**Supplemental Appendix S1-- Example calculation: Step-by-Step Calculation and Spreadsheet Tools for Predicting Stressor Levels that Extirpate Genera and Species**

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# INTRODUCTION

Supplemental Appendix S1 describes, in detail, the arithmetic operations that can be performed by hand or, more expeditiously, by using the Biological Extirpation Analysis Tools (BEAT) v1.3 (Supplemental Appendices S2 and S3). Two tools are provided, one to estimate an XC95 (95th centile extirpation concentration; XC95-BEAT) and another to estimate an HC05 (hazardous concentration extirpating 5% of taxa; HC05-BEAT). The example calculations use a paired data set from Central Appalachia (Supplemental Appendices S4-S9). For the HC05 BEAT (Supplemental Appendix S3), we include XC95 specific conductivity (SC) values for 176 distinct aquatic benthic invertebrates to calculate the HC05.

# METHODS AND EXAMPLES

## Data set used in the Example Calculation

The Central Appalachians (Ecoregion 69) and Western Allegheny Plateau (Ecoregion 70) in West Virginia (Supplemental Appendix S1 Figure S1) include all of the state west of the Ridge and Valley (Ecoregion 67) (USEPA. 2010, 2013; Omernik, 1987; Woods et al., 1996). The hills and mountains are composed of sandstone, shale, conglomerate, limestone outcroppings, and coal. Underground and surface bituminous coal mines are common (Woods et al., 1999, 1996). Background specific conductivity (SC) is among the lowest in the United States (USEPA 2011, Griffith 2014). Wheeling and Charleston are the two largest cities, each with populations of about 50,000.

The data used in the example calculations are from perennial streams collected by the West Virginia Department of Environmental Protection (WVDEP). We used chemical (1996−2011) and biological data (1997−2010) from the WVDEP in‑house Watershed Assessment Branch database (WABbase) as described in USEPA (2016). The WABbase contains data from a mixed sampling design that collects measurements from long‑term monitoring stations, targeted sites within watersheds on a rotating basin schedule, randomly selected sample sites (Smithson, 2007), and sites chosen to further define impaired stream segments in support of total maximum daily load development (WVDEP, 2008a). Most sites are sampled once during an annual sampling period. The data set contains water quality, habitat, watershed characteristics, macroinvertebrate data (both raw data and calculated metrics), and geographic location (WVDEP, 2008a). A wide range of SC levels were sampled, which is useful for modeling the response of organisms to different levels of ionic concentration.

Macroinvertebrate records in the data set are based on collections from a total of 1 m2 area of a 100 m reach at each site. Using a 0.5 m wide rectangular kicknet (595 μm mesh), four 0.25 m2 riffle areas were sampled. In narrow streams, nine areas were sampled with a 0.33 m wide D‑frame dipnet of the same mesh size. Composited samples were preserved in 95% denatured ethanol. A random subsample of 200 individuals (±20%) was identified in the laboratory. All contracted analyses for chemistry and macroinvertebrate identification followed WVDEP’s internal quality control and quality‑assurance protocols (WVDEP, 2006, 2008b).

Several data filters, were applied prior to finalization of the data set and analyses. Samples were removed from the data set with pH ≤ 6 or a high proportion of chloride ions ([HCO3−] + [SO42−] ≤ [Cl−]). A total of 3,734 paired samples were identified with SC measurements and biological samples from Ecoregions 69 and 70. SC ranged from 15−11,646 μS/cm. A total of 176 genera occurred at 25 or more sampling locations and at least once at reference sites identified in the WABbase. The full data set used to derive the example benchmark is available in Supplemental Appendix S5. The statistical package R, Version 3.1.1 was used for some statistical analyses (R Development Core Team, 2014). The R Hmisc package (Harrell et al., 2015) is the basis for cumulative distribution of XC95 values (XCD) calculation.



Figure S1. The geographical locations sampled in Ecoregions 69 (yellow area in southeast) and Ecoregion 70 (green area in northwest).

## Calculations

All calculations follow the methods found in USEPA 2011 and 2016. For each genus meeting data‑selection conditions listed above, an empirical cumulative frequency distribution (CFD) was weighted to correct for any potential bias from the unequal distribution of sampling of sites across the range of stressor exposures such as SC. This weighted CFD represents the proportion of observations of a taxon that occurs below given exposure levels. The extirpation effect threshold for a taxon is defined as the 95th centile point on the CFD of the total observations of the taxon. The two exposure levels bracketing the 95th centile are linearly interpolated to give an XC95 for a taxon.

