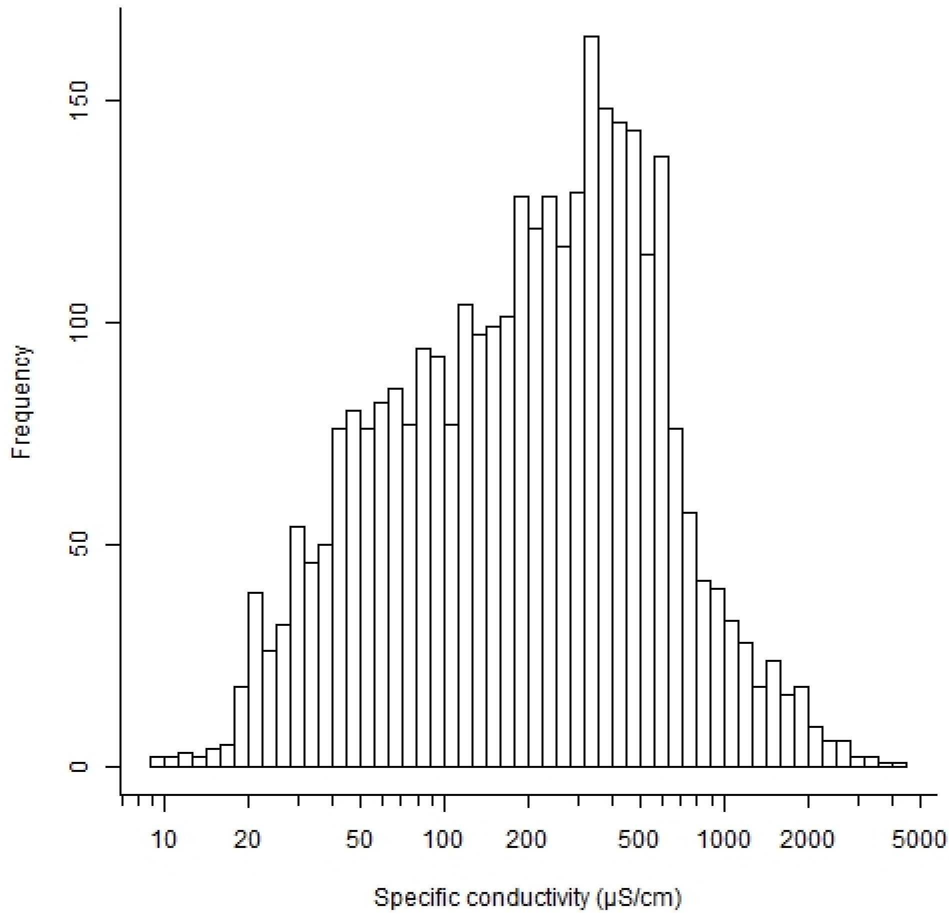


Figure G-2. Histogram of the overall sampling frequencies of observed specific conductivity values in samples from Ecoregions 67, 68, 69, and 70 from March through November. Histograms were customized for each fish species prior to assigning weights. More of the sampled sites were near the median than at the extremes. Specific conductivity values are corrected to 25°C.

G.2.3. Inclusion of Reference Sites

If high-quality (i.e., reference) sites were not included in the data set, effects on salt-intolerant species would not be incorporated into the HC_{05}, because the lower end of the XCD would be excluded. In this case example, the data sets contained an uncertain number of reference sites; but there are at least 134 sites with >90% forest cover which are more likely to be representative of good to high quality stream systems than those with less forest cover.

G.2.4. Inclusion of Listed Species

A number of species were observed that are listed as threatened or endangered by the states in the region (CP, 2013; KDFWR, 2013; MDNR-NHP, 2010; ODNR-DW, 2014; VADGIF, 2014; WVDNR, 2012). One federally-listed species, blackside dace (*Chrosomus cumberlandensis*), was observed at 10 sites. Among species observed at ≥25 sites, 6 are state-listed as threatened: *Chrosomus erythrogaster* (Pennsylvania), *Cyprinella whipplei* (Virginia), *Minytrema melanops* (Pennsylvania), *Notropis atherinoides* (Virginia), *Percina caprodes* (Maryland), and *Salvelinus fontinalis* (Ohio). Five are state-listed as endangered: *Etheostoma variatum* (Virginia), *Lepomis gulosus* (Pennsylvania), *Lepomis megalotis* (Pennsylvania), *Lythrurus umbratilis* (Pennsylvania), and *Noturus flavus* (Maryland). Although neither West Virginia nor Kentucky state-list species as threatened or endangered, these states list *Percina macrocephala* (Kentucky) as critically imperiled and *Clinostomus elongatus* (West Virginia), *Cottus carolinae* (West Virginia), *Etheostoma olmstedi* (West Virginia), and *Luxilus cornutus* (West Virginia) as imperiled.

G.2.5. Ionic Composition

The fish HC_{05} was calculated for a relatively uniform mixture of ions in those streams with salts generally dominated by SO_4^{2-} plus HCO_3^- anions (mg/L) at circumneutral to mildly alkaline pH (6−10). Although Cl^- may represent more than half of the anions in the mixture at some sites, the use of the fish HC_{05} value in Cl-dominated waters is untested and may or may not be appropriate. However, for the circumneutral to alkaline streams, chloride was rarely the dominant anion and the four primary ions (Ca^{2+}, Mg^{2+}, SO_4^{2-}, and HCO_3^-) are highly correlated with SC (see Figures G-3−G-5). In these figures, Spearman rank correlation was used because no assumptions were made about the distributions of these variables. For the same reason, a nonparametric method, locally weighted scatter plot smoothing line, was used to visualize the relationship between each pair of variables. Span is the proportion of the data points used to define the regression weight functions used to determine the smoothed values.

G.2.6. Matrices of Scatter Plots and Absolute Spearman Correlation Coefficients

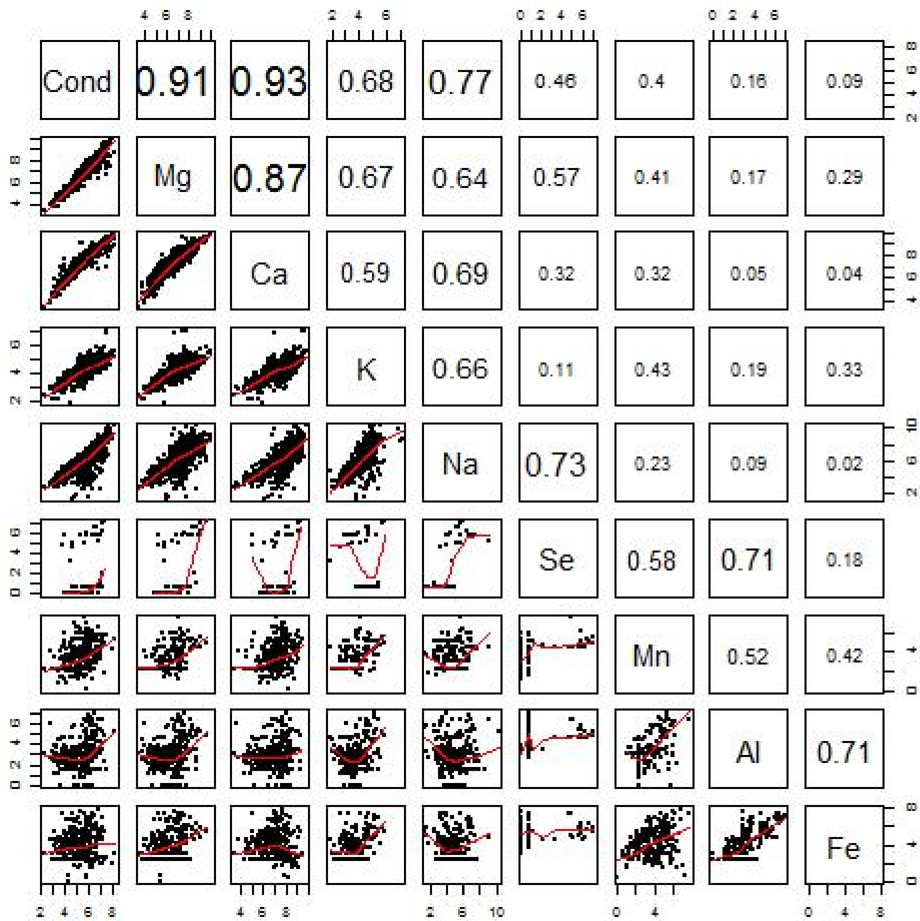


Figure G-3. Cations and metals. Matrix of scatter plots and absolute Spearman rank correlation coefficients between specific conductivity (µS/cm), calcium (Ca, µeq/L), magnesium (Mg, µeq/L), sodium (Na, µeq/L), potassium (K, µeq/L), total aluminum (Al, mg/L), total manganese (Mn, mg/L), total iron (Fe, mg/L), and total selenium (Se, mg/L) in the streams of Ecoregions 67, 68, 69, and 70 in the Appalachians. Each variable is transformed by its natural logarithm. The red lines are the locally weighted scatter plot smoothing lines with a span of 0.67.

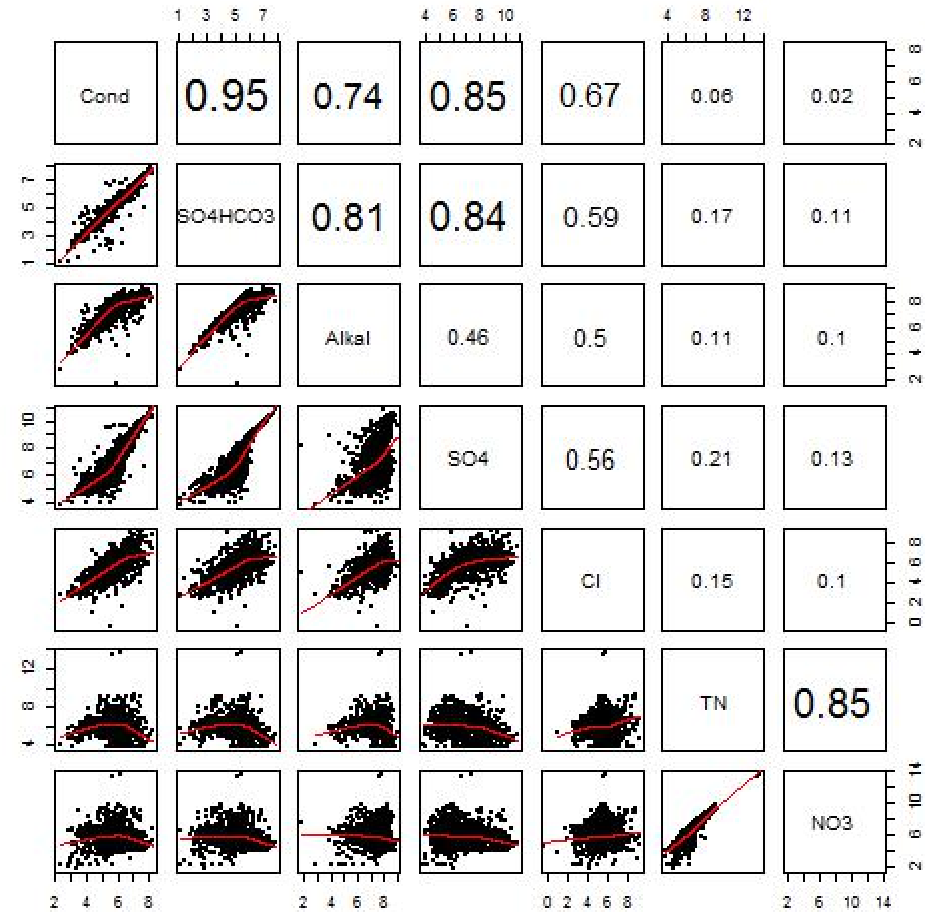


Figure G-4. Anions and nutrients. Matrix of scatter plots and absolute Spearman rank correlation coefficients between specific conductivity (µS/cm), chloride (Cl, µeq/L), sulfate (SO_4, µeq/L), nitrate (NO_3, µg/L), total nitrogen (TN, µg/L), alkalinity (alkal, µeq $CaCO_3$/L), and sulfate + bicarbonate (SO_4HCO_3, mg/L) in the streams of Ecoregions 67, 68, 69, and 70 in the Appalachians. Each variable is transformed by its natural logarithm. The red lines are the locally weighted scatter plot smoothing lines with a span of 0.67.

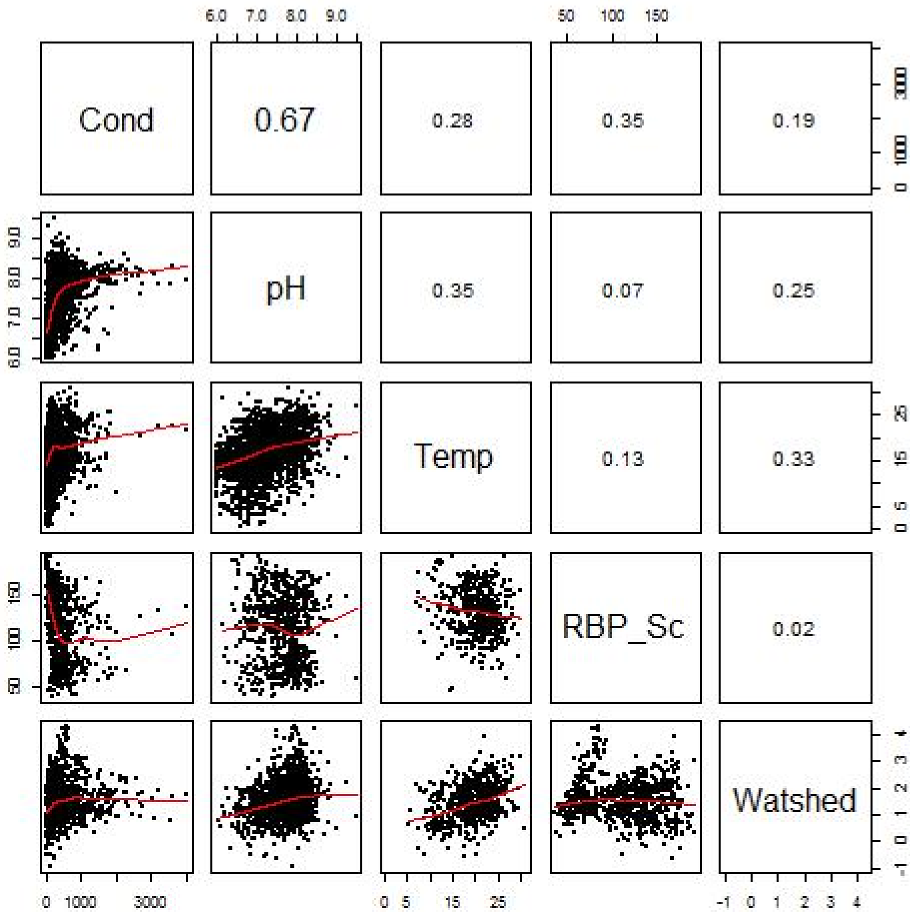


Figure G-5. Other water quality variables. Matrix of scatter plots and absolute Spearman rank correlation coefficients between specific conductivity (μS/cm) and other environmental variables in the streams of Ecoregions 67, 68, 69, and 70 in the Appalachians. The red lines are locally weighted scatter plot smoothing lines with a span of 0.67. The RBP_Sc is the rapid bioassessment protocol habitat score (possible range from 0 to 200); watshed is the logarithm transformed catchment area (km^2), temperature is in °C, and pH is in standard units.

G.3. ANALYTICAL METHODS

G.3.1. Derivation of Hazardous Concentration (HC_{05}) Values

The derivation of the HC_{05} value for fish follows the draft field-based method for macroinvertebrates for SC (see Section 3.1 and U.S. EPA [2011a]). First, the effect endpoint value (XC_{95} value) for each fish species and genus is derived. Second, the XC_{95} values are used to generate a species or genus XCD, and the HC_{05} value is derived from the XCD. The statistical package R, Version 3.1.2 (October 2014), was used for all statistical analyses (R Development Core Team, 2011).

G.3.1.1. *Estimating Confidence Bounds for the Hazardous Concentration (HC_{05})*

The purpose of this analysis is to characterize the uncertainty by calculating confidence bounds on the HC_{05} value. The draft field-based method described in Section 3.1 for deriving SC criteria was modified for fish because the sample size and weights were different for each species. Bootstrap estimates of the XC_{95} were derived for each species used in the derivation of the HC_{05} by resampling 3,277 times (the number of sites in the data set) with replacement (see Figure G-6; Efron and Tibshirani, 1993). For each bootstrap sample, the XC_{95} for each species and the HC_{05} were calculated by the same method applied to the original data (see Section 3.1). That process was repeated 1,000 times to create distributions of XC_{95} and HC_{05} values. These distributions were used to calculate a two-tailed, 95% confidence interval (CI) for each fish species.

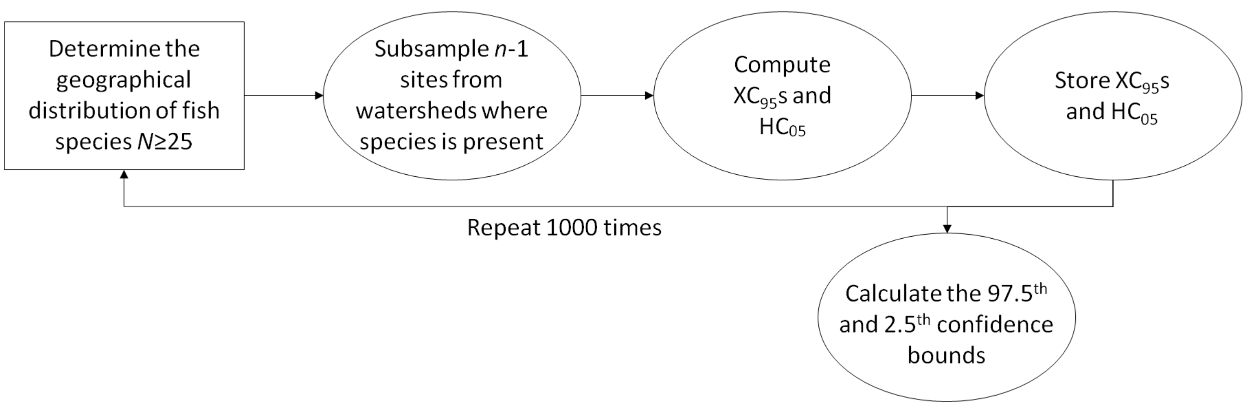


Figure G-6. Flowchart of bootstrapping procedure used to derive confidence limits for the specific conductivity hazardous concentration (HC_{05}) for fish only. Here, a watershed is as a four-digit HU from which a species was collected at least once in these data sets within the range of watershed areas where the species was collected.

G.3.2. Sensitivity Analyses

HC_{05} values and their associated uncertainties are influenced by the number of species and by the number of sites sampled. The number of sites that are sampled affects the number of species that occur with a sufficient number of observations to reliably estimate an XC_{95}. More species helps ensure representativeness of salt-intolerant taxa in the XCD and hence a protective HC_{05}.

G.3.3. Effect of Minimum Number of Observations for Inclusion of a Species

The HC_{05} was calculated using different sample size requirements for inclusion of a species. As the minimum number of observations of a species required for inclusion in the data set increases, fewer species are included in the XCD and the HC_{05} increases (see Figure G-7). The HC_{05} may increase greatly when a taxon in the lower 5th centile is removed because it does not meet the minimum number of samples. Then, the HC_{05} decreases as species with XC_{95} values greater than the 5th centile are removed because they do not meet the minimum number of samples. The number of samples in the data set affect the number of a species included in the XCD and therefore it affects the HC_{05} in the same way that the number of observations does. In order to have >90 species and reliable estimate the XC_{95}, a minimum of 25 observations was selected.

G.3.4. Effect of Minimum Number of Sampled Sites

To evaluate the effect of the number of sites that were sampled and its effect on the number of species and consequently its effect on the HC_{05}, 1,000 XCDs, the number of species in each XCD, and their median from 1,000 HC_{05}s were estimated by bootstrapping for data set sample sizes of 100 to 3,000 site. This process is similar to the method used to calculate confidence bounds on the HC_{05} values (see Figure G-6). For data set sample size, data sets with 100 to 3,000 sites (1,000 samples each) were randomly picked with replacement from the original 3,277 samples. From each bootstrap data set, the XC_{95} was calculated for each species by the same method applied to the original data, and the HC_{05} was calculated. The uncertainty in the HC_{05} value was evaluated by repeating the sampling and HC_{05} calculation 1,000 times for each data set sample size. The distribution of 1,000 HC_{05} values was used to generate a median HC_{05} and two-tailed, 95% confidence bounds on these bootstrap-derived values.

As shown in Figure G-8 for this data set, the CI for the HC_{05} decreases with increasing data set sample sizes, and the range of the number of species also increases. Therefore, the original data set was considered adequate for estimating the example criterion continuous concentration (CCC) with 101 species represented in the XCD. The larger number of samples may be required because there are fewer species in a sample than in invertebrate samples.

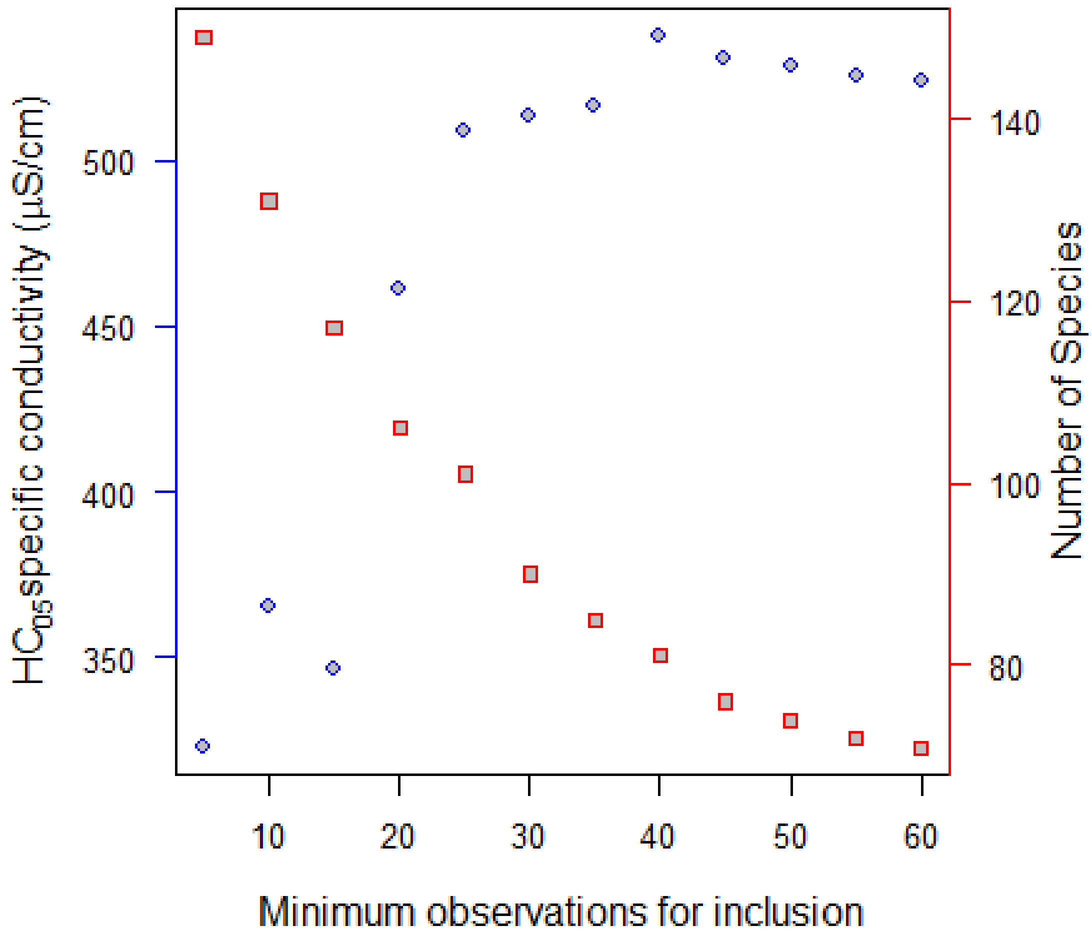


Figure G-7. Relationship of the minimum number of observations for inclusion of a species on the number of species included in the extirpation concentration distribution (XCD) and on the hazardous concentration (HC_{05}) based on the fish data set. Estimates of HC_{05} values (blue circles, left *y*-axis) and the number of species in the XCD (red squares, right *y*-axis) based on minimum number of observations required for inclusion in the XCD (5−60, *x*-axis). As the minimum number of observations of a species increases, fewer are included in the XCD and the HC_{05} rapidly increases to a temporary plateau at approximately 25.

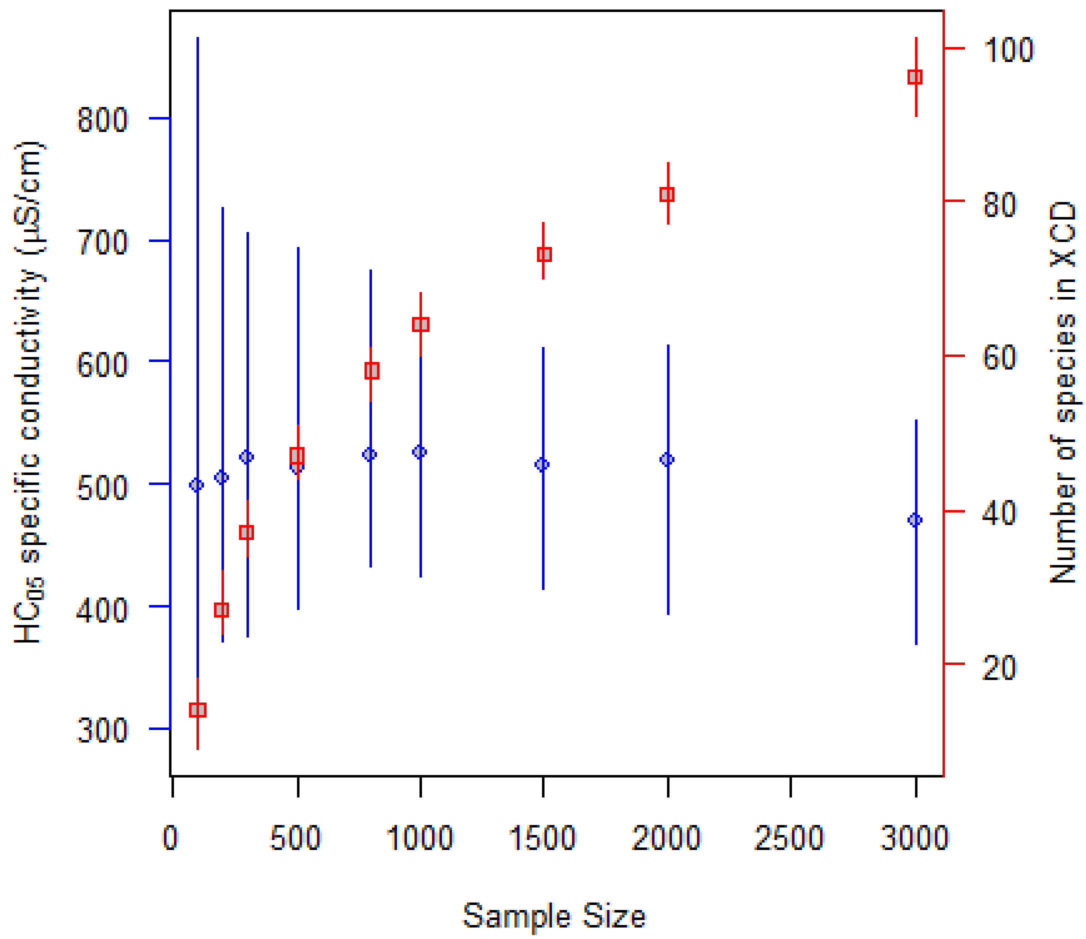


Figure G-8. Adequacy of the number of samples used to model the hazardous concentration (HC_{05}). As sample size increases the number of species included in the extirpation concentration distribution (XCD) increases (squares). As sample size increases, the confidence bounds on the HC_{05} decrease, and the mean HC_{05} confidence interval becomes fairly constant at ≥1,000 sites (circles) and 75−90 species evaluated (squares). Specific conductivity values are corrected to 25°C.

G.4. RESULTS

G.4.1. Extirpation Concentrations

Table G-5 presents the XC_{95} values for all fish species that were observed at a minimum of 25 sampling sites in the combined four ecoregions. That table also presents the genus XC_{95} values for those fish genera for which there were more than one species. Species that were observed at least once in an ecoregion are designated by the ecoregion's number in Tables G-4 and G-5. Multiple SC samples from stations were not available to evaluate whether the HC_{05} represents an annual or a maximum SC value.

Section G.8 provides the Generalized Additive Model (GAM) plots that show the distributions of observations with respect to SC for each species of fish, and Section G.9 presents the cumulative distribution functions (CDFs) used to derive the XC_{95} values. Each GAM plot was used to model the likelihood of a taxon being observed with increasing SC (Hastie and Tibshirani, 1986), and the GAM confidence bounds were used to assign qualifying designations of “approximately” or “greater than” to the calculated values (see Section 3.1.2.1).

Of the 101 XC_{95} values calculated from the combined regional data set, 86 species were observed in the analyzed data set in Ecoregion 70, 97 in Ecoregion 69, 47 in Ecoregion 68, and 76 in Ecoregions 67. The higher density of sites in Ecoregions 69 and 70 may account for some of these differences. One fish species had an XC_{95} value less than the macroinvertebrate derived example CCC in Case Example II, Ecoregion 70.

G.4.2. Extirpation Concentration Distributions (XCDs) and Hazardous Concentration (HC_{05})

An XCD for fish is derived from XC_{95} values for 101 species (see Figure G-9). The HC_{05} is 509 μS/cm (95% CI 355−534 μS/cm).

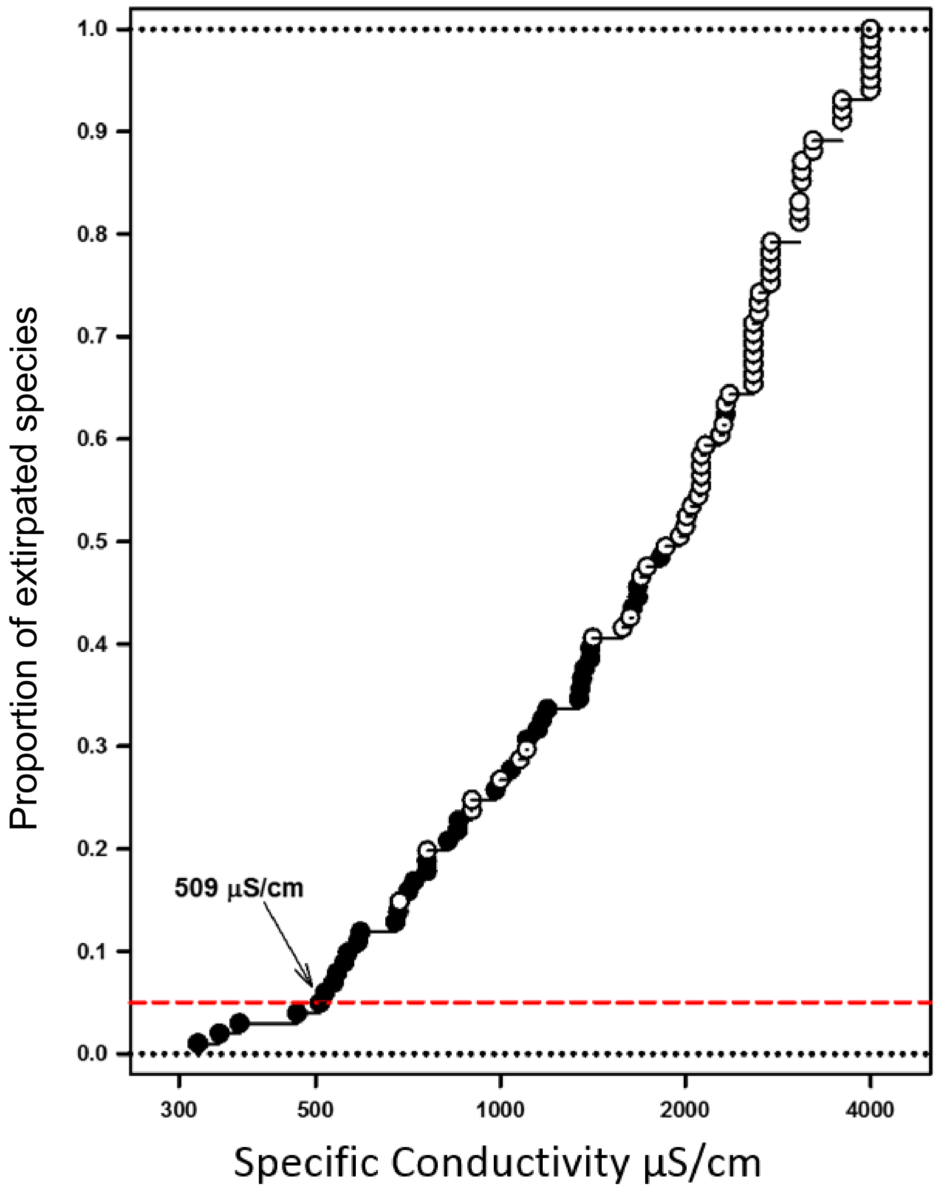


Figure G-9. The extirpation concentration distribution for fish using a combined data set from Ecoregions 67, 68, 69, and 70. The hazardous concentration (HC_{05}; 509 μS/cm, 95% confidence interval [CI] 355−534 μS/cm) is the specific conductivity value at the intercept of the extirpation concentration distribution (XCD) with the horizontal, hashed, red line at the 5th centile. Extirpation concentration (XC_{95}) with an approximate or greater than designation are shown as open circles.

G.4.3. Validation of the extirpation concentration distributions (XCD) Model

The XCD model was validated and uncertainty around the HC_{05} values was estimated using bootstrapping, as recommended by the EPA Science Advisory Board in their review of the

EPA *Benchmark Report* (U.S. EPA, 2011b). The similarity between the two HC_{05} values suggests that a similar model would be generated using an independent data set (see Figure G-10). However, validation with an independent data set is preferred.

Confidence bounds represent the potential range of HC_{05} values using the XCD approach, given the data and the model. Conceptually, these confidence bounds may be thought of as representing the potential range of HC_{05} values that one might obtain by returning to the region and resampling the streams. The contributors to this uncertainty include measurement variance in SC and sampling variance in the location for monitoring, collecting, and enumerating fish. Variance due to differences in stream reaches, weather, and other random factors is also included. Unlike the bootstrapped XCDs for macroinvertebrates (e.g., see Sections 4.5.2 and 5.5.2), the confidence bounds in the analyses for fish characterizes some additional potential systematic sources of variance, such as differences between geographic areas and different organizations performing the sampling.

Significant variation is observed in the salt-intolerance of different species within fish genera. For example, the XC_{95} values among the ten species of the darter genus, *Etheostoma*, range from 322 μS/cm (*E. baileyi*) to >4,000 μS/cm (*E. caeruleum*; see Figure G-11, 41st genus in XCD). The macroinvertebrate data used to develop the example criteria (see Case Studies I and II) were identified to genus because of practical difficulties with the identification of insect nymphs to the species level; as a result, macroinvertebrate species variability within a genus could not be assessed. The genus level XC_{95} tends toward the high end of the range of XC_{95} values for species, suggesting that the XC_{95} at the genus level represents the XC_{95} among the most salt-tolerant species in the genus.

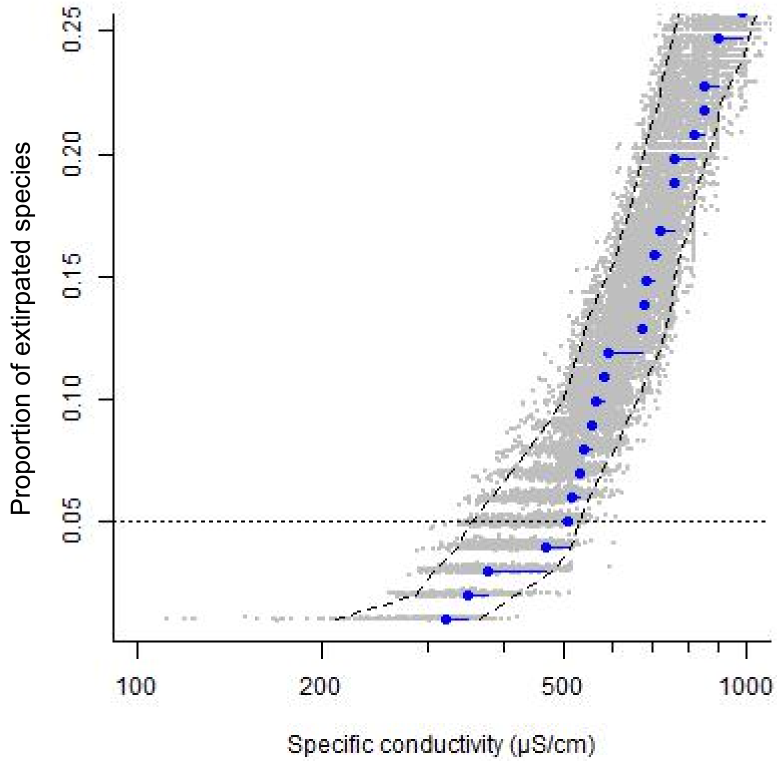


Figure G-10. Cumulative distribution of the extirpation concentration (XC_{95}) values for the 25 most salt-intolerant fish species (blue circles) and 95% confidence intervals (CI) (dotted lines) based on 1,000 extirpation concentration distributions (XCD) bootstrapping results. Each small gray dot represents an XC_{95} value for a bootstrapping iteration (note that the species in each percentage varies with each XCD iteration). Each larger dark dot represents the calculated XC_{95} of the XCD. The median bootstrapped hazardous concentration (HC_{05}) is 456 µS/cm (95% confidence interval is 355−534 µS/cm).

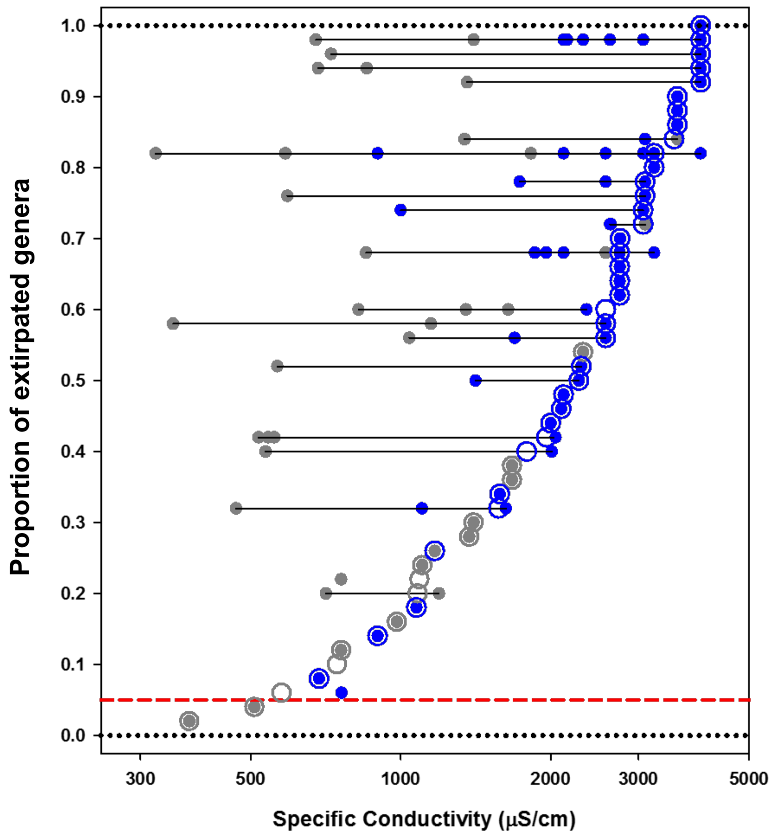


Figure G-11. The genus-level extirpation concentration distribution (XCD) for fish for March through November. The genus-level extirpation concentrations (XC_{95}) of the 50 fish genera observed ≥25 times (open circles) are depicted with the species-level XC_{95} value for the 101 fish species observed ≥25 times (small solid circles), although some species are obscured by plotting at the same location. For visualization, the horizontal lines connect fish species in the same genus. In the case of the 6th genus, none of its constituent species were observed ≥25 times. The XC_{95} values for many of the 19 genera with 2 or more species observed ≥25 times are close to the constituent species with the greatest XC_{95} value. The gray solid circles indicate *species* XC_{95} values assigned without special designation or as an approximation to the specific conductivity value, while blue solid circles indicate a species XC_{95} value that is greater than the assigned value. The gray open circles indicate a *genus* XC_{95} value assigned without special designation as an approximate XC_{95} value, while blue open circles indicate a genus XC_{95} that is greater than the assigned value. Genera with a solid circle nested inside an open circle with no line were represented by only one fish species. The horizontal dashed red line is at the 5th centile (545 µS/cm) of the genus-level XC_{95} values. Genera XC_{95} values are higher than species.

G.4.4. Geographic Applicability

Extirpation of fish associated with ionic stress was assessed in four adjoining ecoregions (Ecoregions 67, 68, 69, and 70). The water chemistry in these four ecoregions is similar because of the underlying sedimentary rock formations and the unglaciated geological history of the region (Griffith, 2014). Although the analysis for fish is from a composite data set of several ecoregions, identification to species ensures that the XC_{95} values are not influenced by different sensitivities of species of a genus occurring in different geographical locations. Therefore, an XC_{95} value and its confidence bounds represent the effect level of a species regardless of where it is exposed to sulfate plus bicarbonate dominated waters. The XCD from which the HC_{05} is derived, is a model of how freshwater fish species, in general, respond to ionic stress. The HC_{05}, therefore, estimates the SC at which 5% of fish species are extirpated (509 µS/cm, 95% CI 355−534 µS/cm) in geographic areas with similar low natural background SC, in this example, 84 µS/cm (95% CI 80−90 µS/cm).

G.4.4.1. *Seasonality and Life History*

Fish have multiple-year life spans, and adults, at least, can be captured by electrofishing or seines and will be present throughout the year. As a result, most fish species are likely to be detected in all seasons if present in observable numbers in a stream.

G.4.5. Treatment of Potential Confounders

The analysis of confounding begins by identifying environmental variables that are possible confounders that can be analyzed. Possible confounding stressors for the fish XCD include: pH, catchment area, habitat, organic enrichment/nutrients, temperature, dissolved oxygen (DO), selenium, and metals. Low pH, was known to cause effects and was controlled by removing sites with pH <6 (see also Section 3.1.1.2.6). Metals were not analyzed because data were available only for total concentration. Selenium was not analyzed because most measurements were below detection limit and the number of Se measurements was small. The other possible confounders either were evaluated by removing samples with levels of a potential confounder that may cause adverse effects and then developing XC_{95}s and HC_{05} values. Potential confounding was evaluated by the position of the XCD and the overlap of the HC_{05} CIs of the constrained data set relative to the original fish data set.

G.4.5.1. *Influence of Catchment Area, Habitat, Dissolved Oxygen and Temperature on the Hazardous Concentration (HC_{05})*

To assure that the XCD model was detecting effects from SC and not a response to poor habitat or small catchment area, samples with potentially harmful levels of four potential confounders were removed from the example criterion data set: a rapid bioassessment protocol (RBP) score <135, catchments <10 km^2, DO <4 mg/L, and temperature >22°C. The threshold of RBP <135 was the same thresholds as for invertebrates in Case Study I, Appendix A (Gerritsen et al., 2000). Because the samples sizes would be too small for simultaneous analysis, four constrained data sets were prepared.