For each genus, a probability plot was calculated. The y‑axis shows the probability of observing a taxon, in this instance, the fraction of sampling sites within a given bin of SC values in which this genus was observed. This probability plot was used to assess whether an XC95 is defined (i.e., determined to be equal to the value) or undefined (greater than the XC95 calculated by the CFD). The XC95 was judged as accepted when a genus probability plot decreased at the higher SC ranges and some probabilities were zero. The XC95 was judged to be undefined when a genus probability plot increased or was static at the higher SC ranges and some probabilities were greater than zero.

Calculation of the probability plot, the empirical weighted CFD, and the resulting XC95 involves several steps, all of which are automated in the XC95 BEAT. First, equally‑sized bins are defined to compute weights for each sample. The selection of bin size depends on the size of the data set and requires balancing the requirements of sufficient observations in a bin to define the proportion and sufficient bins to define the form of the response. The default number of bins in the XC95 BEAT is 60. For the example, 60 bins are defined and each bin was assigned a width equal to 0.017 (1/60) multiplied by the range of the log10 transformed SC values within the data set. The user can choose to change the number of bins on the data input worksheet.

### Example calculation using the XC95 BEAT

The details of manual calculation of the XC95 are shown in this example. The sample data is used to calculate the XC95 with SC as the stressor and *Acentrella* as the genus. These calculations are automated in the spreadsheet tool. (Note: to make it easier to follow the process, we have rounded some numbers to describe the example; however, this will not occur in the actual calculation by the Excel® macro). The number of bins can be set on the data input worksheet.

### Calculating weights

The XC95 BEAT calculates base-10 logarithms of SC for each sample. Next it divides the range of SC (i.e., minimum to maximum) by 60. The result is the width of a SC bin in log units. For the example: Assuming that the minimum and maximum are 15.4 and 11,646 µS/cm, respectively. These are converted to Log10 scale.

Max = log10 [11,646 µS/cm] ≈ 4.07 µS/cm

Min = log10 [15.4 µS/cm] = 1.19 µS/cm

The range is 4.07 µS/cm − 1.19 µS/cm = 2.88 µS/cm Eq. 1

The calculation for the standard bin widths on a Log10 scale for 60 bins for the data set is:

2.88 µS/cm / 60 = 0.048 µS/cm Eq. 2

The upper bound of each bin interval is calculated by multiplying the rank (1 to 60) of the interval by the standard bin width, plus the minimum stressor value.

The calculation for the first bin is as follows:

Minimum observed SC (log10 1.19 µS/cm) + standardized bin width (log10 0.048 µS/cm) = upper bin range (log10 1.24 µS/cm). The range for bin ranked #1 is therefore 1.19 to 1.24 µS/cm log units. The bin ranked #2 is > 1.24 to 1.28 µS/cm log units, and the process is repeated until the maximum observed SC is included in a bin which will be the 60th bin (Table S1). To convert back to real numbers: the lower bin limit is 101.19 = 15.4 µS/cm and the upper limit of the bin is 101.24 = 17.2µS/cm. So, the first bin limits are 15.4 – 17.2 µS/cm and the second bin is >17.2−19.2 µS/cm.

Next, the XC95 BEAT assigns each sample to a bin and counts the number of samples within each bin. Then it normalizes the sampling probability in each bin to 1 by weighting. The total number of sites in a bin multiplied by its bin weight (*w*) equals 1. To do this, it calculates the weight for each bin by taking the inverse of the count of sites that occur in each bin for the whole data set. Finally, it calculates normalized weights (Table S1, Column 7) by dividing each weight by the sum of all weights.

For example, if there are 3 samples within a bin (Table S1, Rank 1), the weight for that bin is ⅓ (Table S1, Column 6). The sum of all weights in Column 6 is 7.37 (Table S1). So, the normalized weight for the first bin (rank 1) is 0.33 / 7.37 = 0.045.