Removal of samples with poor habitat, small catchments, low DO, and higher temperature sites from the data set had little effect on the XCD model or HC_{05}. With the data set constrained to sites with an RBP >135, the HC_{05} was 464 µS/cm (95% CI 368−582 µS/cm). A lower HC_{05} is converse to what is predicted with less combined stress (see Figure G-12). When the data set was constrained to catchment area >10 km^2, the HC_{05} was 519 µS/cm (95% CI 360−578 µS/cm) which is very similar to the HC_{05} from the unconstrained data set (see Figure G-13). Removing samples with dissolved oxygen <4 mg/L, resulted in an HC_{05} values of 509 µS/cm (95% CI 358−534 µS/cm) which is very similar to the HC_{05} from the unconstrained data set (see Figure G-14). After removing samples with a temperature >22°C, the HC_{05} was 548 µS/cm (95% CI 435−610 µS/cm; see Figure G-15). The slightly higher value in the predicted direction for less stress suggests that there is potentially some confounding by temperature. For more precise XC_{95} values and analysis and correction for temperature may be useful, however, the correction itself may create error. The confidence intervals for all constrained data sets included the HC_{05} for fish from the entire data set (509 µS/cm 95% CI 355−534 µS/cm). Therefore, no correction was made for habitat quality, catchment area, low DO, or temperature.

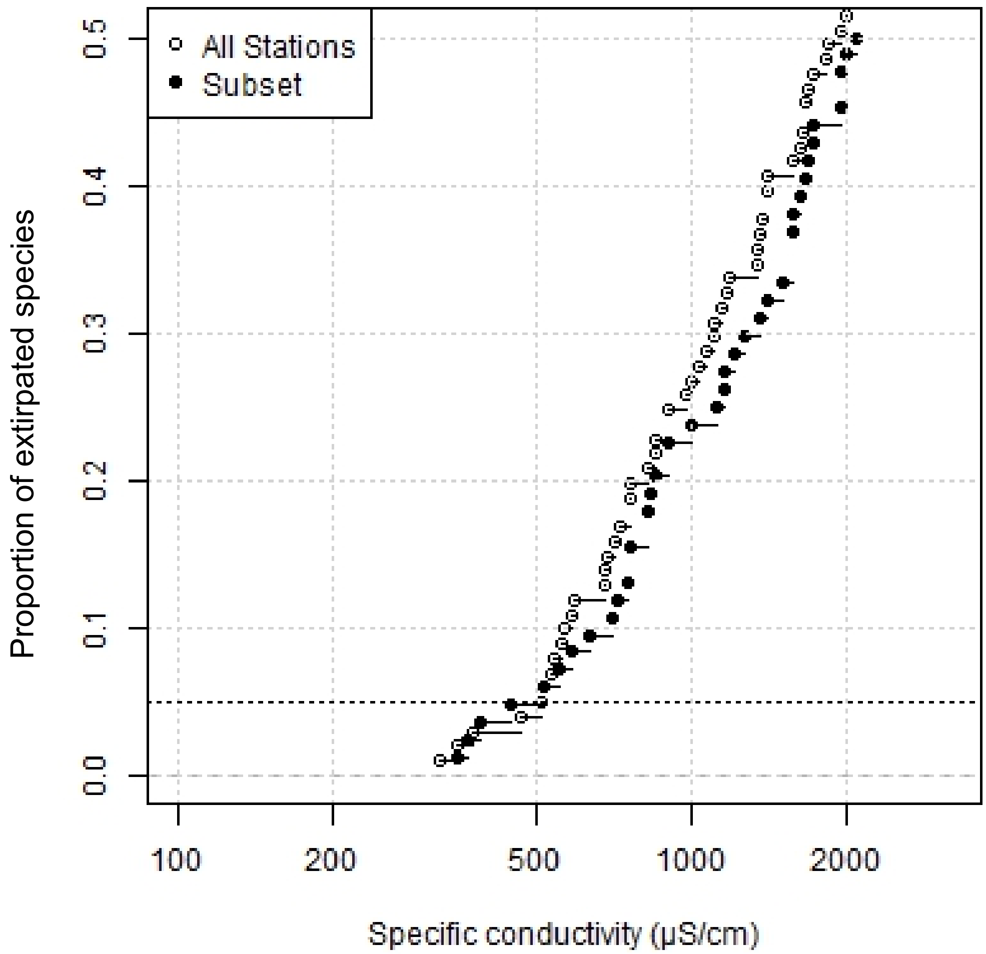


Figure G-12. Extirpation concentration distributions for sites with Rapid Bioassessment Protocol score <135 removed (closed circles) and for all sites (open circles). Sites with pH ≤6 were also removed. The example criterion (unconstrained) data set (N = 3,277) has 101 species (open circles) and the constrained data set (N = 2,714) has 84 species (closed circles). Habitat quality has little influence; the hazardous concentration (HC_{05}) for the constrained data set is 464 µS/cm.

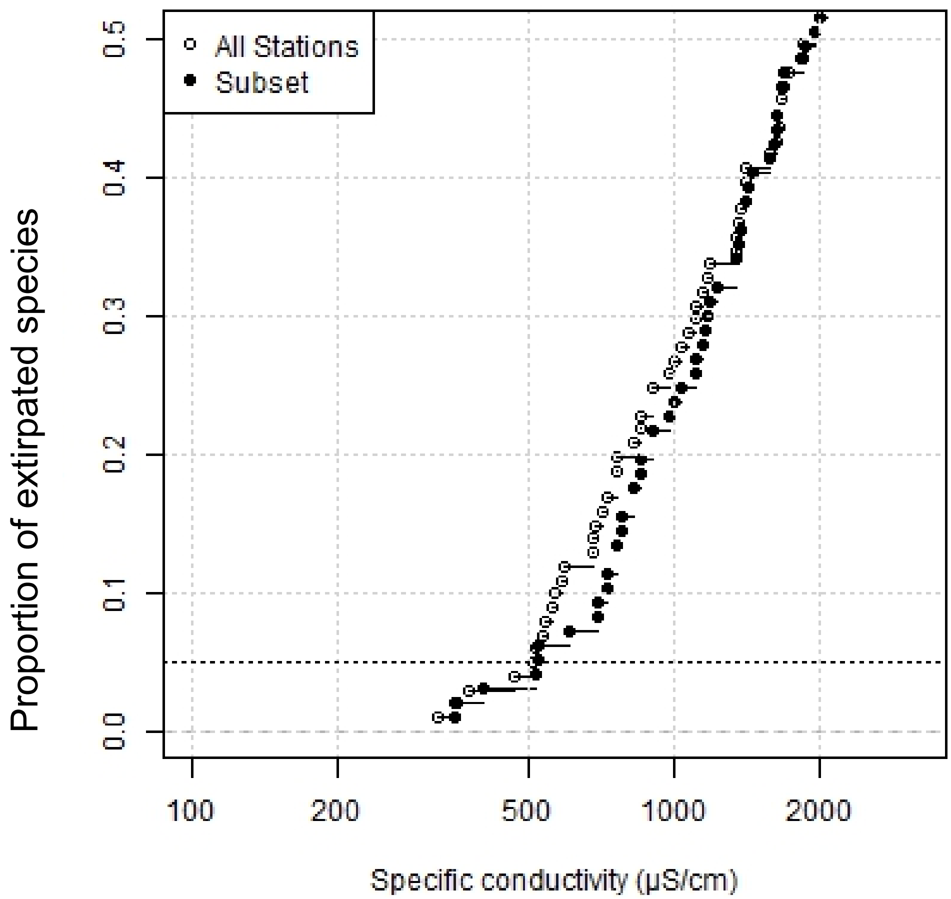


Figure G-13. Extirpation concentration distributions for sites with catchment area <10 km² removed (closed circles) and for all sites (open circles). Sites with pH ≤6 were also removed. The example criterion (unconstrained) data set (*N* = 3,277) has 101 species (open circles) and the constrained data set (*N* = 3,011) has 97 species (closed circles). Catchment area has little influence; the hazardous concentration (HC_{05}) for the constrained data set is 519 µS/cm.

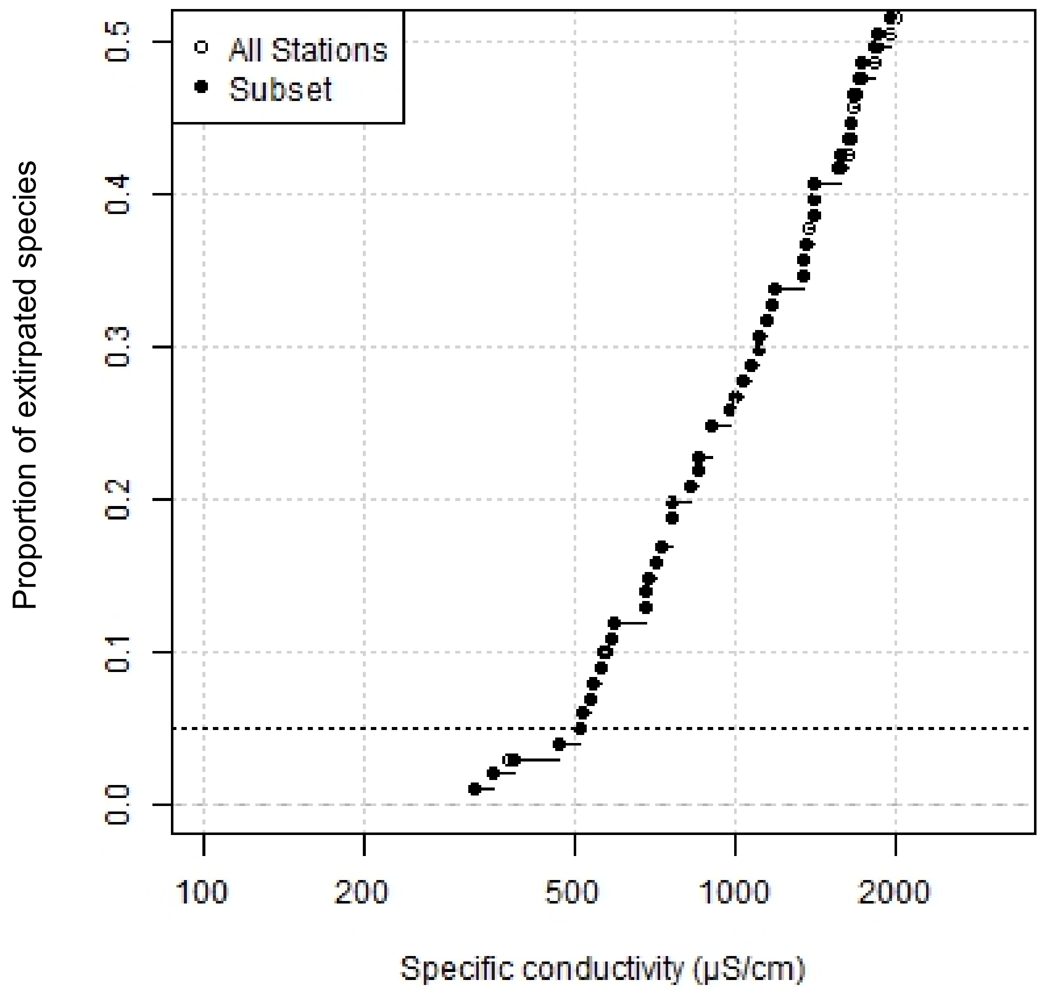


Figure G-14. Extirpation concentration distributions for sites with dissolved oxygen (DO) <4 mg/L removed (closed circles) and for all sites (open circles). Sites with pH ≤6 were also removed. The example criterion (unconstrained) data set (*N* = 3,277) has 101 species (open circles) and the constrained data set (*N* = 3,259) has 87 species (closed circles). Low DO has little influence; the hazardous concentration (HC_{05}) for the constrained data set is 509 µS/cm.

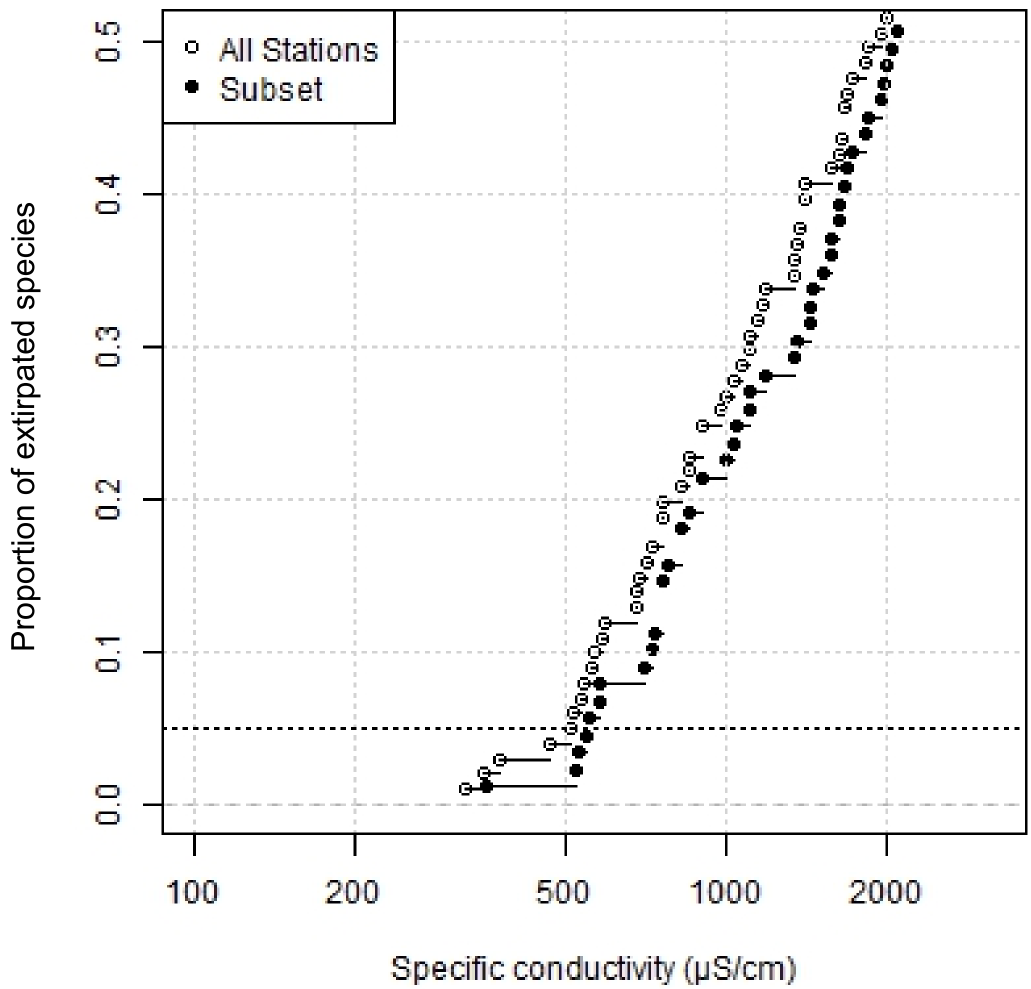


Figure G-15. Extirpation concentration distributions for sites with temperature >22°C removed (closed circles) and for all sites (open circles). Sites with pH ≤6 were also removed. The example criterion (unconstrained) data set (*N* = 3,277) has 101 species (open circles) and the constrained data set (*N* = 2,942) has 89 species (closed circles). Temperature has a little influence; the hazardous concentration (HC_{05}) for the constrained data set is 548 µS/cm.

G.4.6. Comparison of Fish and Benthic Macroinvertebrates

Because fish are identified to species, their calculated XC_{95} values are independent of geographic bounds within their biogeographical range. For macroinvertebrates, the taxonomic resolution is at the genus level, and there may be different species within a given genus in different ecoregions. This is one of the reasons that the case example criteria using macroinvertebrate data were calculated for separate ecoregions (see Case Studies I and II).

Although fish appear to be generally more salt-tolerant than macroinvertebrates, this result may be due to the dates of sampling and the difference in life history of salt-intolerant fish and aquatic insects rather than to actual differences in salt-intolerance. Most salt-intolerant benthic macroinvertebrates are univoltine, reproducing and surviving over a single year; whereas, fish are longer lived. Although aquatic insects do move, there is a tendency to drift downstreams rather than move upstream except during the aerial life stage. Fish are highly mobile within an unobstructed watershed and may be observed at SC where reproduction may not be possible. Therefore, direct comparison of the fish and macroinvertebrate XC_{95} values and XCDs are intended to be illustrative and should be interpreted cautiously.

G.5. CONCLUSION

This analysis demonstrates that fish species are either directly or indirectly affected by increased SC associated with salts dominated by Ca^{2+} plus Mg^{2+}, and HCO_3^- plus SO_4^{2-} (see Figures G-4 and G-5). XC_{95} values for fish fall within the range of XC_{95} values calculated for benthic macroinvertebrates. Only one fish XC_{95} value (i.e., 322 μS/cm for *Etheostoma baileyi*) was less than 340 μS/cm for Case Study II, the case example ecoregional criteria based on macroinvertebrates. Furthermore, the confidence intervals for the HC_{05} for fish (509 μS/cm, 955 CI 355−544 μS/cm) overlaps with the CI of the macroinvertebrates HC_{05} for Ecoregion 70 (338 μS/cm, 95% CI 272−365 μS/cm) but not with Ecoregion 69 (305 μS/cm, 95% CI 233−329 μS/cm).

Although fish species appear to be somewhat more salt-tolerant than macroinvertebrates, this may be due differences in the probability of capturing, observing, and enumerating fish and aquatic insects in a sample. Additional analyses are needed to validate this analysis. In particular, additional analyses and appropriate data sets are needed to evaluate the relevant frequency and duration parameters and whether the HC_{05} for fish represents an annual average annual or maximum concentration. For these reasons and because both groups are salt-intolerant and ecologically important assemblages, the HC_{05} for fish does not supplant the case example ecoregional SC criteria for macroinvertebrates described in Cases I and II.

This example fish HC_{05} is directly relevant to Ecoregions 67, 68, 69, and 70. The fish HC_{05} may also be appropriate for streams in other nearby regions with the same ionic mixture and similar background SC. However, this example HC_{05} based on fish species would not apply when the relative concentrations of dissolved ions are different (see Table G-2) or when the natural background is greater than the background in these ecoregions.

G.6. CATCHMENT SIZE AND HYDROLOGIC UNIT CODE (HUC) INCLUSION FOR DEVELOPING SAMPLING DATA SETS FOR FISH

Table G-4. Geographic constraints for inclusion of sites that were used to develop data sets and influenced the weights within specific conductivity bins used to estimate extirpation concentration (XC_{95}) values for each species of fish

Rank	Species	Ecoregions observed at least once	Minimum catchment area (km^2)	Maximum catchment area (km^2)	Basins represented in data set HUC
1	*Etheostoma baileyi*	68,69,70	1.8	353	0500
2	*Noturus insignis*	67,69	19.6	901	0204, 0205, 0207, 0208, 0301, 0500, 0601
3	*Erimyzon oblongus*	67,69,70	4.4	493	0204, 0205, 0207, 0208, 0500
4	*Esox niger*	67,69	3.5	743	0204, 0205, 2027, 0500
5	*Salvelinus fontinalis*	67,69,70	1.4	395	0204, 0205, 0207, 0500
6	*Cottus girardi*	67	2.0	594	0205, 0207
7	*Clinostomus funduloides*	67,69,70	1.1	259	0204, 0205, 0207, 0208, 0301, 0500
8	*Cottus carolinae*	67,68,69	1.7	1,461	0208, 0500, 0601
9	*Cottus cognatus*	67,69	3.7	395	0204, 0205, 0207, 0500
10	*Nocomis leptocephalus*	67,69	0.5	594	0207, 0208, 0301, 0500
11	*Etheostoma kennicotti*	68,69	3.6	131	0500
12	*Chrosomus oreas*	67,69	1.1	207	0207, 0208, 0301, 0500
13	*Notropis telescopus*	67,68,69	4.8	1,460	0500, 0601
14	*Cyprinella analostana*	67,69,70	3.7	506	0204, 0205, 0207, 0500, 0601
15	*Margariscus margarita*	67,70	6.0	76.6	0205, 0207,0500
16	*Lythrurus fasciolaris*	68,69,70	3.9	707	0500
17	*Luxilus cornutus*	67,69,70	3.1	3,859	0204, 0205, 0207, 0208, 0500
--[a]	*Erimystax* spp.	67,68,69,70	405	18,638	0500, 0601
18	*Fundulus diaphanus*	67,69	4.8	391	0204, 0205, 0207, 0500
19	*Salmo trutta*	67,69,70	3.7	64	0204, 0205, 0207, 0500

Table G-4. Geographic constraints for inclusion of sites that were used to develop data sets and influenced the weights within specific conductivity bins used to estimate extirpation concentration (XC_{95}) values for each species of fish (continued)

Rank	Species	Ecoregions observed at least once	Minimum catchment area (km²)	Maximum catchment area (km²)	Basins represented in data set (HUC)
20	*Exoglossum maxillingua*	67,69	4.4	901	0204, 0205, 0207, 0208
21	*Percina peltata*	67,69	26.9	901	0204, 0205
22	*Lepomis auritus*	67,68,69,70	3.3	8,336	0405, 0205, 0207, 0208, 0301, 0500, 0601
23	*Cyprinella whipplei*	67,69,70	24.4	15,990	0207, 0500
24	*Etheostoma olmstedi*	67,69	3.2	901	0204, 0205, 0207
25	*Anguilla rostrata*	67	1.3	8,337	0204, 0205, 0207
26	*Hybopsis amblops*	67,69,70	16	15,649	0207, 0500, 0601
27	*Semotilus corporalis*	67,69	3.3	8,337	0204, 0205, 0207, 0208
28	*Moxostoma carinatum*	69,70	19.7	16,638	0500
29	*Oncorhynchus mykiss*	67,69,70	0.1	668	0204, 0205, 0207, 0301, 0500, 0601
30	*Esox lucius*	69,70	18.0	15,522	0500
31	*Percopsis omiscomaycus*	69,70	9.4	5,840	0500
32	*Noturus miurus*	69,70	12.3	429	0500
33	*Lepisosteus osseus*	68,69,70	6.3	18,638	0500
34	*Lythrurus umbratilis*	69,70	8.0	1,251	0500
35	*Rhinichthys cataractae*	67,69,70	2.1	1,011	0204, 0205, 0207, 0208, 0500
36	*Percina macrocephala*	67,69,70	48.1	709	0500, 0601
37	*Ameiurus nebulosus*	67,69,70	7.8	646	0204, 0205, 0207, 0500 0601
38	*Minytrema melanops*	69,70	10.5	1,518	0500
39	*Notemigonus crysoleucas*	67,68,69,70	3.3	1,518	0204, 0205, 0207, 0500
40	*Notropis hudsonius*	67,69,70	13.8	668	0204, 0205, 0207, 0500
41	*Pomoxis nigromaculatus*	67,69,70	7.9	18,638	0204, 0205, 0500
42	*Perca flavescens*	67,69,70	22.0	2,826	0204, 0205, 0207, 0500

Table G-4. Geographic constraints for inclusion of sites that were used to develop data sets and influenced the weights within specific conductivity bins used to estimate extirpation concentration (XC_{95}) values for each species of fish (continued)

Rank	Species	Ecoregions observed at least once	Minimum catchment area (km^2)	Maximum catchment area (km^2)	Basins represented in data set (HUC)
43	*Esox americanus*	67,69,70	7.0	2,440	0204, 0205, 0301, 0500
44	*Percina maculata*	68,69,70	1.6	4,079	0500
45	*Carpiodes cyprinus*	69,70	11.1	18,638	0500
46	*Ictiobus bubalus*	69,70	6.3	18,638	0500
47	*Moxostoma anisurum*	69,70	35	17,742	0500
48	*Pimephales promelas*	67,68,69,70	4.5	250	0204, 0205, 0207, 0500
49	*Etheostoma spectabile*	68,70	4.0	1,238	0500
50	*Lepomis microlophus*	67,69,70	17.0	6,724	0204, 0207, 0500
51	*Lepomis gulosus*	67,68,69,70	11.5	1,950	0207, 0500
52	*Phenacobius mirabilis*	69,70	7.0	15,991	0500
53	*Clinostomus elongatus*	67,69,70	1.5	1,011	0205, 0500
54	*Cottus bairdii*	67,69,70	0.1	2,826	0204, 0205, 0207, 0208, 0301, 0500, 0601
55	*Aplodinotus grunniens*	69,70	6.3	18,638	0500
56	*Etheostoma camurum*	67,68,69,70	36.1	14,885	0500, 0601
57	*Lepomis gibbosus*	67,69,70	0.3	2,826	0204, 0205, 0207, 0208, 0500, 0601
58	*Notropis volucellus*	67,68,69,70	8.0	18,638	0205, 0500, 0601
59	*Pylodictis olivaris*	69,70	6.3	18,638	0500
60	*Notropis atherinoides*	67,69,70	6.2	18,638	0205, 0207, 0500
61	*Pomoxis annularis*	67,69,70	16.4	14,885	0205, 0500
62	*Nocomis micropogon*	67,68,69,70	4.9	8,336	0205, 0207, 0208, 0500, 0601
63	*Lampetra aepyptera*	67,68,69,70	0.3	1,189	0205, 0500
64	*Notropis buccatus*	67,68,69,70	1.1	1,518	0207, 0500
65	*Percina caprodes*	67,68,69,70	6.8	18,638	0500, 0601

Table G-4. Geographic constraints for inclusion of sites that were used to develop data sets and influenced the weights within specific conductivity bins used to estimate extirpation concentration (XC_{95}) values for each species of fish (continued)

Rank	Species	Ecoregions observed at least once	Minimum catchment area (km^2)	Maximum catchment area (km^2)	Basins represented in data set (HUC)
66	*Lepomis megalotis*	67,68,69,70	1.9	10,010	0207, 0500, 0601
67	*Etheostoma nigrum*	67,68,69,70	0.5	6,695	0205, 0207, 0301, 0500
68	*Etheostoma variatum*	67,69,70	5.0	17,742	0500, 0601
69	*Moxostoma duquesnei*	67,68,69,70	4.3	4,079	0205, 0500, 0601
70	*Moxostoma erythrurum*	67,68,69,70	1.7	18,638	0205, 0207, 0301, 0500, 0601
71	*Notorus flavus*	67,68,69,70	11.3	18,638	0205, 0500
72	*Pimephales vigilax*	69,70	25.0	18,638	0500
73	*Micropterus salmoides*	67,68,69,70	1.3	15,649	0204, 0205, 0207, 0500, 0601
74	*Notropis rubellus*	67,68,69,70	1.7	15,522	0204, 0205, 0207, 0500, 0601
75	*Micropterus dolomieu*	67,68,69,70	3.3	18,638	0404, 0205, 0207, 0208, 0301, 0500, 0601
76	*Dorosoma cepedianum*	67,68,69,70	8.0	18,638	0205, 0500
77	*Ictaluruws punctatus*	67,69,70	16.0	18,638	0204, 0205, 0207, 0500
78	*Labidesthes sicculus*	68,69,70	8.1	15,649	0500
79	*Lepomis macrochirus*	67,68,69,70	1.1	18,638	0204, 0205, 0207, 0208, 0500, 0601
80	*Catostomus commersoni*	67,68,69,70	1.5	3,859	0204, 0205, 0207, 0208, 0301, 0500, 0601
81	*Etheostoma zonale*	67,68,69,70	3.9	17,742	0205, 0207, 0500, 0601
82	*Semotilus atromaculatus*	67,68,69,70	0.2	15,991	0204, 0205, 0207, 0208, 0301, 0500, 0601
83	*Notropis photogenis*	67,68,69,70	1.7	15,522	0500, 0601
84	*Micropterus punctulatus*	68,69,70	1.7	18,638	0500
85	*Chrosomus erythrogaster*	68,69,70	1.1	123	0500
86	*Pimephales notatus*	67,68,69,70	1.7	15,991	0204, 0205, 0207, 0500, 0601
87	*Rhinichthys obtusus*	70	2.0	14,885	0500

Table G-4. Geographic constraints for inclusion of sites that were used to develop data sets and influenced the weights within specific conductivity bins used to estimate extirpation concentration (XC_{95}) values for each species of fish (continued)

Rank	Species	Ecoregions observed at least once	Minimum catchment area (km^2)	Maximum catchment area (km^2)	Basins represented in data set (HUC)
88	*Ambloplites rupestris*	67,68,69,70	1.7	15,649	0204, 0205, 0207, 0208, 0301, 0500, 0601
89	*Etheostoma flabellare*	67,68,69,70	0.5	8,336	0205, 0207, 0208, 0301, 0500, 0601
90	*Lepomis cyanellus*	67,68,69,70	0.3	15,522	0204, 0205, 0207, 0500, 0601
91	*Rhinichthys atratulus*	67,68,69,70	0.2	668	0204, 0205, 0207, 0208, 0301, 0500, 0601
92	*Campostoma anomalum*	67,68,69,70	0.5	17,742	0205, 0207, 0208, 0301, 0500, 0601
93	*Cyprinus carpio*	67,69,70	11.2	18,638	0204, 0205, 0207, 0301, 0500, 0601
94	*Hypentelium nigricans*	67,68,69,70	1.6	18,638	0204, 0205, 0207, 0208, 0301, 0500, 0601
95	*Ameiurus natalis*	67,68,69,70	3	4,079	0204, 0205, 0207, 0500, 0601
96	*Cyprinella spiloptera*	67,68,69,70	5.1	18,638	0204, 0205, 0207, 0500, 0601
97	*Etheostoma blennioides*	67,68,69,70	3.3	15,522	0205, 0207, 0500, 0601
98	*Etheostoma caeruleum*	67,68,69,70	1.5	8,336	0207, 0500, 0601
99	*Luxilus chrysocephalus*	67,68,69,70	0.5	13,289	0207, 0500, 0601
100	*Notropis stramineus*	69,70	2	17,742	0500
101	*Sander canadensis*	69,70	27	15,991	0500

[a]Only a genus XC_{95} was calculated for *Erimystax* spp., because none of the four species collected in the combined data set, *E. cahni*, *E. insignis*, *E. x-punctatus*, or *E. dissimilis*, were observed in ≥25 samples, but together, they were observed in 38 samples. All the other information is for the four species combined.

G.7. EXTIRPATION CONCENTRATION

Table G-5. Extirpation concentration (XC_{95}) values for fish that were observed at greater than or equal to 25 sites. N_{total} is the number of samples in the combined data set where the fish species potentially occurred and $N_{observed}$ is the number of those samples where the fish species was observed. Rank is the order of the fish species from smallest to greatest species XC_{95} in the extirpation concentration distribution. The XC_{95} is listed as approximate (≈) if the Generalized Additive Model (GAM) mean curve at maximum specific conductivity is greater than 0 but the lower confidence limit is approximately 0 (<1% of the maximum mean modeled probability). The XC_{95} is listed as greater than (>), if the GAM lower confidence limit is greater than 0. Ecoregions observed are the ecoregions where the species was collected in the combined data set.

Rank	Species	Common name	Species XC_{95}	$N_{observed}$	N_{total}	Genus XC_{95}	Ecoregions observed
1	*Etheostoma baileyi*	emerald darter	322	38	1,744	>3,226	68,69,70
2	*Noturus insignis*	margined madtom	349	208	3,277	>2,578	67,69
3	*Erimyzon oblongus*	creek chubsucker	376	27	3,249	376	67,69,70
4	*Esox niger*	chain pickerel	≈467	63	1,505	>1,572	67,69
5	*Salvelinus fontinalis*	brook trout	508	1,361	3,232	508	67,69,70
6	*Cottus girardi*	Potomac sculpin	≈518	31	1,087	>1,961	67
7	*Clinostomus funduloides*	rosyside dace	535	79	3,253	>1,790	67,69,70
8	*Cottus carolinae*	banded sculpin	542	29	1,785	--[a]	67,68,69
9	*Cottus cognatus*	slimy sculpin	≈557	303	3,232	--	67,69
10	*Nocomis leptocephalus*	bluehead chub	≈565	29	1,851	>2,303	67,69
11	*Etheostoma kennicotti*	stripetail darter	≈586	27	1,744	--	68,69
12	*Chrosomus oreas*	mountain redbelly dace	592	27	1,851	≈3,094	67,69
13	*Notropis telescopus*	telescope shiner	675	36	1,768	>4,000	67,68,69
14	*Cyprinella analostana*	satinfin shiner	682	28	3,256	>4,000	67,69,70
15	*Margariscus margarita*	pearl dace	>685	34	2,831	>685	67,70
16	*Lythrurus fasciolaris*	scarlet shiner	707	115	1,744	1,081	68,69,70
17	*Luxilus cornutus*	common shiner	724	443	3,249	>4,000	67,69,70
--	*Erimystax* spp.	chub	--[b]	33	1,768	744	67,68,69,70
18	*Fundulus diaphanus*	banded killifish	759	42	3,232	1,090	67,69
19	*Salmo trutta*	brown trout	≈759	1,485	3,232	≈759	67,69,70
20	*Exoglossum maxillingua*	cutlips minnow	>760	447	1,505	576	67,69

Table G-5. Extirpation concentration (XC_{95}) values for fish that were observed at greater than or equal to 25 sites. N_{total} is the number of samples in the combined data set where the fish species potentially occurred and $N_{observed}$ is the number of those samples where the fish species was observed. Rank is the order of the fish species from smallest to greatest species XC_{95} in the extirpation concentration distribution. The XC_{95} is listed as approximate (≈) if the Generalized Additive Model (GAM) mean curve at maximum specific conductivity is greater than 0 but the lower confidence limit is approximately 0 (<1% of the maximum mean modeled probability). The XC_{95} is listed as greater than (>), if the GAM lower confidence limit is greater than 0. Ecoregions observed are the ecoregions where the species was collected in the combined data set. (continued)

Rank	Species	Common name	Species XC_{95}	$N_{observed}$	N_{total}	Genus XC_{95}	Ecoregions observed
21	*Percina peltata*	shield darter	≈822	80	1,402	>2,578	67,69
22	*Lepomis auritus*	redbreast sunfish	851	139	3,277	>2,750	67,68,69,70
23	*Cyprinella whipplei*	steelcolor shiner	854	29	1,830	--	67,69,70
24	*Etheostoma olmstedi*	tessellated darter	>898	530	1,488	--	67,69
25	*Anguilla rostrata*	American eel	>898	182	1,488	>898	67
26	*Hybopsis amblops*	bigeye chub	≈982	69	1,854	≈982	67,69,70
27	*Semotilus corporalis*	fallfish	>1,000	279	1,505	>3,066	67,69
28	*Moxostoma carinatum*	river redhorse	1,040	28	1,744	>2,578	69,70
29	*Oncorhynchus mykiss*	rainbow trout	>1,075	574	3,260	>1,075	67,68,70
30	*Esox lucius*	northern pike	>1,103	27	1,744	--	69,70
31	*Percopsis omiscomaycus*	trout-perch	≈1,105	66	1,744	≈1,105	69,70
32	*Noturus miurus*	brindled madtom	1,150	31	1,744	--	69,70
33	*Lepisosteus osseus*	longnose gar	≈1,170	30	1,744	≈1,170	68,69,70
34	*Lythrurus umbratilis*	redfin shiner	≈1,193	40	1,744	--	69,70
35	*Rhinichthys cataractae*	longnose dace	≈1,343	878	3,249	>3,535	67,69,70
36	*Percina macrocephala*	longhead darter	1,351	27	1,768	--	67,69,70
37	*Ameiurus nebulosus*	brown bullhead	≈1,358	75	3,256	>4,000	67,69,70
38	*Minytrema melanops*	spotted sucker	≈1,372	50	1,744	≈1,372	69,70
39	*Notemigonus crysoleucas*	golden shiner	≈1,400	85	3,232	≈1,400	67,68,69,70
40	*Notropis hudsonius*	spottail shiner	≈1,400	87	3,232	≈1,400	67,69,70
41	*Pomoxis nigromaculatus*	black crappie	>1,413	70	3,146	>2,278	67,69,70
42	*Perca flavescens*	yellow perch	>1,580	56	3,232	>1,580	67,69,70
43	*Esox americanus*	redfin pickerel	>1,625	113	3,150	--	67,69,70
44	*Percina maculata*	blackside darter	≈1,643	221	1,744	--	68,69,70
45	*Carpiodes cyprinus*	quillback	1,672	54	1,744	1,672	69,70

Table G-5. Extirpation concentration (XC_{95}) values for fish that were observed at greater than or equal to 25 sites. N_{total} is the number of samples in the combined data set where the fish species potentially occurred and $N_{observed}$ is the number of those samples where the fish species was observed. Rank is the order of the fish species from smallest to greatest species XC_{95} in the extirpation concentration distribution. The XC_{95} is listed as approximate (≈) if the Generalized Additive Model (GAM) mean curve at maximum specific conductivity is greater than 0 but the lower confidence limit is approximately 0 (<1% of the maximum mean modeled probability). The XC_{95} is listed as greater than (>), if the GAM lower confidence limit is greater than 0. Ecoregions observed are the ecoregions where the species was collected in the combined data set. (continued)

Rank	Species	Common name	Species XC_{95}	$N_{observed}$	N_{total}	Genus XC_{95}	Ecoregions observed
46	*Ictiobus bubalus*	smallmouth buffalo	1,672	67	1,744	1,672	69,70
47	*Moxostoma anisurum*	silver redhorse	>1,693	101	1,744	--	69,70
48	*Pimephales promelas*	fathead minnow	>1,732	38	3,232	>3,094	67,68,69,70
49	*Etheostoma spectabile*	orangethroat darter	1,824	103	1,744	--	68,70
50	*Lepomis microlophus*	redear sunfish	>1,858	35	2,231	--	67,69,70
51	*Lepomis gulosus*	warmouth	>1,958	40	1,830	--	67,68,69,70
52	*Phenacobius mirabilis*	suckermouth minnow	>2,000	40	1,744	>2,000	69,70
53	*Clinostomus elongatus*	redside dace	>2,009	170	2,745	--	67,69,70
54	*Cottus bairdii*	mottled sculpin	>2,046	878	3,277	--	67,69,70
55	*Aplodinotus grunniens*	freshwater drum	>2,099	79	1,744	>2,099	69,70
56	*Etheostoma camurum*	bluebreast darter	>2,122	32	1,768	--	67,68,69,70
57	*Lepomis gibbosus*	pumpkinseed	>2,157	447	3,273	--	67,69,70
58	*Notropis volucellus*	mimic shiner	>2,122	183	2,769	--	67,68,69,70
59	*Pylodictis olivaris*	flathead catfish	>2,122	28	1,744	>2,122	69,70
60	*Notropis atherinoides*	emerald shiner	>2,157	157	2,831	--	67,69,70
61	*Pomoxis annularis*	white crappie	>2,278	41	2,745	--	67,69,70
62	*Nocomis micropogon*	river chub	>2,303	309	2,872	--	67,68,69,70
63	*Lampetra aepyptera*	least brook lamprey	2,323	143	2,745	2,323	67,68,69,70
64	*Notropis buccatus*	silverjaw minnow	>2,323	516	1,830	--	67,68,69,70
65	*Percina caprodes*	logperch	>2,359	296	1,768	--	67,68,69,70
66	*Lepomis megalotis*	longear sunfish	2,578	343	1,324	--	67,68,69,70
67	*Etheostoma nigrum*	Johnny darter	>2,578	818	2,835	--	67,68,69,70
68	*Etheostoma variatum*	variegate darter	>2,578	113	1,768	--	67,69,70
69	*Moxostoma duquesnei*	black redhorse	>2,578	156	2,769	>2,578	67,68,69,70

Table G-5. Extirpation concentration (XC_{95}) values for fish that were observed at greater than or equal to 25 sites. N_{total} is the number of samples in the combined data set where the fish species potentially occurred and $N_{observed}$ is the number of those samples where the fish species was observed. Rank is the order of the fish species from smallest to greatest species XC_{95} in the extirpation concentration distribution. The XC_{95} is listed as approximate (≈) if the Generalized Additive Model (GAM) mean curve at maximum specific conductivity is greater than 0 but the lower confidence limit is approximately 0 (<1% of the maximum mean modeled probability). The XC_{95} is listed as greater than (>), if the GAM lower confidence limit is greater than 0. Ecoregions observed are the ecoregions where the species was collected in the combined data set. (continued)

Rank	Species	Common name	Species XC_{95}	$N_{observed}$	N_{total}	Genus XC_{95}	Ecoregions observed
70	*Moxostoma erythrurum*	golden redhorse	>2.578	404	2,859	--	67,68,69,70
71	*Notorus flavus*	stonecat	>2,578	96	2,745	--	67,68,69,70
72	*Pimephales vigilax*	bullhead minnow	>2,578	49	1,744	--	69,70
73	*Micropterus salmoides*	largemouth bass	>2,630	514	3,256	>3,066	67,68,69,70
74	*Notropis rubellus*	rosyface shiner	>2,630	342	3,256	--	67,68,69,70
75	*Micropterus dolomieu*	smallmouth bass	>2,641	718	3,277	--	67,68,69,70
76	*Dorosoma cepedianum*	gizzard shad	>2,750	127	2,745	>2,750	67,68,69,70
77	*Ictalurus punctatus*	channel catfish	>2,750	128	3,763	>2,750	67,69,70
78	*Labidesthes sicculus*	brook silverside	>2,750	80	1,744	>2,750	68,69,70
79	*Lepomis macrochirus*	bluegill	>2,750	943	3,273	--	67,68,69,70
80	*Catostomus commersoni*	white sucker	>2,755	1,984	3,277	>2,755	67,68,69,70
81	*Etheostoma zonale*	banded darter	>3,066	328	2,855	--	67,68,69,70
82	*Semotilus atromaculatus*	creek chub	>3,066	2,024	3,277	--	67,68,69,70
83	*Notropis photogenis*	silver shiner	>3,066	223	1,768	--	67,68,69,70
84	*Micropterus punctulatus*	spotted bass	≈3,094	161	1,744	--	68,69,70
85	*Chrosomus erythrogaster*	southern redbelly dace	>3,094	161	1,744	--	68,69,70
86	*Pimephales notatus*	bluntnose minnow	>3,094	1,028	3,256	--	67,68,69,70
87	*Rhinichthys obtusus*	western blacknose dace	>3,094	326	1,744	--	69,70
88	*Ambloplites rupestris*	rock bass	>3,266	922	3,277	>3,266	67,68,69,70
89	*Etheostoma flabellare*	fantail darter	>3,266	919	2,876	--	67,68,69,70
90	*Lepomis cyanellus*	green sunfish	>3,266	789	3,256	--	67,68,69,70
91	*Rhinichthys atratulus*	eastern blacknose dace	≈3,590	1,108	1,857	--	67,68,69,70
92	*Campostoma anomalum*	central stoneroller	>3,590	1,211	2,876	>3,590	67,68,69,70