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| --- | --- | --- | --- | --- | --- | --- |
| Table S1. Bin intervals and number of sites in each specific conductivity bin, along with the weights and normalized weights for each bin. Only a portion of table is shown to illustrate the process. | | | | | | |
| **Rank** | **Upper Limit of Bin, Log10 µS/cm** | **Upper Limit of Bin, Real Numbers µS/cm** | **Rounded Bin Intervals (µS/cm)** | **# Sites per Bin** | **Weight**  **(1/# sites)** | **Normalized Weight** |
| 1 | 1.24 | 17.2 | 15.4−17.2 | 3 | 1/3 | 0.045 |
| 2 | 1.28 | 19.2 | >17.2−19.2 | 3 | 1/3 | 0.045 |
| 3 | 1.33 | 21.5 | >19.2−21.5 | 5 | 1/5 | 0.027 |
| 4 | 1.38 | 24 | >21.5−24 | 7 | 1/7 | 0.019 |
| 5 | 1.43 | 26.8 | >24−26.8 | 13 | 1/13 | 0.010 |
| 6 | 1.48 | 29.9 | >26.8−29.9 | 7 | 1/7 | 0.019 |
| … | | | | | | |
| 35 | 2.87 | 736 | >659-736 | 98 | 1/98 | 0.001 |
| 36 | 2.91 | 822 | >736-822 | 81 | 1/81 | 0.002 |
| 37 | 2.96 | 918 | >822-918 | 74 | 1/74 | 0.002 |
| 38 | 3.01 | 1,025 | >918-1,025 | 88 | 1/88 | 0.002 |
| 39 | 3.06 | 1,145 | >1,025-1,145 | 66 | 1/66 | 0.002 |
| 40 | 3.11 | 1,278 | >1,145-1,278 | 75 | 1/75 | 0.002 |
| 41 | 3.15 | 1,428 | >1,278-1,428 | 68 | 1/68 | 0.002 |
| 42 | 3.20 | 1,594 | >1,428-1,594 | 63 | 1/63 | 0.002 |
| 43 | 3.25 | 1,781 | >1,594-1,781 | 47 | 1/47 | 0.003 |
| 44 | 3.30 | 1,989 | >1,781-1,989 | 31 | 1/31 | 0.004 |
| 45 | 3.35 | 2,221 | >1,989-2,221 | 8 | 1/8 | 0.017 |
| 46 | 3.39 | 2,480 | >2,221-2,480 | 13 | 1/13 | 0.010 |
| 47 | 3.44 | 2,770 | >2,480-2,770 | 12 | 1/12 | 0.011 |
| … | | | | | | |
| 59 | 4.02 | 10,428 | >9,337-10,428 | 3 | 1/3 | 0.045 |
| 60 | 4.07 | 11,646 | >10,428-11,646 | 2 | 1/2 | 0.068 |

### Assigning weights to observations of a selected taxon within a bin

The XC95 BEAT assigns weights to observations of a taxon by sorting the data set by presence and then by SC. Then, it deletes all sites where the taxon was not observed. After counting the number of samples with the same SC (Table S2, Column 3), the XC95 BEAT removes duplicate rows.

Table S2 illustrates the calculations performed by the XC95 BEAT. In the 4th column, the “Normalized Bin Weights” (from Table S1, Column 7) have been entered that were assigned to the bin interval (Table S1, Column 4) that includes the SC value of those sites. This operation was repeated for each row. Next, the “Normalized Bin Weight” (Table S2, Column 4) was multiplied by the “Count” of samples (Table S2 Column 3) within a SC within that bin interval and then entered into the 5th column as the “Total Bin Weight”. For example in Table S2, Rank 1, “Total Bin Weight” = “Bin Weight\*Count” = (1 × 0.045 = 0.045).