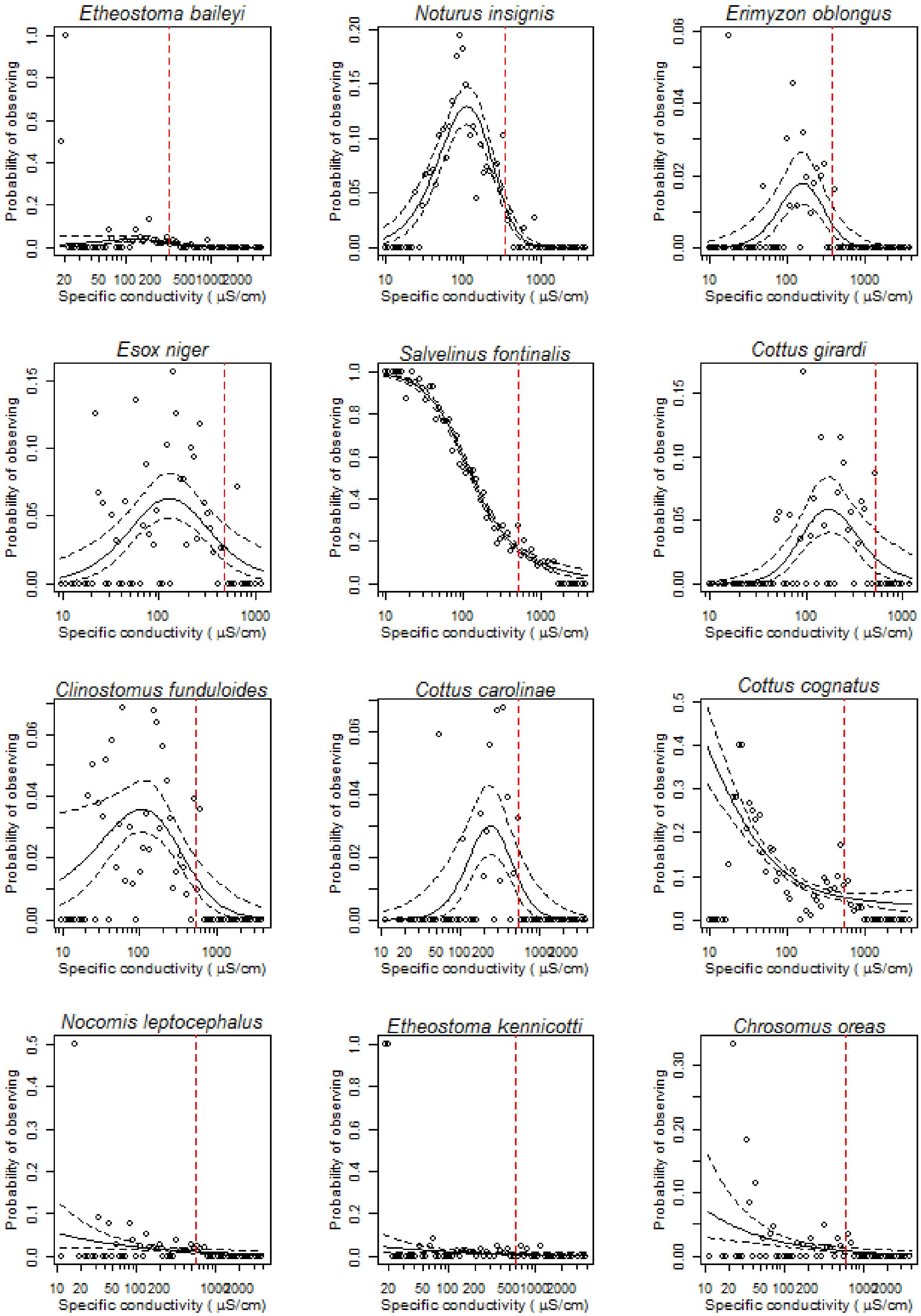
Table G-5. Extirpation concentration (XC_{95}) values for fish that were observed at greater than or equal to 25 sites. N_{total} is the number of samples in the combined data set where the fish species potentially occurred and $N_{observed}$ is the number of those samples where the fish species was observed. Rank is the order of the fish species from smallest to greatest species XC_{95} in the extirpation concentration distribution. The XC_{95} is listed as approximate (≈) if the Generalized Additive Model (GAM) mean curve at maximum specific conductivity is greater than 0 but the lower confidence limit is approximately 0 (<1% of the maximum mean modeled probability). The XC_{95} is listed as greater than (>), if the GAM lower confidence limit is greater than 0. Ecoregions observed are the ecoregions where the species was collected in the combined data set. (continued)

Rank	Species	Common name	Species XC_{95}	$N_{observed}$	N_{total}	Genus XC_{95}	Ecoregions observed
93	*Cyprinus carpio*	common carp	>3,590	200	3,260	>3,590	67,69,70
94	*Hypentelium nigricans*	northern hog sucker	>3,590	1,169	3,277	>3,590	67,68,69,70
95	*Ameiurus natalis*	yellow bullhead	>4,000	364	3,256	--	67,68,69,70
96	*Cyprinella spiloptera*	spotfin shiner	>4,000	410	3,256	--	67,68,69,70
97	*Etheostoma blennioides*	greenside darter	>4,000	740	2,855	--	67,68,69,70
98	*Etheostoma caeruleum*	rainbow darter	>4,000	634	1,854	--	67,68,69,70
99	*Luxilus chrysocephalus*	striped shiner	>4,000	707	1,854	--	67,68,69,70
100	*Notropis stramineus*	sand shiner	>4,000	354	1,744	--	69,70
101	*Sander canadensis*	walleye	>4,000	48	1,744	>4,000	69,70

[a]A long dash indicates fish species where the genus XC_{95} is provided for a congeneric species above it in the table.
[b]Only a genus XC_{95} was calculated for *Erimystax* spp., because none of the four species collected in the combined data set, *E. cahni*, *E. insignis*, *E. x-punctatus*, or *E. dissimilis*, were observed in ≥25 samples, but together, they were observed in 38 samples. All the other information is for the four species combined.

G.8. GRAPHS OF OBSERVATION PROBABILITIES FOR EACH FISH SPECIES

The purpose of this section is to help visualize the changes in the observations of each species as SC increases. Each figure depicts a GAM of the relationship between capture probability of the species and SC. Species are ordered from the smallest to the greatest XC_{95} value. Open circles are the probabilities of observing the species within a SC. Circles at zero probability indicate no individuals at any sites were found at these conductivities. The GAM line (solid line) fitted to the probabilities is for visualization and the dashed lines are the 90% confidence bounds. The vertical dotted red line indicates the XC_{95} as listed in Table G-5. Note that different species respond differently to increasing salinity. For example, *Notropis telescopus, Chrosomus oreas*, and *Salvelinus fontinalis* decline; *Esox niger*, *Cottus carolinae*, *Cyprinella whipplei,* and *Semotilus corporalis* have optima; and *Notropis rubellus, Etheostoma caeruleum*, and *Campostoma anomalum* increase. The fitted lines and confidence bounds were used to assign qualifiers to the XC_{95} values in Table G-5.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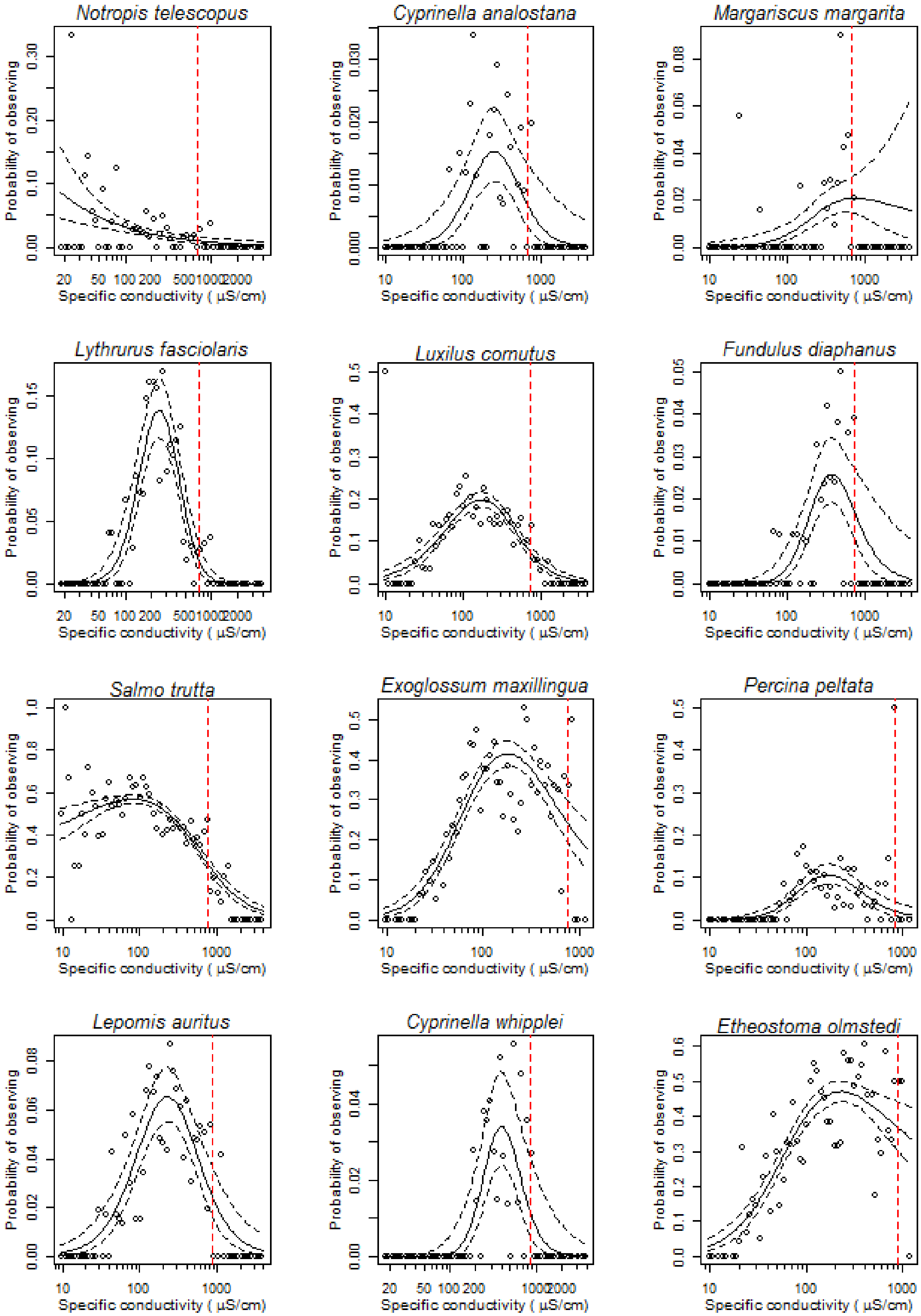
Noturus insignis
Erimyzon oblongus
Esox niger
Salvelinus fontinalis
Cottus girardi
Clinostomus funduloides
Cottus carolinae
Cottus cognatus
Nocomis leptocephalus
Etheostoma kennicotti
Chrosomus oreas
Probability of observing
Specific conductivity (µS/cm)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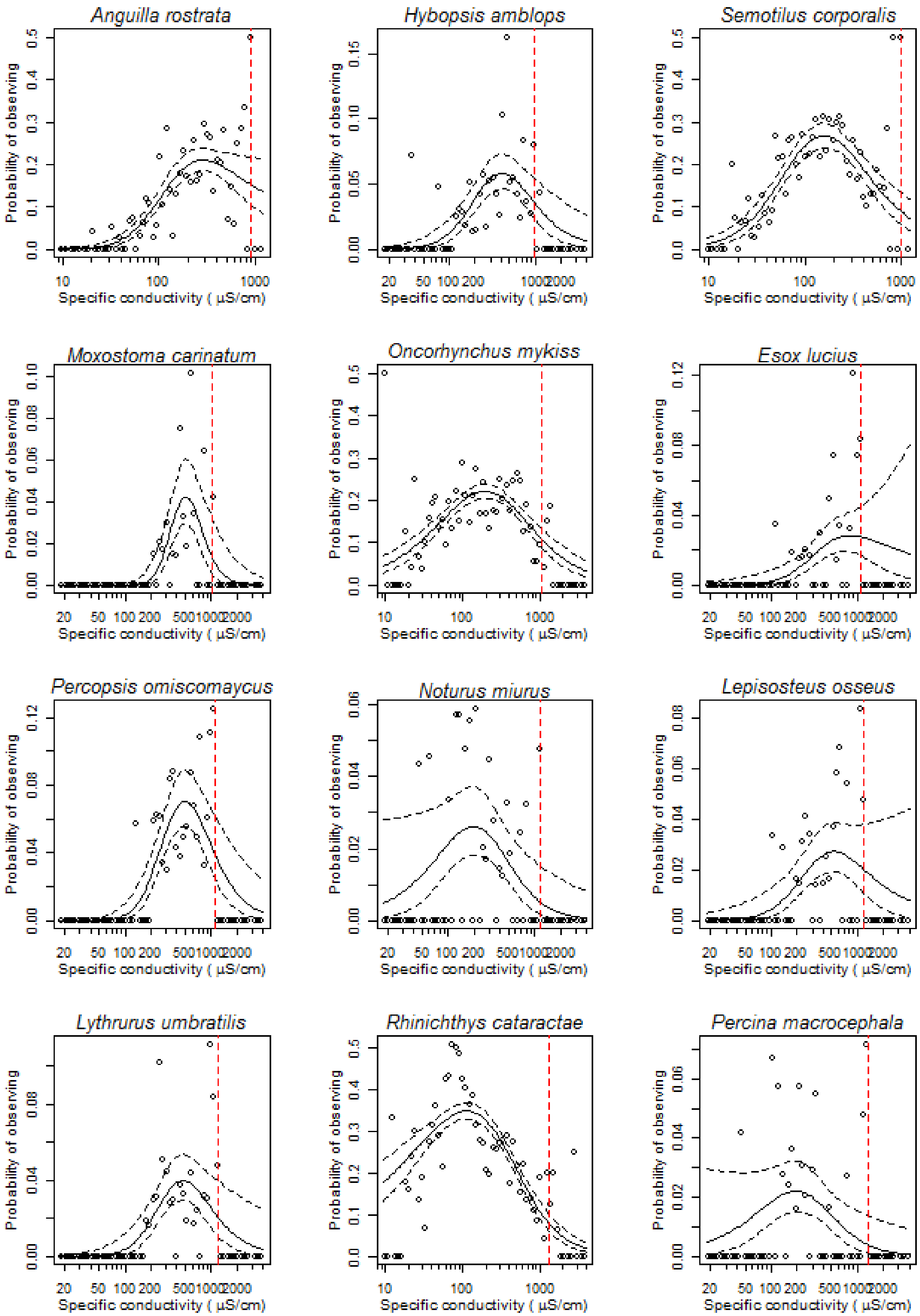
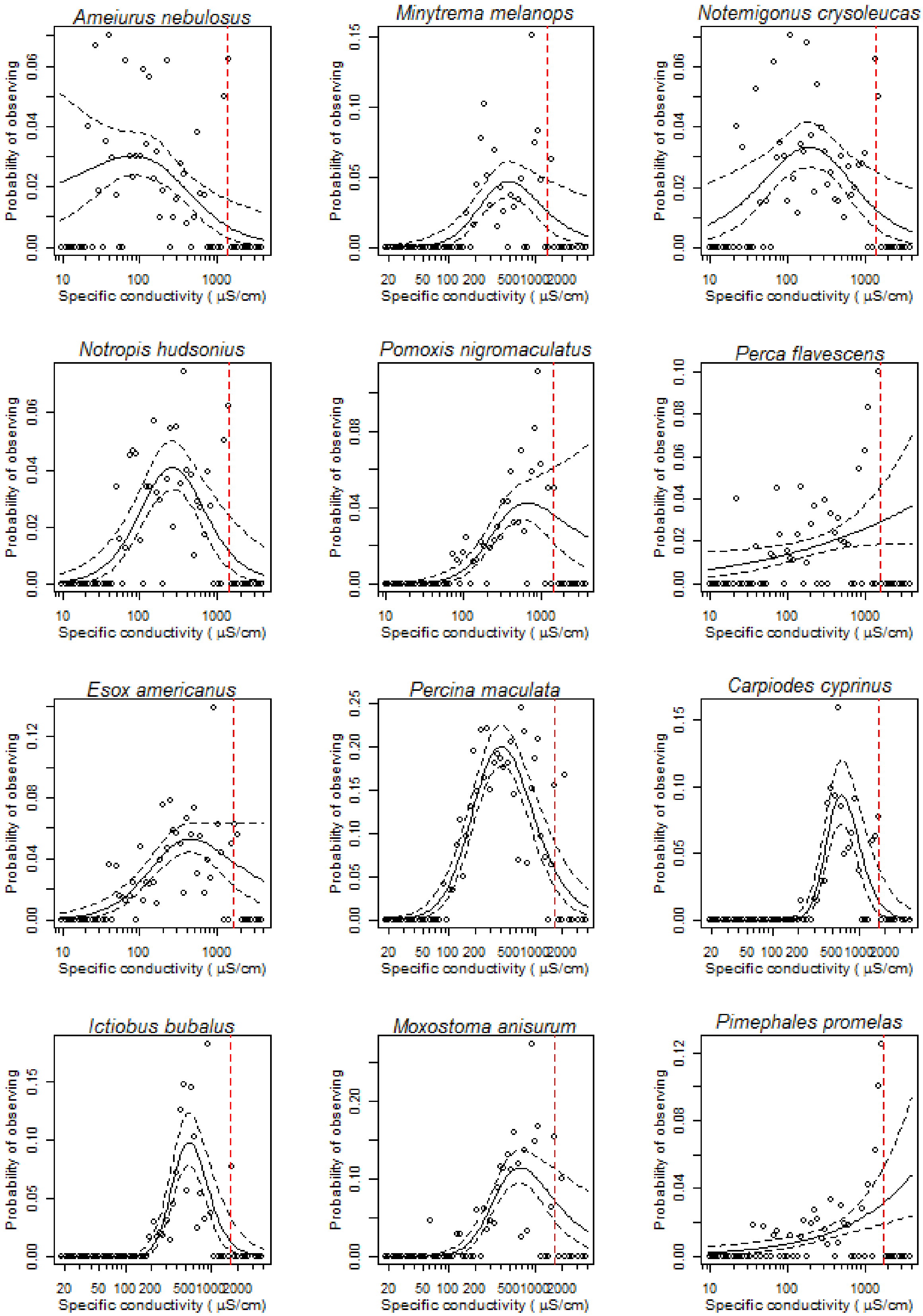
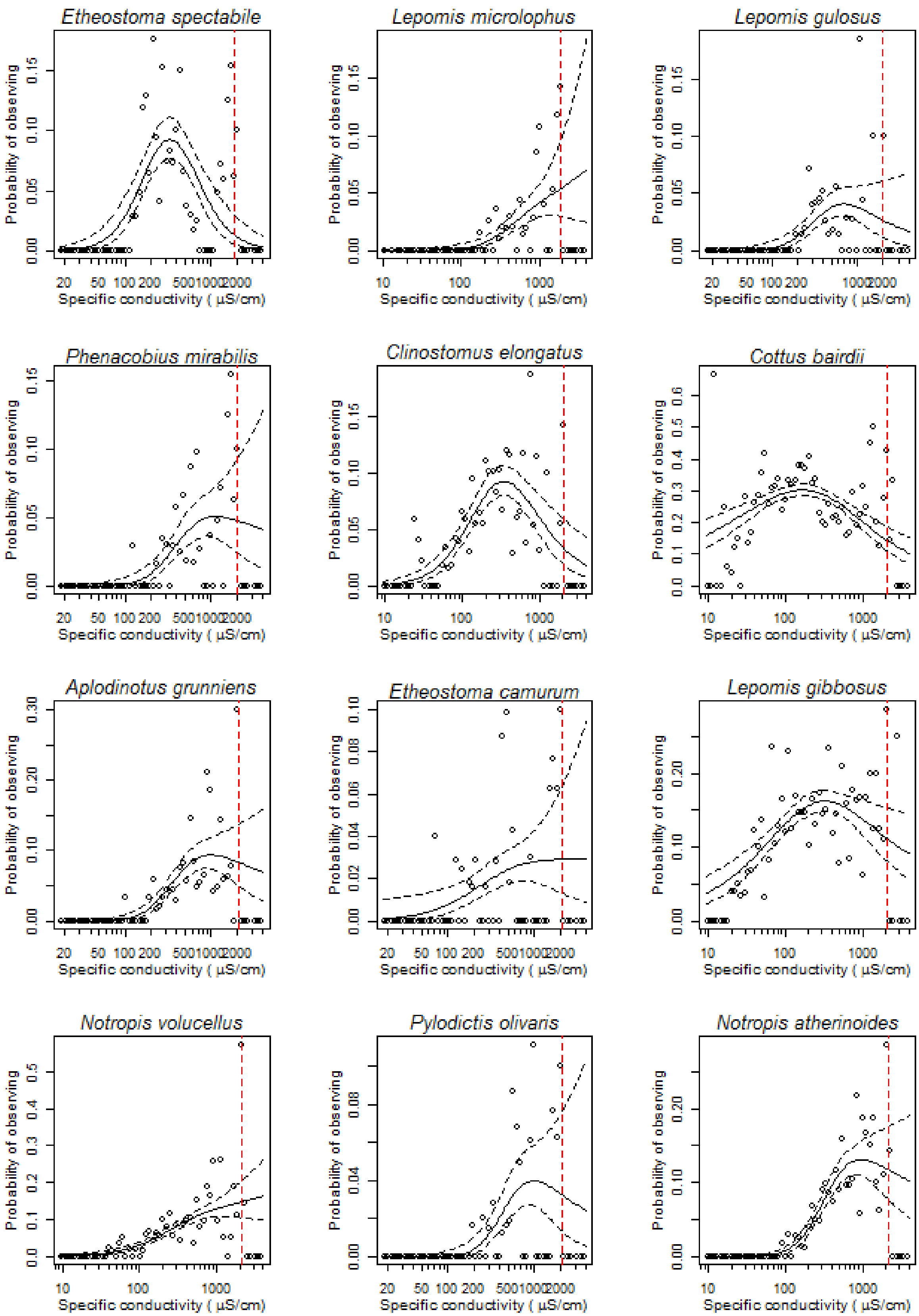
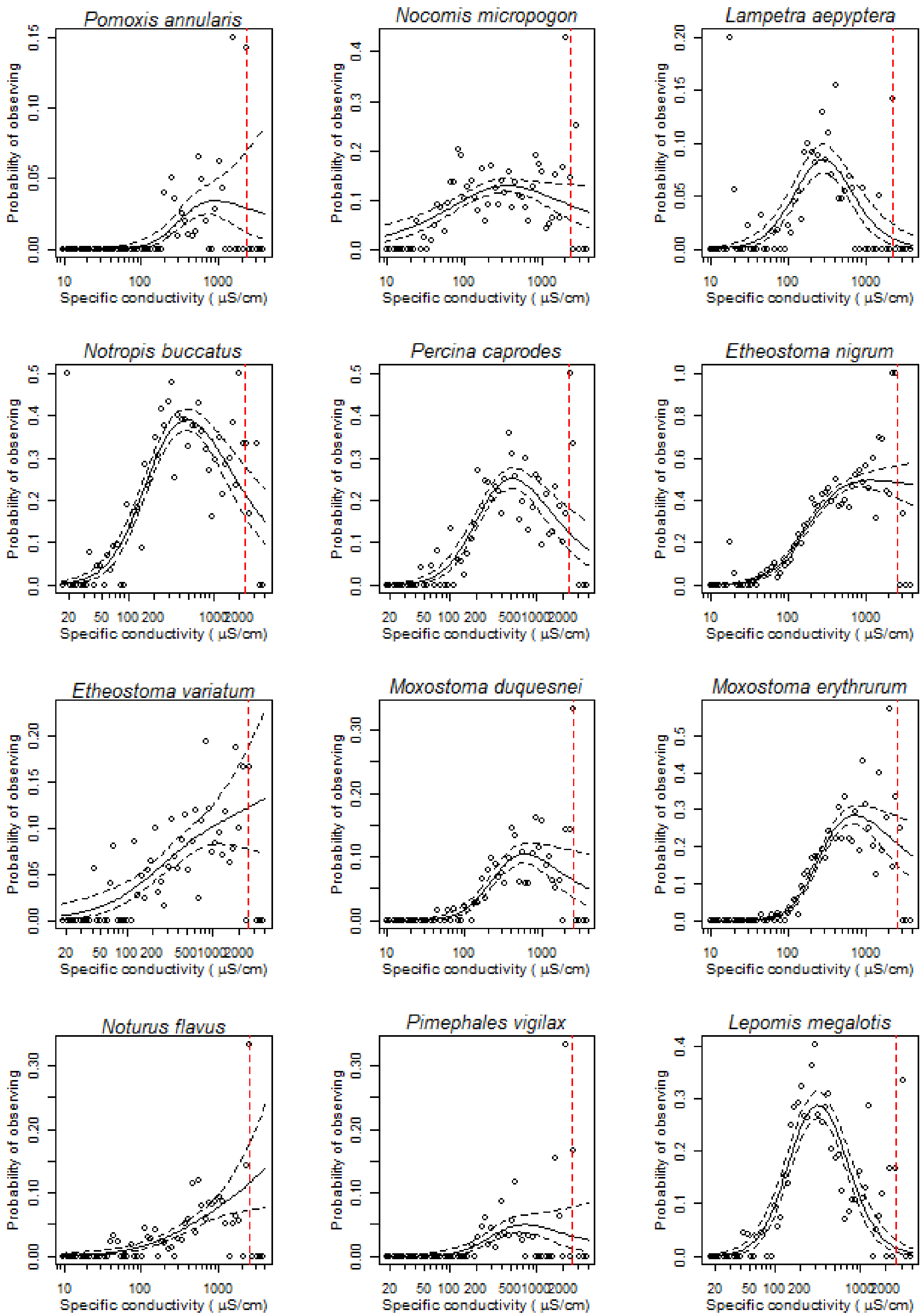
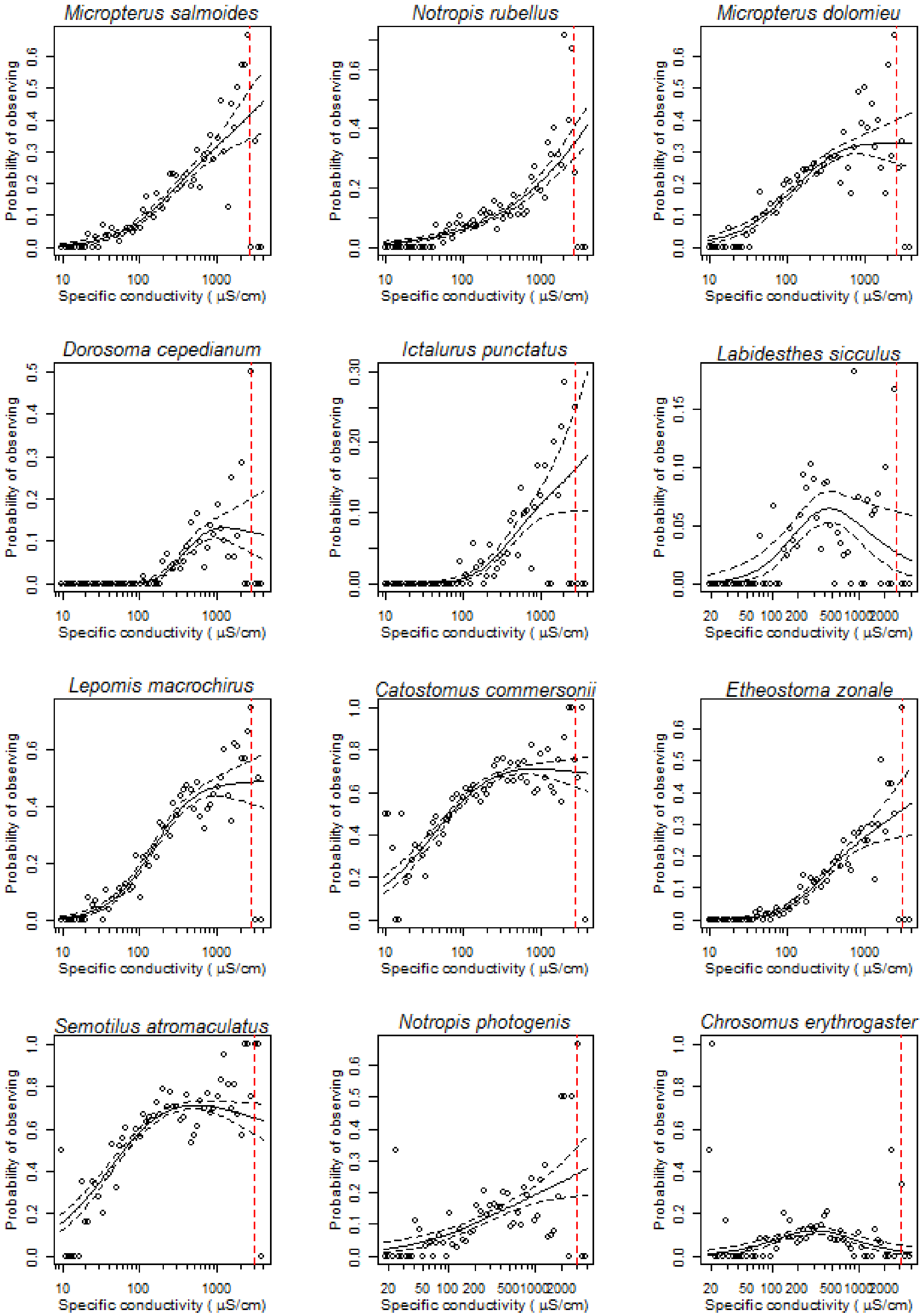
Cyprinella analostana
Margariscus margarita
Lythrurus fasciolaris
Luxilus cornutus
Fundulus diaphanus
Salmo trutta
Exoglossum maxillingua
Percina peltata
Lepomis auritus
Cyprinella whipplei
Etheostoma olmstedi
Probability of observing
Specific conductivity (µS/cm)

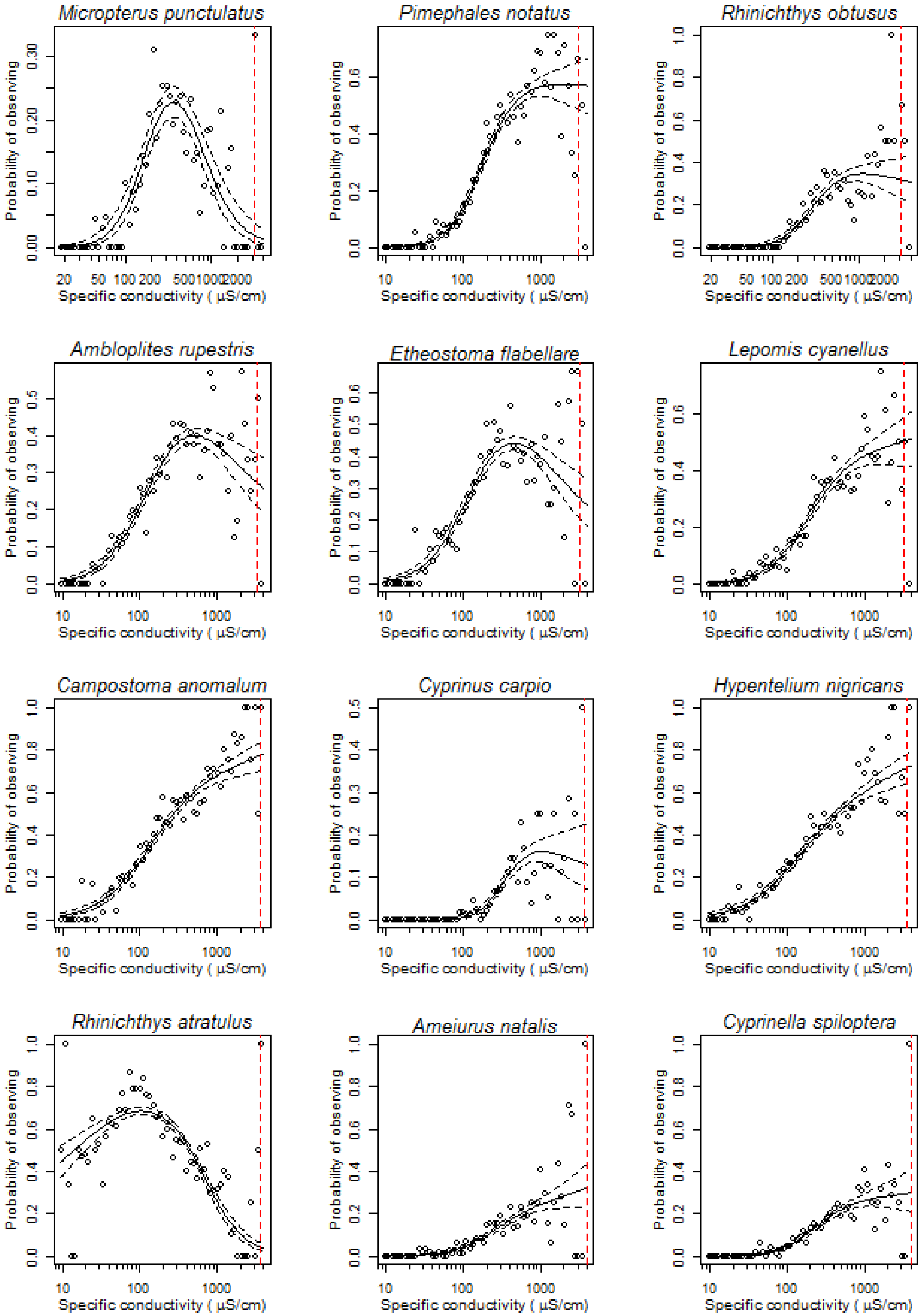

Anguilla rostrata
Hybopsis amblops
Semotilus corporalis
Moxostoma carinatum
Oncorhynchus mykiss
Esox lucius
Percopsis omiscomaycus
Noturus miurus
Lepisosteus osseus
Lythrurus umbratilis
Rhinichthys cataractae
Percina macrocephala
Probability of observing
Specific conductivity (µS/cm)

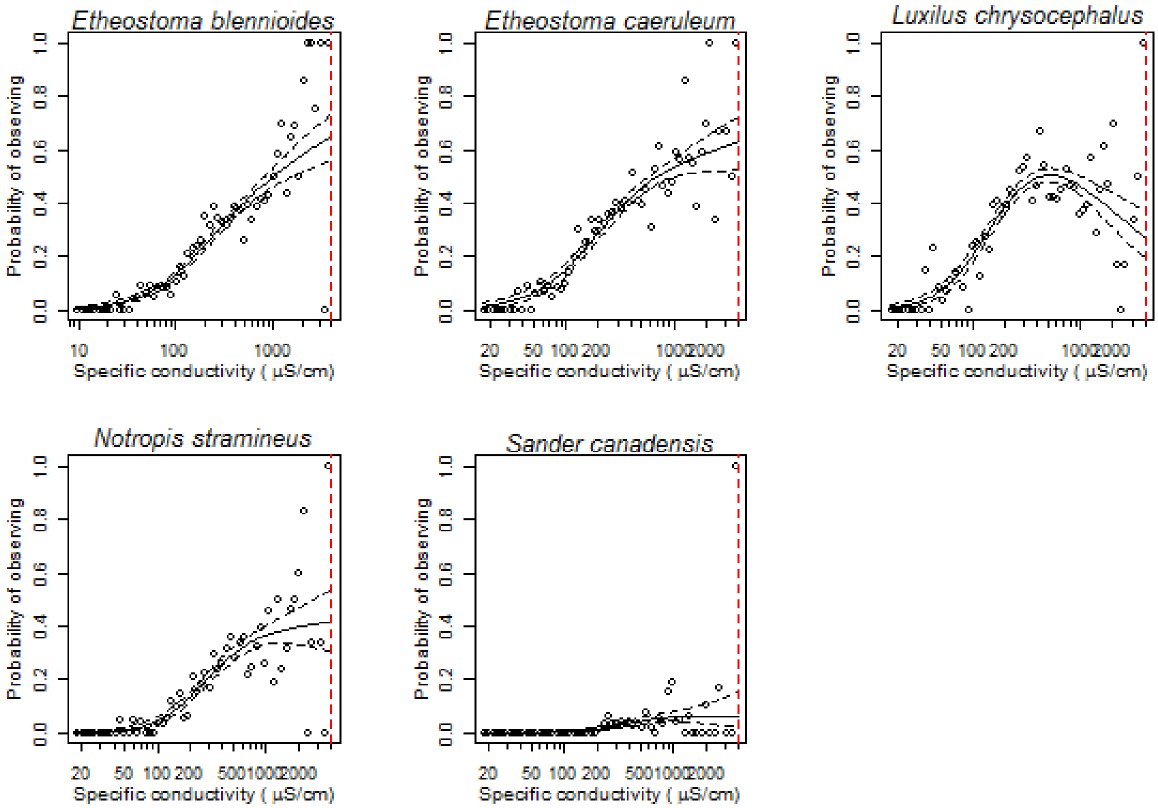

Ameiurus nebulosus
Minytrema melanops
Notemigonus crysoleucas
Notropis hudsonius
Pomoxis nigromaculatus
Perca flavescens
Esox americanus
Percina maculata
Carpiodes cyprinus
Ictiobus bubalus
Moxostoma anisurum
Pimephales promelas
Probability of observing
Specific conductivity (µS/cm)


Etheostoma spectabile
Lepomis microlophus
Lepomis gulosus
Phenacobius mirabilis
Clinostomus elongatus
Cottus bairdii
Aplodinotus grunniens
Etheostoma camurum
Lepomis gibbosus
Notropis volucellus
Pylodictis olivaris
Notropis atherinoides
Probability of observing
Specific conductivity (µS/cm)


Pomoxis annularis
Nocomis micropogon
Lampetra aepyptera
Notropis buccatus
Percina caprodes
Etheostoma nigrum
Etheostoma variatum
Moxostoma duquesnei
Moxostoma erythrurum
Noturus flavus
Pimephales vigilax
Lepomis megalotis
Probability of observing
Specific conductivity (µS/cm)

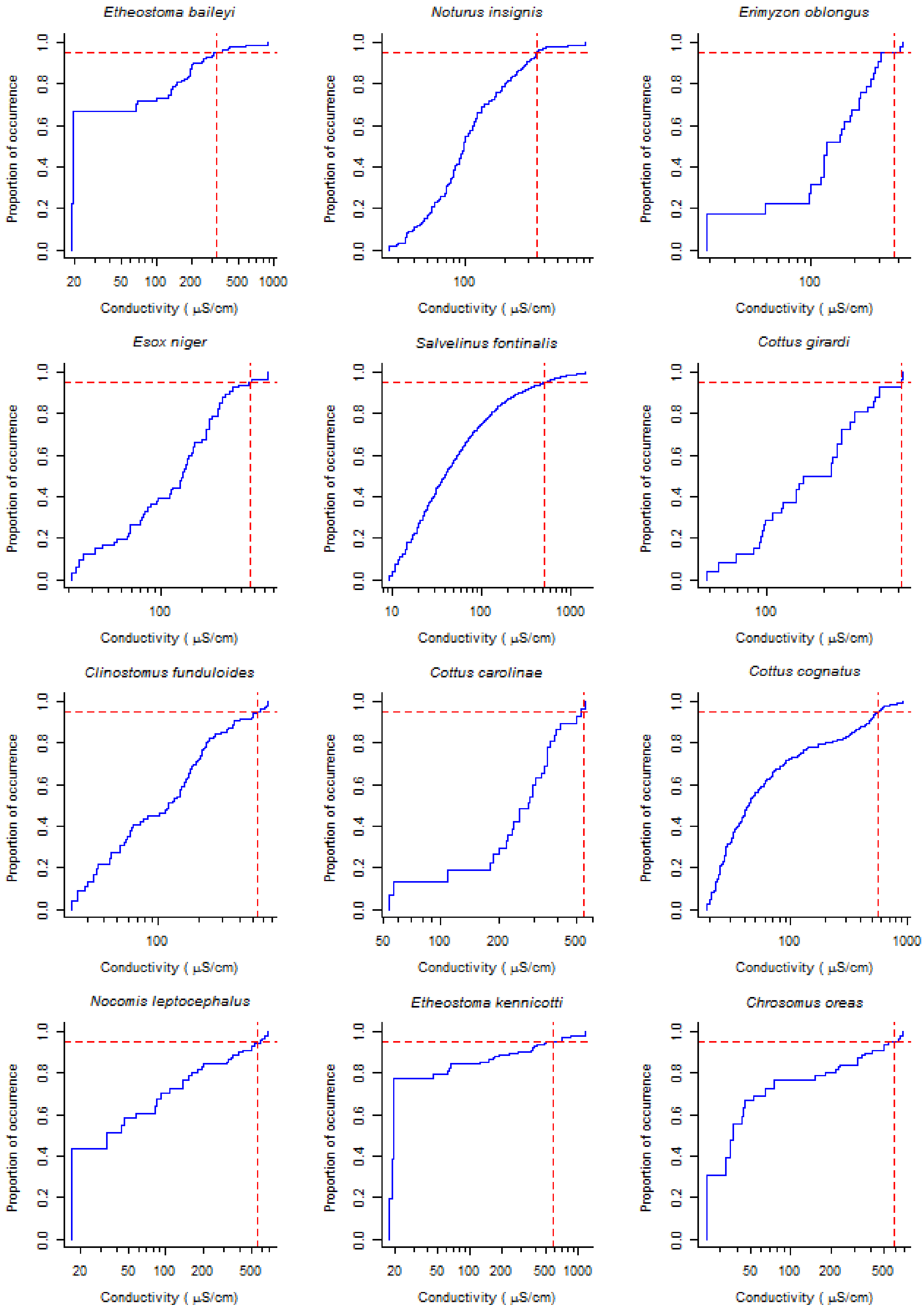

Micropterus salmoides
Notropis rubellus
Micropterus dolomieu
Dorosoma cepedianum
Ictalurus punctatus
Labidesthes sicculus
Lepomis macrochirus
Catostomus commersonii
Etheostoma zonale
Semotilus atromaculatus
Notropis photogenis
Chrosomus erythrogaster
Probability of observing
Specific conductivity (µS/cm)

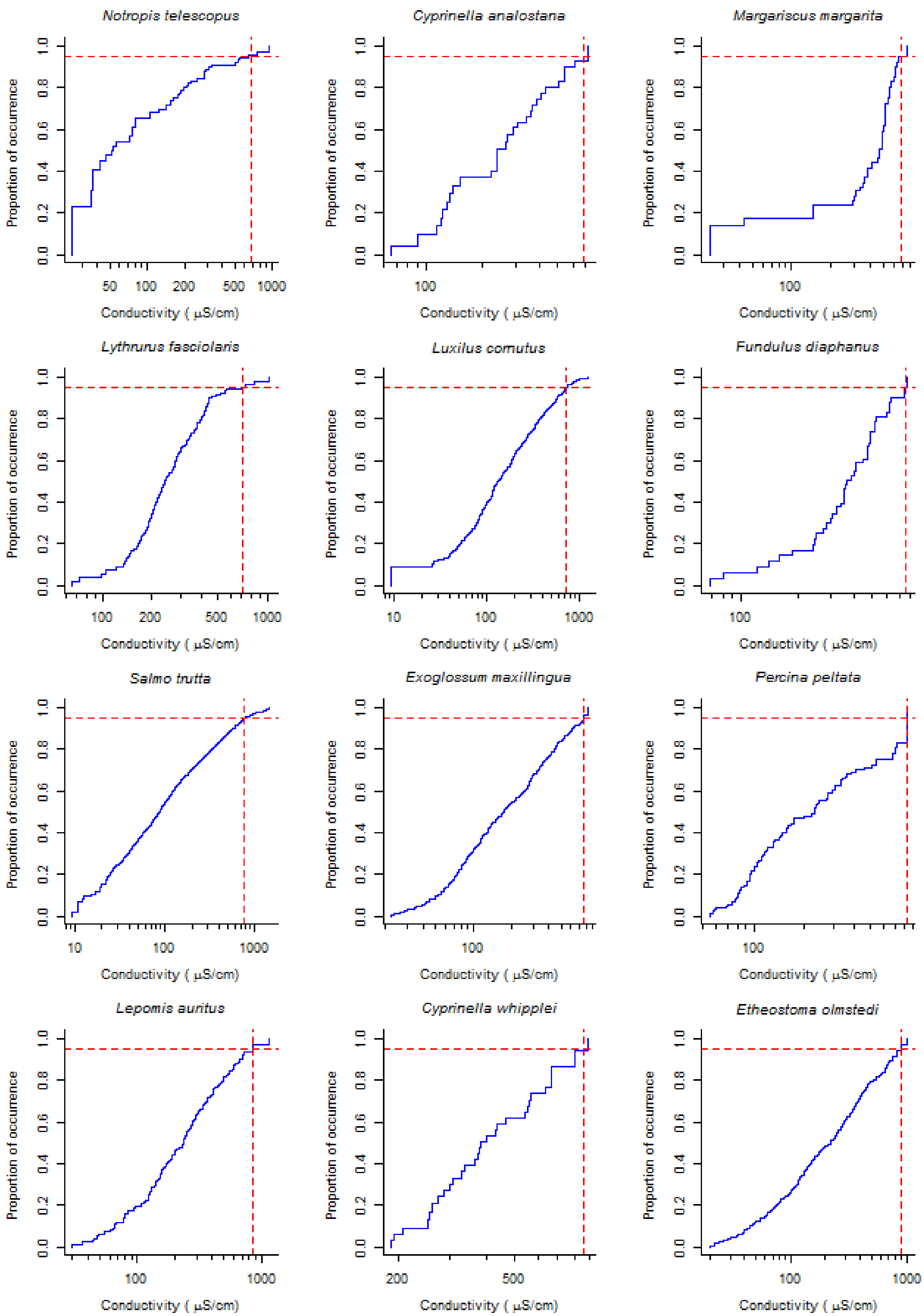

Micropterus punctulatus
Pimephales notatus
Rhinichthys obtusus
Ambloplites rupestris
Etheostoma flabellare
Lepomis cyanellus
Campostoma anomalum
Cyprinus carpio
Hypentelium nigricans
Rhinichthys atratulus
Ameiurus natalis
Cyprinella spiloptera
Probability of observing
Specific conductivity (µS/cm)