Next, the “Rescaled Bin Weight” was calculated by dividing the “Total Bin Weight” by the sum of the “Total Bin Weight” (Column 5) (2.094) and multiplying by the total number of sites where the taxon was observed (which is equal to the sum of the “Count” (Column 3), 1,291). The “Rescaled Bin Weight” was entered into the 6th column. For example in Table S2, Rank 1 the “Rescaled Bin Weight” = (0.045/2.094 × 1,291) = 27.882. Finally, in the 7th column, the cumulative sum of the rescaled weights was calculated by sequentially adding “Rescaled Bin Weights” and entering into the 7th column. For example, in Table S2 Column 7, the Rank 3 “Cumulative Rescaled Weight” = 16.729 + 55.765 = 72.494.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table S2. Bin weights and cumulative sum bin weights for one genus, *Acentrella*. Each unique specific conductivity value was assigned a weight according to its bin and cumulative weights for all bins were calculated. Only a portion of table is shown to illustrate the process. | | | | | | | |
| **Rank** | **SC (μS/cm)** | **Count** | **Normalized Bin Weight** | **Total Bin Weight = Bin Weight \* Count** | **Rescaled Bin Weight** | **Cumulative Rescaled Weights** | **Weighted Incremental Proportion** |
| 1 | 16 | 1 | 0.045 | 0.045 | 27.882 | 27.882 | 0.0216 |
| 2 | 19 | 1 | 0.045 | 0.045 | 27.882 | 55.765 | 0.0432 |
| 3 | 21 | 1 | 0.027 | 0.027 | 16.729 | 72.494 | 0.0562 |
| 4 | 23 | 2 | 0.019 | 0.039 | 23.899 | 96.394 | 0.0747 |
| 5 | 24 | 1 | 0.010 | 0.010 | 6.434 | 102.828 | 0.0796 |
| … | | | | | | | |
| 518 | 937 | 1 | 0.0015 | 0.0015 | 0.95 | 1,226.8 | 0.9503 |
| 519 | 956 | 1 | 0.0015 | 0.0015 | 0.95 | 1,227.8 | 0.9510 |
| … | | | | | | | |
| 546 | 1,620 | 1 | 0.003 | 0.003 | 1.780 | 1,259.337 | 0.9755 |
| 547 | 1,746 | 1 | 0.003 | 0.003 | 1.780 | 1,261.116 | 0.9769 |
| 548 | 1,831 | 1 | 0.004 | 0.004 | 2.698 | 1,263.815 | 0.9789 |
| 549 | 2,087 | 1 | 0.017 | 0.017 | 10.456 | 1,274.271 | 0.9870 |
| 550 | 3,162 | 1 | 0.027 | 0.027 | 16.729 | 1,291.000 | 1.0000 |

### Calculating the XC95

The XC95 for the taxon is calculated using the rescaled weights. The steps and an example are provided here. First, the XC95 BEAT calculates the “order” = 1 + (*n* ‒1) × 0.95, where *n* is the total number of sites where the taxon was observed. In this example the total number of sites is 1,291 and the order at the 95th centile is 1,226.5. Because the order is not among the available “Cumulative Rescaled Weights”, the SC is interpolated from the low and high integers above and below 1,226.5, that is, the sites ranked 1,226.8 and 1,227.8. Next the XC95 BEAT identifies the values (e.g., log10 SC) that have “Cumulative Rescaled Weights” just above the “low” and “high” integers. In this example, the closest cumulative weights are 1,226.8 and 1,227.8. These correspond to log10 (SC) of 2.972 and 2.980. The macro interpolates linearly between these values of log10 (SC), to calculate the order. For example, XC95 log 10 = (1,226.5 − 1,226) × 2.972 log10 µS/cm + (1,227 − 1,226.5) × 2.980 log10 µS/cm = 2.976 log10 µS/cm. For convenience, the XC95 BEAT transforms the SC value back to base 10. For example, XC95 = 102.976 = 947 µS/cm.

### Plotting a cumulative proportion of observations of a taxon over a range of SC

Although a cumulative proportion of observations and the probability of observing a taxon could be plotted by hand, the spreadsheet tool does this very efficiently. To produce a plot, the XC95 BEAT performs the two operations described below. First, the XC95 BEAT divides each “Cumulative Rescaled Weight” in Table S2 by the maximum “Cumulative Rescaled Weight” (1,291 in Table S2), and multiplies by 100. In the example, this yields 550 “Incremental Ranked Proportions” (Table S2, Column 8) that form the y-axis of a cumulative proportion of observations of a genus over a range of SC measurements. Next, the XC95 BEAT plots each “Incremental Ranked Proportion” from 0−1 on the y-axis (from Table S2, Column 8) versus SC on a logarithmic scale x‑axis (from Table S2, Column 2). It should be noted that the “Incremental Ranked Proportion” for the XC95 is at (1 + (*n*‒ 1) × 0.95). In the example, *n*= 0.9508, so the XC95 is between 937 and 955 µS/cm as in Table S2.

### Plotting the probability of observing a taxon over a range of SC

Plots of the probability of observing a species or genus at any particular SC are also more easily plotted using the XC95 BEAT, but can be created by hand. To produce a plot, the XC95 BEAT performs the two operations described below. First, the XC95 BEAT calculates the *Probability of Observing* a taxon by dividing the *Count* of sites where a genus was observed in a bin (Table S2, Column 3) by the “# of Sites per Bin” (Table S1, Column 5) and multiply by 100. This is the probability of observing a taxon in that SC bin. This operation is repeated for each SC bin. Next, the XC95 BEAT plots the capture probabilities on the y‑axis versus the geometric mean of SC sites within each SC bin on the x-axis.

# CALCULATING AN HC05

The HC05 BEAT calculates a XCD and the HC05 for the stressor. The XCD is a plot showing the proportion of taxa extirpated as a function of the stressor value. The HC05 is the estimated value of the stressor at which 5% of species are predicted to become extirpated.

## Example calculation using the HC05 BEAT

Details of the manual calculation of the XCD and HC05 are shown in this example. The steps below are performed automatically by the spreadsheet tool. A simple quantile‑based method is used. Users may load XC95 values of their own that they have calculated using the XC95 BEAT. They may also use the preloaded values in the BEAT from Ecoregions 69 and 70, being aware that the species comprising the genera in their region may differ and may result in higher or lower XC95 values and may only represent situations with similar background SC.

The sample data set includes 176 genera. Each genus has an associated XC95 for SC (μS/cm). The “Present” column holds a “TRUE” or “FALSE*”* value, indicating whether the taxon will or will not be included in the analysis. For example, if the analysis is run at the species level and the taxon is only identified to genus level, the “Present”column entry would be “FALSE”. For this example, we have designated all genera as present, i.e., *Present*= *TRUE*. Also, given for each genus are the Order, Family, number of observations for that genus, and the probability plot designation (e.g., =, ~, <). These symbols indicate that the XC95 is equal to (=), approximate (~) or greater than (>) the calculated value. These designations are not used in the derivation of the HC05. They may be useful in other applications, for example ensuring that it is or is not valid at a selected SC level to predict extirpation of a particular taxon or of the percentage of taxa. The extirpation of taxa with > designation cannot be reliably estimated with the tested SC range. Predictions of the proportion from an XCD in the SC range where taxa are identified as > may not be reliable. In this particular example, the > symbols were calculated using a software script in *R* (R Core Team 2014) as described in Cormier and Suter (2013). However, these designations can also be estimated by examining plots of the probability of observing a taxon over a range of SC (Supplemental Appendix S7).

## Steps to calculate HC05 using the HC05 BEAT

The HC05 BEAT performs several tasks after the user selects which taxa will be included by selecting “Present” as “TRUE”. First, it copies the columns with “TaxaName” (genus), “*XC95*”, and “Present” into a new sheet. Then it sorts taxa by “Present”, then by XC95 from lowest to highest. Next it deletes all rows with *Presen*t = FALSE (none in this example). The HC05 BEAT then creates a column and calculates the “Cumulative Proportion” as (*i*/(*N*+ 1)), where *i* is the rank of the genus (*i*= 1 for lowest XC95), and *N* is the total number of taxa (i.e., 176 genera). For the example (Table S3), the cumulative proportion ranges from 1/177 to 176/177. The HC05 BEAT calculates the 5th centile of the pseudo-index using Eq. 3 where.

*p5*= (*N*+ 1) × 0.05 Eq. 3

*N* is the total number of taxa in the XCD and *p5* is the 5th centile of the distribution of XC95 values. In this example, the index value at p5 is 8.85((176 + 1) × 0.05). The HC05 BEAT then identifies the XC95 values bracketing the p5. In this example, the lower index, 8, is *Tallaperla* with an XC95 = 243 μS/cm. The upper index, 9, is *Alloperla* with an XC95 = 245 μS/cm. The HC05 is then estimated by linear interpolation, which is the value of XC95 at the 5th centile as described in Eq. 4.

HC05 = XC95 lower index + (XC95 upper index – XC95 lower index) ×  
 (*p*5 – rank of lower index) Eq. 4.

In this example: HC05 = 243 μS/cm + (245 μS/cm – 243 μS/cm) × (8.85 – 8) = 244.7 μS/cm. The HC05 BEAT reports the HC05 and provides a plot of the cumulative proportion of genera versus XC95.

Table S3: Taxa, XC95 (specific conductivity, μS/cm), and calculated cumulative proportion of taxa. Genera indexed at 8 and 9 bracket the 5th centile. Only a portion of table is shown to illustrate the process.