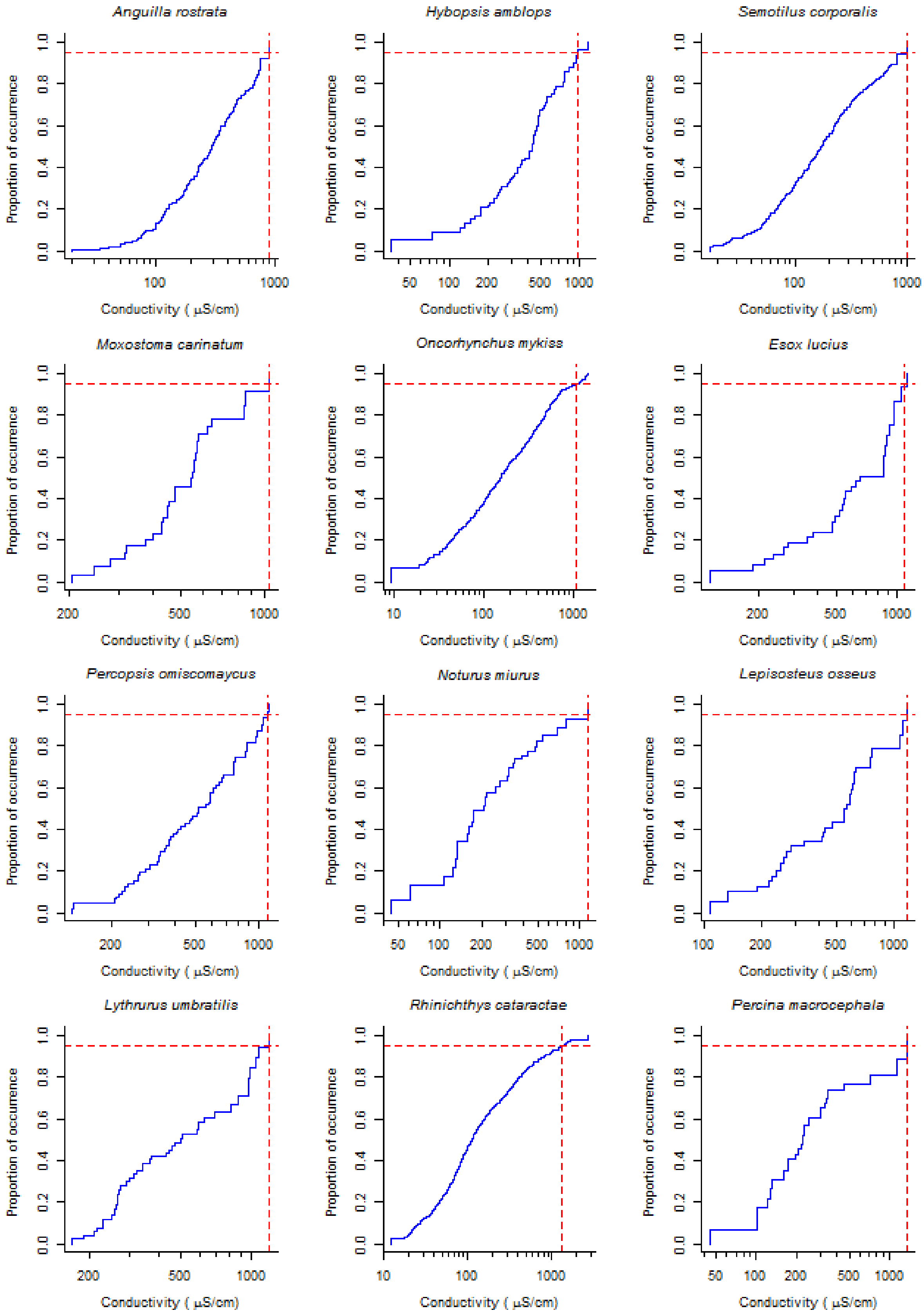

Etheostoma blennioides
Etheostoma caeruleum
Luxilus chrysocephalus
Notropis stramineus
Sander canadensis
Probability of observing
Specific conductivity (µS/cm)

G.9. GRAPHS OF CUMULATIVE DISTRIBUTION FUNCTION FOR EACH FISH SPECIES

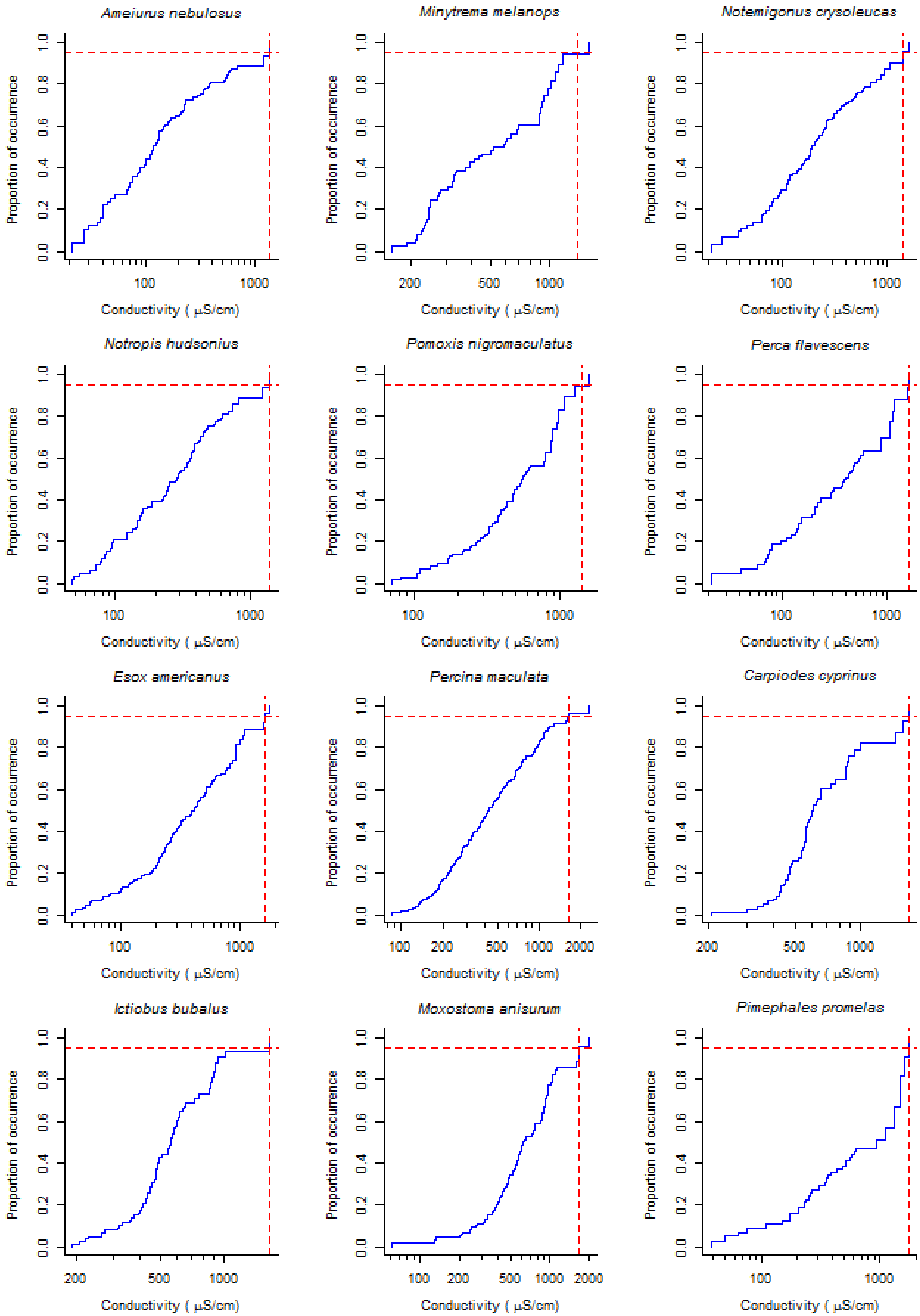
The purpose of this section is to help visualize the changes in the observations of each fish species as SC increases and to understand how the XC_{95} values are derived. Each plot contains the weighted CDF for the observations of a fish species with respect to SC and the associated XC_{95} value. The species are ordered from those having the smallest to the greatest XC_{95} value. For each species, the points in the CDF represent the weighted proportion of observations of each species in samples less than the indicated SC value (μS/cm), calculated using eq 3-1 in Section 3. The CDF was calculated from data collected in March through November. In a CDF, species that are most affected by increasing salinity (e.g., *Etheostoma baileyi*, *Lythrurus fasciolaris*) show a steep slope and asymptote below the measured range of exposures, whereas species unaffected by increasing salinity (e.g., *Semotilus atromaculatus*, *Etheostoma blennioides*) have a steady increase over the entire range of measured exposure and do not reach a perceptible asymptote. The 95^{th} centile is found at the intersection of the dashed horizontal line with the CDF. The SC at the 95^{th} centile is the XC_{95} value, which is found at the intersection of the vertical line and the *x*-axis.

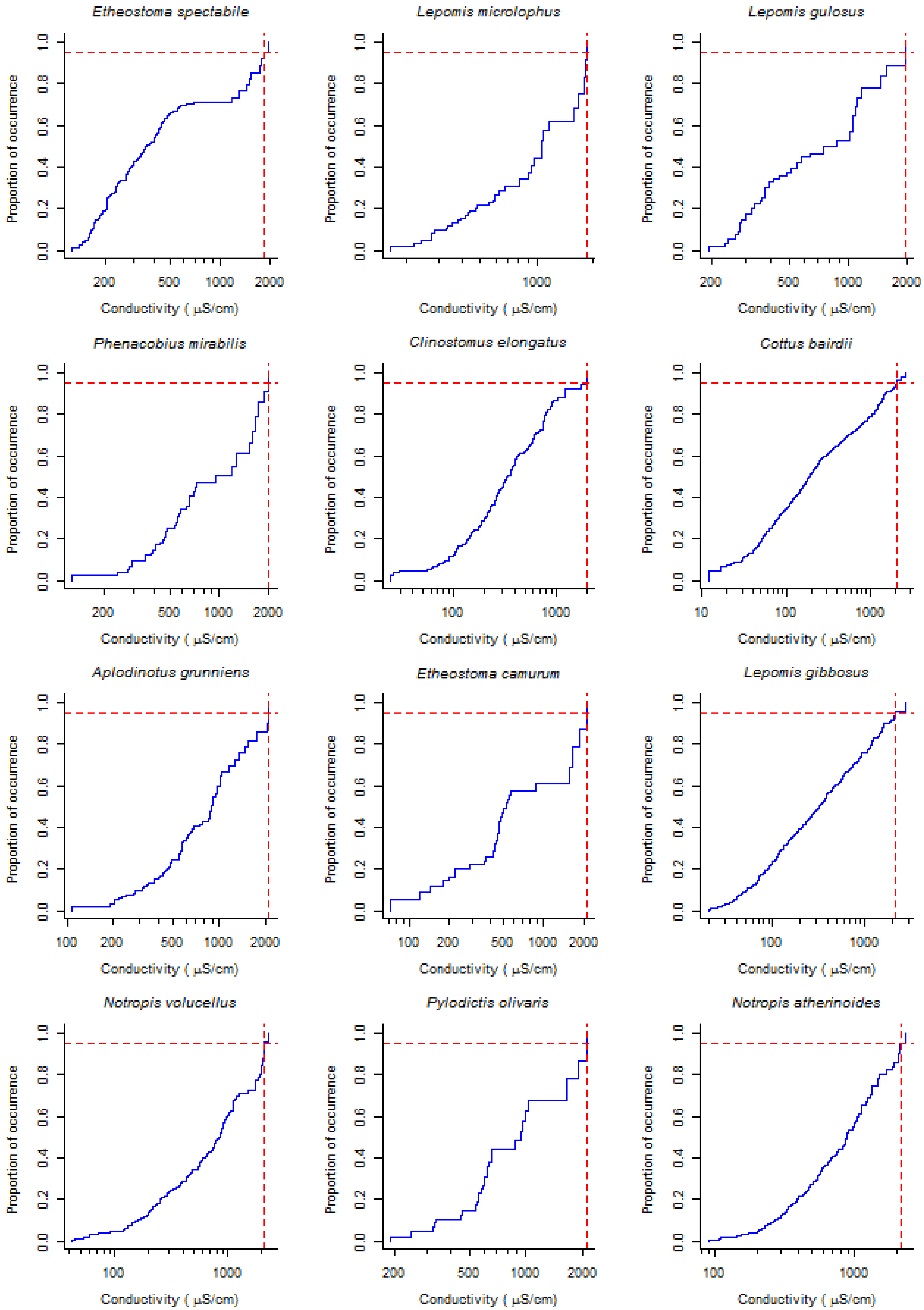

Etheostoma baileyi
Noturus insignis
Erimyzon oblongus
Esox niger
Salvelinus fontinalis
Cottus girardi
Clinostomus funduloides
Cottus carolinae
Cottus cognatus
Nocomis leptocephalus
Etheostoma kennicotti
Chrosomus oreas
Proportion of occurrence
Conductivity (µS/cm)

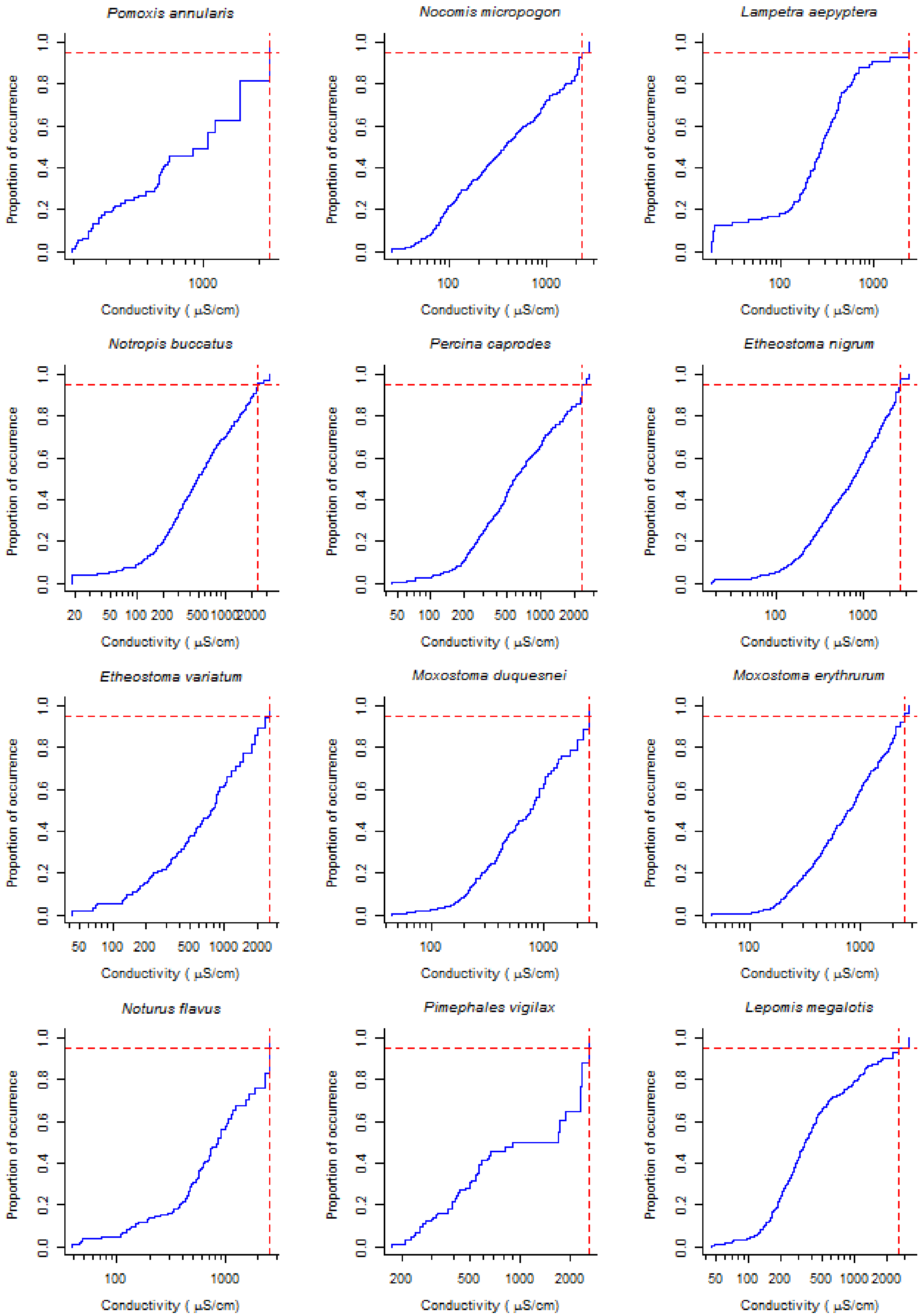

Notropis telescopus
Cyprinella analostana
Margariscus margarita
Lythrurus fasciolaris
Luxilus cornutus
Fundulus diaphanus
Salmo trutta
Exoglossum maxillingua
Percina peltata
Lepomis auritus
Cyprinella whipplei
Etheostoma olmstedi
Proportion of occurrence
Conductivity (µS/cm)

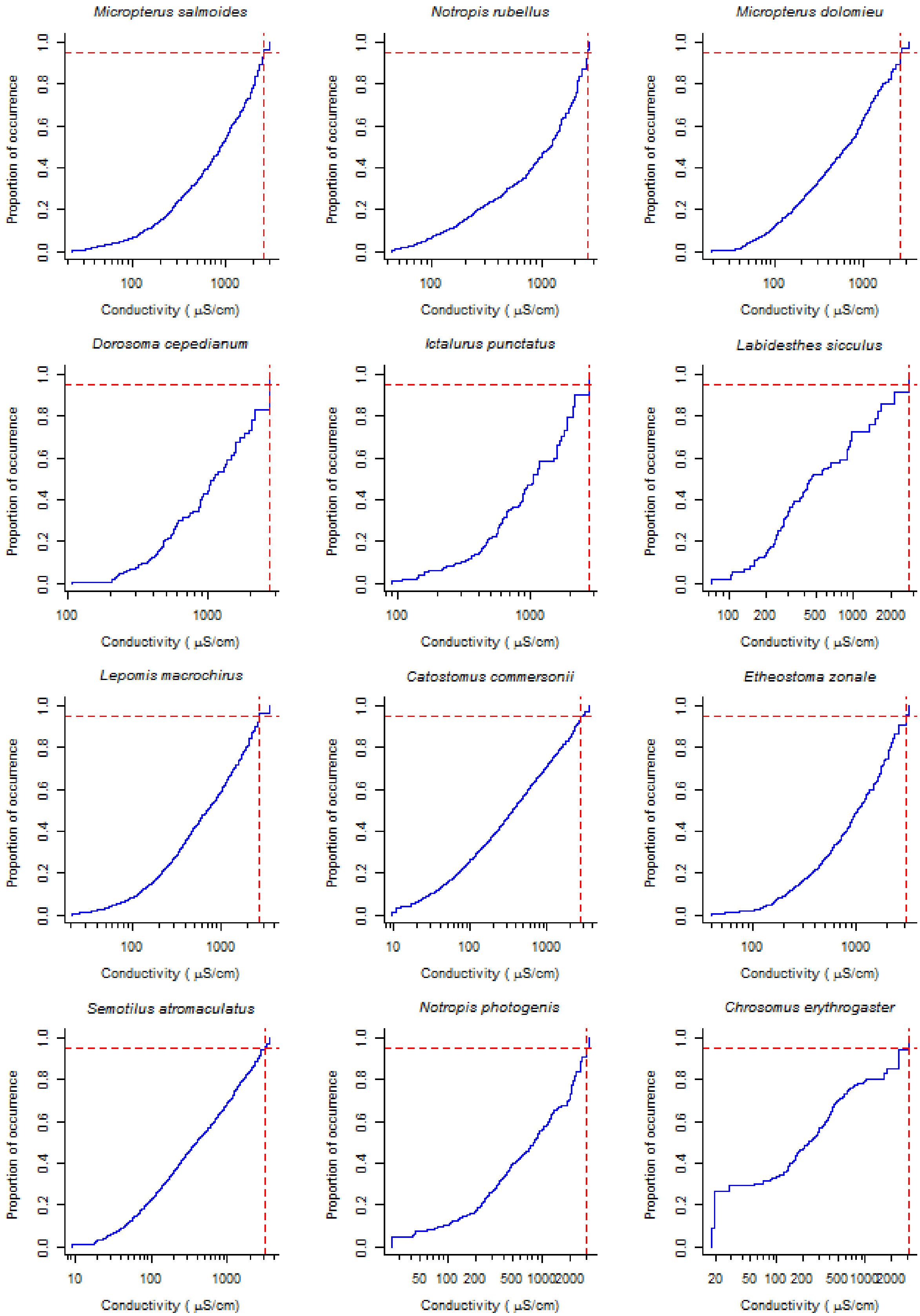


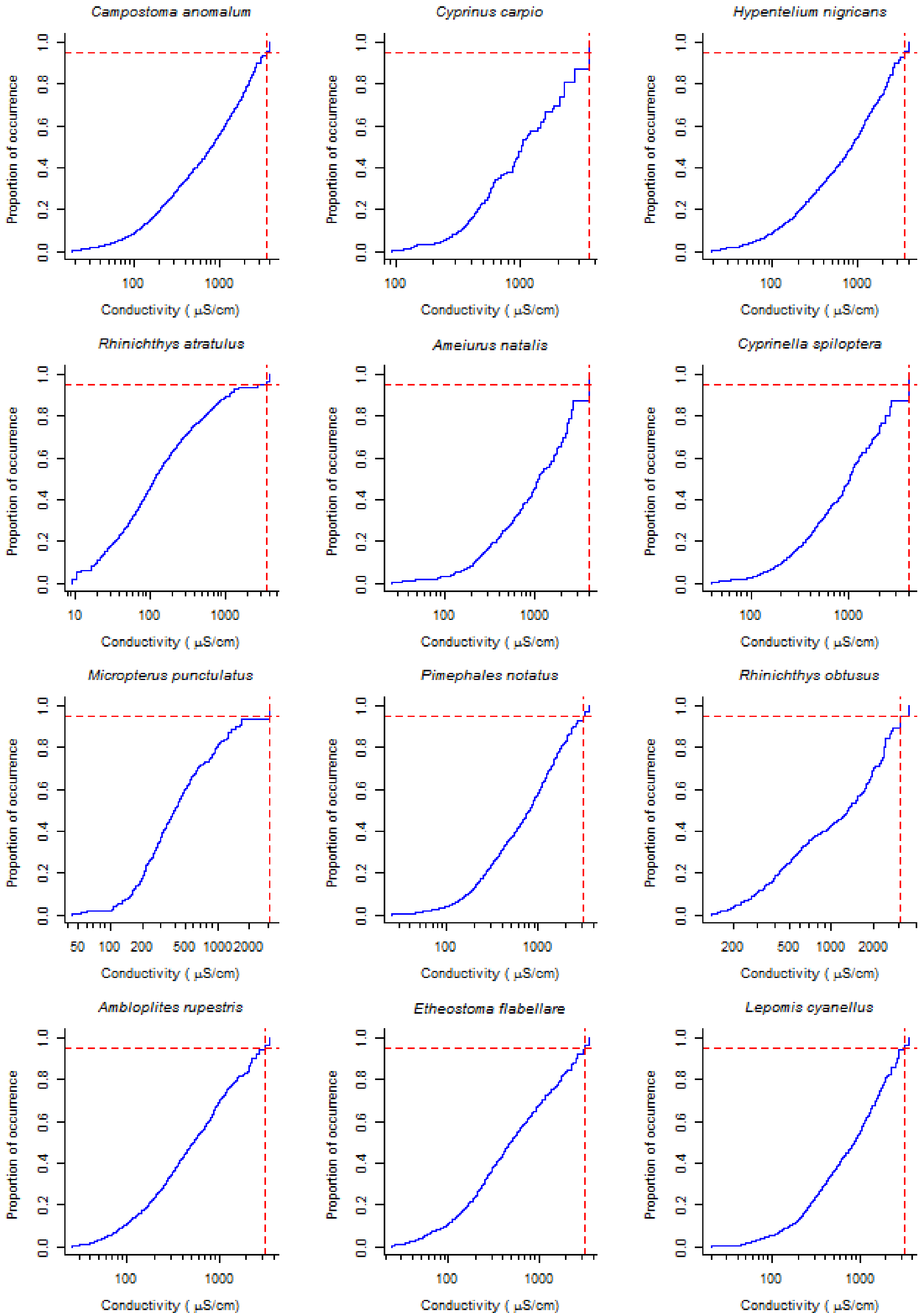
Anguilla rostrata
Hybopsis amblops
Semotilus corporalis
Moxostoma carinatum
Oncorhynchus mykiss
Esox lucius
Percopsis omiscomaycus
Noturus miurus
Lepisosteus osseus
Lythrurus umbratilis
Rhinichthys cataractae
Percina macrocephala
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
Conductivity (µS/cm)
10 50 100 200 500 1000

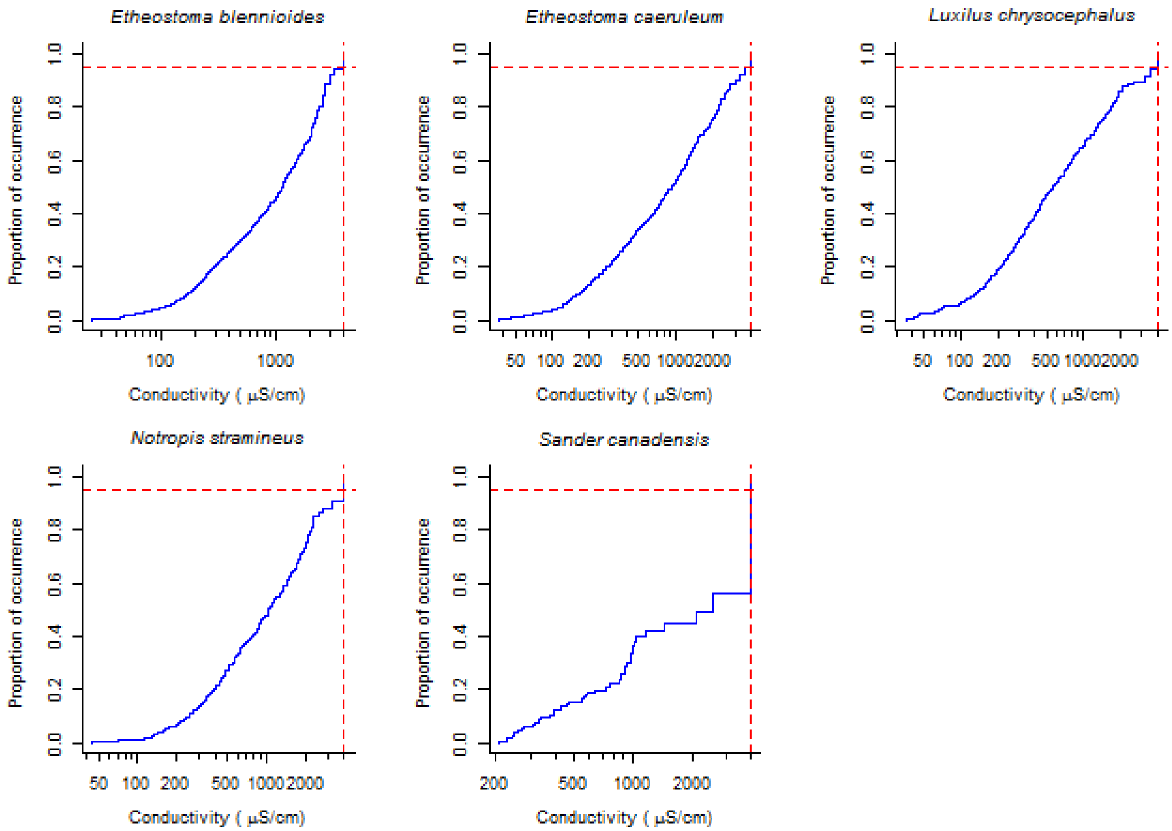

Ameiurus nebulosus
Minytrema melanops
Notemigonus crysoleucas
Notropis hudsonius
Pomoxis nigromaculatus
Perca flavescens
Esox americanus
Percina maculata
Carpiodes cyprinus
Ictiobus bubalus
Moxostoma anisurum
Pimephales promelas
Proportion of occurrence
Conductivity (µS/cm)


Etheostoma spectabile
Lepomis microlophus
Lepomis gulosus
Phenacobius mirabilis
Clinostomus elongatus
Cottus bairdii
Aplodinotus grunniens
Etheostoma camurum
Lepomis gibbosus
Notropis volucellus
Pylodictis olivaris
Notropis atherinoides
Proportion of occurrence
0.0
0.2
0.4
0.6
0.8
1.0
Conductivity (µS/cm)
10
100
200
500
1000
2000


Pomoxis annularis
Nocomis micropogon
Lampetra aepyptera
Notropis buccatus
Percina caprodes
Etheostoma nigrum
Etheostoma variatum
Moxostoma duquesnei
Moxostoma erythrurum
Noturus flavus
Pimephales vigilax
Lepomis megalotis
Proportion of occurrence
Conductivity (µS/cm)


Micropterus salmoides
Notropis rubellus
Micropterus dolomieu
Dorosoma cepedianum
Ictalurus punctatus
Labidesthes sicculus
Lepomis macrochirus
Catostomus commersonii
Etheostoma zonale
Semotilus atromaculatus
Notropis photogenis
Chrosomus erythrogaster
Proportion of occurrence
Conductivity (µS/cm)


Campostoma anomalum
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)
Cyprinus carpio
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)
Hypentelium nigricans
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)
Rhinichthys atratulus
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
10 100 1000
Conductivity (µS/cm)
Ameiurus natalis
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)
Cyprinella spiloptera
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)
Micropterus punctulatus
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
50 100 200 500 1000 2000
Conductivity (µS/cm)
Pimephales notatus
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)
Rhinichthys obtusus
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
200 500 1000 2000
Conductivity (µS/cm)
Ambloplites rupestris
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)
Etheostoma flabellare
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)
Lepomis cyanellus
Proportion of occurrence
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
Conductivity (µS/cm)


Etheostoma blennioides
Etheostoma caeruleum
Luxilus chrysocephalus
Notropis stramineus
Sander canadensis
Proportion of occurrence
Conductivity (µS/cm)
0.0 0.2 0.4 0.6 0.8 1.0
100 1000
50 100 200 500 1000 2000
200 500 1000 2000

G.10. REFERENCES

Allan, JD. (1981) Determinants of diet of brook trout (Salvelinus fontinalis) in a mountain stream. Can J Fish Aqua Sci 38:184−92.

Barbour, MT; Gerritsen, J; Snyder, BD; Stribling, JB. (1999) Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. 2nd ed. EPA/841/B-99/002. Washington, DC: U.S. Environmental Protection Agency, Office of Water. Available online at http://www.epa.gov/owow/monitoring/rbp/wp61pdf/rbp.pdf.

Bradley, TJ. (2009) Animal osmoregulation. Oxford, U.K: Oxford University Press.

Bryant, G; McPhilliamy, S; Childers, H. (2002) A survey of the water quality of stream in the primary region of mountaintop/valley fill coal mining, October 1999 to January 2001. In: Draft programmatic environmental impact statement on mountaintop mining/valley fills in Appalachia—2003. Appendix D. Philadelphia, PA: U.S. Environmental Protection Agency, Region 3. Available online at http://www.cet.edu/pdf/mtmvfchemistry.pdf.

Cada, GF; Loar, JM; Sale, MJ. (1987) Evidence for food limitation of rainbow and brown trout in Southern Appalachian soft-water streams. Trans Am Fish Soc 116:692−702.

CP (Commonwealth of Pennsylvania). (2013) Chapter 75. Endangered species. Pennsylvania code title 58. Recreation, Part II. Fish and Boat Commission, Subpart B. Fishing. Harrisburg, PA: Commonwealth of Pennsylvania. Available online at http://www.pacode.com/secure/data/058/chapter75/chap75toc.html.

Detenbeck, NE; Cincotta, D; Denver, JM; Greenlee, SK; Olsen, AR; Pitchford, AM. (2005) Watershed based survey designs. Environ Monitor Assess 103(1−3):59−81.

Efron, B; Tibshirani, R. (1993) An introduction to bootstrap. Monographs on statistics and applied probability 57. Boca Raton, FL: Chapman & Hall/CRC Press.

Evans, DH. (2008a) Osmotic and ionic regulation: cells and animals. Boca Raton, FL: CRC Press. 606 pp.

Evans, DH. (2008b) Teleost fish osmoregulation: what have we learned since August Krogh, Homer Smith, and Ancel Keys? Am J Physiol Regul Intgr Comp Physiol 295(2):R704−R713.

Fenneman, NM (1938) Physiography of the Eastern United States. New York, NY: McGraw-Hill Book Company.

Gerritsen, J; Burton, J; Barbour, MT. (2000) A stream condition index for West Virginia wadeable streams. Prepared by: Tetra Tech, Inc. Owing Mills, MD. Prepared for: U.S. EPA Region 3 Environmental Services Division, and U.S. EPA Office of Science and Technology, Office of Water. Available online at http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/WVSCI.pdf.

Griffith, MB. (2014) Natural variation and current reference for specific SC and major ions in wadeable streams of the coterminous U.S. Freshw Sci 33(1):1−17.

Griffith, MB. (2016). Toxicological perspective on the osmoregulation and ionoregulation physiology of major ions by freshwater animals: Teleost fish, crustacea, aquatic insects, and Mollusca: Osmoregulation and ionoregulation physiology of major ions. Environ Toxicol Chem. Epub DOI: 10.1002/etc.3676

Hastie, T; Tibshirani, R. (1986) Generalized additive models. Stat Sci 1(3):297−318.

Herlihy, AT; Larsen DP; Paulsen, SG; Urguhart, NS; Rosenbaum, GJ. (2000) Designing a spatially balanced, randomized site selection process for regional stream surveys: The EMAP mid-Atlantic pilot study. Environ Monit Assess 63:95−113.

Hitt, NP; Chambers, DB. (2014) Temporal changes in taxonomic and functional diversity of fish assemblages downstream from mountaintop mining. Freshw Sci 33(3):915−926. Available online at http://www.jstor.org/stable/10.1086/676997.

Hocutt, CH; Wiley, EO. (1986) The zoogeography of North American freshwater fishes. New York, NY: John Wiley & Sons, Inc.

KDEP-DOW (Kentucky Department of Environmental Protection-Division of Water). (2009a) Standard operating procedure: In situ water quality measurements and meter calibration standard operating procedure. Frankfort, KY: Kentucky Department of Environmental Protection, Division of Water. Available online at http://water.ky.gov/permitting/Documents/InsituwaterqualitymeasurementsandmetercalibrationSOP.pdf.

KDEP-DOW (Kentucky Department of Environmental Protection-Division of Water). (2009b) Standard operating procedure: Sampling surface water quality in lotic systems. Frankfort KY: Kentucky Department of Environmental Protection, Division of Water.

KDEP-DOW (Kentucky Department of Environmental Protection-Division of Water). (2009c) Standard operation procedure: Laboratory procedures for fish processing and taxonomic identification. Frankfort, KY: Kentucky Department of Environmental Protection, Division of Water. Available online at http://water.ky.gov/Documents/QA/Surface%20Water%20SOPs/FishLaboratoryProceduresandIdentificationSOP.pdf.

KDEP-DOW (Kentucky Department of Environmental Protection-Division of Water). (2010) Standard operating procedure: Collection methods for fish in wadeable streams. Revision 2.1. Frankfort, KY: Kentucky Department of Environmental Protection, Division of Water. Available online at http://water.ky.gov/Documents/QA/Surface%20Water%20SOPs/SOPCollectionMethodsforfishinwadeablestreams21_FINAL.pdf.

KDFWR (Kentucky Department of Fish and Wildlife Resources). (2013) Kentucky's comprehensive wildlife conservation strategy, Appendix 1.1. Frankfort, KY: Kentucky Department of Fish and Wildlife Resources. Available online at http://fw.ky.gov/WAP/Documents/1.1%20ListSpeciesForTable.pdf.

Kimmel, WG; Argent, DG. (2010) Stream fish community responses to a gradient of specific conductance. Water Air Soil Pollut 206(1−4):49−56.

Lazorchak, JM; Klemm, DJ; Peck, DV; eds. (1998) Environmental monitoring and assessment program—surface waters: Field operations and methods for measuring the ecological condition of wadeable streams. EPA/620/R-94/004F. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. Available online at https://archive.epa.gov/emap/archive-emap/web/html/ws_chap.html.

Marshall, WS. (2002) Na^+, Cl^-, Ca_{2+} and Zn_{2+} transport by fish gills: retrospective review and prospective synthesis. J Exp Zool 293:264−283.

McCormick, FH; Hughes, RM; Kaufmann, PR; Peck, DV; Stoddard, JL; Herlihy, AT. (2001) Development of an index of biotic integrity for the mid Atlantic Highlands region. Trans Am Fish Soc 130(5):857−877.

MDNR-NHP (Maryland Department of Natural Resources, Natural Heritage Program). (2010) Rare, threatened, and endangered animals of Maryland. Annapolis, MD: Maryland Department of Natural Resources. Available online at http://dnr.maryland.gov/wildlife/Documents/rte_Animal_List.pdf.

ODNR-DW (Ohio Department of Natural Resources, Division of Wildlife). (2014) Wildlife that are considered to be endangered, threatened, species of concern, special interest, extirpated, or extinct in Ohio. Publication 5356. Columbus, OH: Ohio Department of Natural Resources. Available online at http://wildlife.ohiodnr.gov/species-and-habitats/state-listed-species.

OEPA-DSW (Ohio Environmental Protection Agency, Division of Surface Water). (1989a) Biological criteria for the protection of aquatic life: Volume III. Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Columbus, OH: Ohio Environmental Protection Agency, Division of Surface Water, Ecological Assessment Section. Available on-line at http://epa.ohio.gov/portals/35/documents/Vol3.pdf.

OEPA-DSW (Ohio Environmental Protection Agency, Division of Surface Water). (1989b) The qualitative habitat evaluation index [QHEI]: Rationale, methods, and application. Columbus, OH: Ohio Environmental Protection Agency, Division of Surface Water, Ecological Assessment Section. Available online at http://epa.ohio.gov/Portals/35/documents/QHEI_1989.pdf.

OEPA-DSW (Ohio Environmental Protection Agency, Division of Surface Water). (2013a) Surface water field sampling manual for water column chemistry, bacteria and flows. Columbus, OH: Ohio Environmental Protection Agency, Division of Surface Water, Ecological Assessment Section. Available online at http://epa.ohio.gov/Portals/35/documents/SW_SamplingManual.pdf.

OEPA-DSW (Ohio Environmental Protection Agency, Division of Surface Water). (2013b) 2013 updates to Biological criteria for the protection of aquatic life: Volume III. Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Columbus, OH: Ohio Environmental Protection Agency, Division of Surface Water, Ecological Assessment Section. Available online at http://epa.ohio.gov/portals/35/documents/BioCrit88_Vol3Updates2013.pdf.

Omernik, JM. (1987). Ecoregions of the conterminous United States. Ann Assoc Am Geograph 77:118−125.

PA DEP (Pennsylvania Department of Environmental Protection). (2013) Wadeable semi-quantitative fish sampling protocol for streams. Harrisburg, PA: Pennsylvania Department of Environmental Protection, Bureau of Point and Non-point Source Management.

R Development Core Team (2011) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available online at http://www.R-project.org.

Richardson, JS. (1993) Limits of productivity of streams: Evidence from studies of macroinvertebrates. Can Spec Pub Fish Aqua Sci 118:9−15.

Smithson, J. (2007) West Virginia stream/river survey design 2007—2111. Charleston, WV. West Virginia Department of Environmental Protection, Division of Water and Waste Management.

Stauffer, JR; Boltz, JM; White, LR. (1995) The fishes of West Virginia. Proc Nat Sci Phila 146:1−389.

Stauffer, JR; Ferreri, CP. (2002) Characterization of stream fish assemblages in selected regions of mountain top removal/valley fill coal mining. In: Draft programmatic environmental impact statement on mountaintop mining/valley fills in Appalachia (2003) Appendix D. Philadelphia, PA: U.S. Environmental Protection Agency, Region 3. Available online at http://www.epa.gov/region03/mtntop/eis2003appendices.htm#appd.

U.S. EPA (Environmental Protection Agency) (1987) Handbook of methods for acid deposition studies: Laboratory analyses for surface water chemistry. EPA/600/4-87/026. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Acid Deposition and Atmospheric Research Division. Available online at http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30000TA0.txt.

U.S. EPA (Environmental Protection Agency). (2007) Level III ecoregions of the continental United States (revision of Omernik, 1987). Corvallis, OR: National Health and Environmental Effects Research Laboratory. Available online at http://kgs.uky.edu/kgsweb/download/geology/useco.pdf.

U.S. EPA (Environmental Protection Agency). (2011a) A field-based aquatic life benchmark for conductivity in Central Appalachian streams. EPA/600/R-10/023F. Washington, DC: Office of Research and Development, National Center for Environmental Assessment. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=233809.

U.S. EPA (Environmental Protection Agency). (2011b) Review of field-based aquatic life benchmark for conductivity in Central Appalachian streams. Washington, DC: Science Advisory Board, Office of the Administrator. Available online at: http://yosemite.epa.gov/sab/sabproduct.nsf/0/EEDF20B88AD4C6388525785E007331F3/$File/EPA-SAB-11-006-unsigned.pdf.

VADGIF (Virginia Department of Game and Inland Fisheries). (2014) Special legal status faunal species in Virginia. Richmond, VA: Wildlife Diversity Division, Virginia Department of Natural Resources. Available online at http://www.dgif.state.va.us/wildlife/virginiatescspecies.pdf.

Wilken, E; Nava, FJ; Griffith, G. (2011) North American terrestrial ecoregions—Level III. Montreal, QC, Canada: Commission for Environmental Cooperation. Available online at http://www3.cec.org/islandora/en/item/10415-north-american-terrestrial-ecoregionslevel-iii-en.pdf .

Wood, CM; Shuttleworth, TJ. (2008) Cellular and molecular approaches to fish ionic regulation. Vol. 14: Fish Physiology. San Diego, CA: Academic Press, Inc.

Woods, Omernik, JM; Moran, BC. (2007) Ecoregions of New Jersey (map and descriptive text, map scale 1:500,000). Corvallis, OR: U.S. Environmental Protection Agency, Office of Research and Development. Available online at https://www.epa.gov/eco-research/ecoregions-publications.

Woods, AJ; Omernik, JM; Martin, WH; Pond, GJ; Andrews, WM; Call, SM; Cornstock, JA; Taylor, DD. (2002) Ecoregions of Kentucky (color poster with map, descriptive text, summary tables, and photographs, map scale 1:1,000,000). Reston, VA: U.S. Geological Survey. Available online at http://www.epa.gov/wed/pages/ecoregions/ky_eco.htm.

Woods, AJ; Omernik, JM; Brown, DD. (1996) Level III and IV ecoregions of Pennsylvania and the Blue Ridge Mountains, the central Appalachian Ridge and Valley, and the central Appalachians of Virginia, West Virginia, and Maryland. EPA/600/R-96/077. Corvallis, OR: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory. http://training.fws.gov/courses/csp/csp3200/resources/documents/epa_region_3_eco_desc.pdf.

WVDEP (West Virginia Department of Environmental Protection). (2009) 2009 Standard operating procedures. Charleston, WV: West Virginia Department of Environmental Protection, Watershed Branch. Available online at http://www.dep.wv.gov/WWE/watershed/wqmonitoring/Documents/SOP%20Doc/WAB%20SOP.pdf.

WVDNR (West Virginia Department of Natural Resources). (2012) Rare, threatened and endangered animals. Charleston, WV: West Virginia Department of Natural Resources. Available online at http://www.wvdnr.gov/Wildlife/PDFFiles/RTE_Animals_2012.pdf.