|  |  |  |  |
| --- | --- | --- | --- |
| **Index** | **TaxaName** | **XC95** | **Cumulative Proportion** |
| 1 | *Remenus* | 108.00 | 0.0056 |
| 2 | *Timpanoga* | 138 | 0.0113 |
| 3 | *Strophopteryx* | 159 | 0.0169 |
| 4 | *Lepidostoma* | 183 | 0.0226 |
| 5 | *Utaperla* | 231 | 0.0282 |
| 6 | *Leptophlebia* | 235 | 0.0339 |
| 7 | *Drunella* | 243 | 0.0395 |
| **8** | ***Tallaperla*** | **243** | **0.0452** |
| **9** | ***Alloperla*** | **245** | **0.0508** |
| 10 | *Cinygmula* | 281 | 0.0565 |
|  | | | |
| 167 | *Hydroptila* | 11,227 | 0.9435 |
| 168 | *Procladius* | 11,227 | 0.9492 |
| 169 | *Sialis* | 11,227 | 0.9548 |
| 170 | *Dicrotendipes* | 11,310 | 0.9605 |
| 171 | *Ablabesmyia* | 11,646 | 0.9661 |
| 172 | *Atherix* | 11,646 | 0.9718 |
| 173 | *Chrysops* | 11,646 | 0.9774 |
| 174 | *Cladotanytarsus* | 11,646 | 0.9831 |
| 175 | *Helichus* | 11,646 | 0.9887 |
| 176 | *Pseudochironomus* | 11,646 | 0.9944 |

# RESULTS

The XC95 values for the 176 genera are listed in the “Biological Extirpation Analysis Tool (HC05)” on the *XC95 Select* tab (Supplemental Appendix S3). For genera with XC95 values >1,500 µS/cm, the occurrence of those genera often increases as SC increases without an apparent optimum in the measured SC range of the data set (e.g., *Gammarus,* Figure 1b, Cormier et al., [c]). XC95 values were also calculated separately for Ecoregions 69 and 70 and XC95 values for genera occurring in both ecoregions (Figure S2). Uncertainty bounds were calculated using a bootstrapping technique described by Cormier and Suter (2013) using an R-script. The XC95-BEAT does not perform this calculation.

In general, the XC95 values for the combined data sets are intermediate to the XC95 values calculated separately for Ecoregion 69 and 70 and the confidence intervals overlap for the results from the data sets (Figure S2). Separately calculated XC95 values for a few genera (e.g., *Drunella*, *Pycnosyche*) do not overlap or have very different confidence intervals. The differences in individual genera cannot be explained by this data set but additional studies of species variations within genera may provide clarification. One potential explanation is that the representation of species comprising the genus may differ between the regions.

In summary, the extirpation of an entire genus is a serious consequence for biological integrity and extirpation over a wide area may lead to extinction. The BEATs provided here need to be used with care and the outputs interpreted judiciously as with any computational tool.

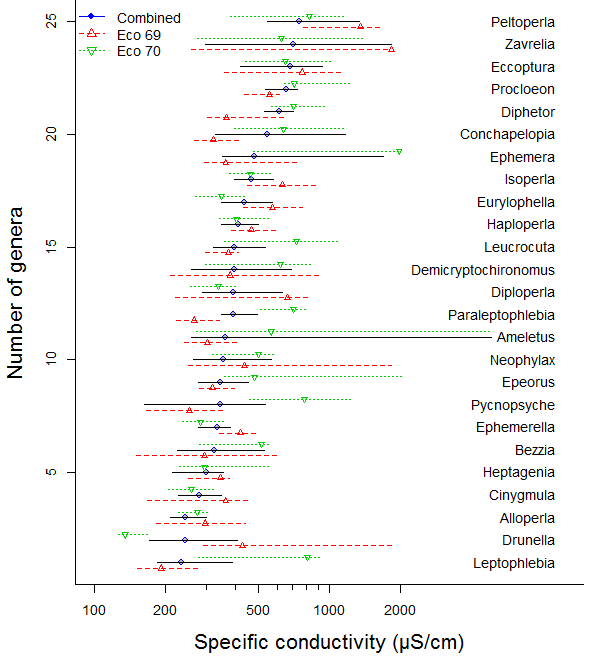
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Figure S2. The extirpation concentration (XC95) values for the 25 most salt-intolerant genera calculated for Ecoregion 69, Ecoregion 70, and both ecoregions combined. Genera not occurring in both ecoregions were not compared. Specific conductivity (SC) is represented on the x‑axis and the rank order starting with the most salt-intolerant genus is represented on the y‑axis. Three XC95 values and associated 95% confidence intervals are shown for each genus. XC95 (open diamond) and 95% confidence intervals (CI) (solid line) are shown in blue for the combined data sets of Ecoregion 69 and 70. XC95 values for Ecoregion 69 are shown in red (triangle) and for Ecoregion 70 are shown in green (inverted triangle) as well as their 95% CI (hashed and dotted lines, respectively).

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