Minnesota wetland plant response to salinity stressors: conductivity, chloride and sulfate

High level recommendations

- Aquatic plants are sensitive to specific conductance, chloride and sulfate and their response should be considered in development of any aquatic life salinity related criteria or standards development
- Salinity criteria or standards development should be stratified at least to a level II ecoregion scale
- Species extirpation analysis; XC95 and HC05 is an appropriate biological response approach
- The current 230 mg/L Class 2 aquatic life chloride standard may not be adequately protective of aquatic plants in Minnesota
- Additional wetland plant data paired in space and time with salinity variables (conductivity, chloride and sulfate from across MN are recommended to improve salinity response benchmark development

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Introduction

Plants are known to be responsive to salinity and ionic gradients, including those associated with sulfate, sulfide, ammonium, chloride, iron and carbonates (Flowers et al 2010, Kinsman-Costello et al 2015). These major ion constituents affect specific conductance or simply “conductivity”, a measure of water’s ability to conduct electricity, which increases with elevated ion concentrations. Thus, conductivity represents concentrations of nonspecific cation and anion mixtures that are present in water (ionic strength). Elevated ionic strength (conductivity) and ion composition due to natural sources, as well as human caused pollutant loading, has long been recognized as affecting plant species structure and community composition (Moyle and Hotchkiss 1945). These mixtures of ions are typically associated with disassociated salts; therefore, “conductivity” and “salinity”, the proportion or concentration of salts in solution, are closely related. Several investigators report aquatic plant taxa responding differently in their sensitivity or tolerance to salinity (Timoney 2015, Klosowski 2006, Miklovic and Galatowitsch 2005, Hammer and Heseltine 1988). U.S. EPA (1988) reported on plant and algae sensitivity thresholds to chloride ranging from 71 mg/L in the algae *Spirogyra setifomis* and *Chlorella luteoviridis* tolerating chloride concentrations up to 36,400 mg/L.

Flowers et al. (2010) reviewed the evolutionary development of plant tolerance to salinity, including those that have high tolerance to salinity known as halophytes. Halophytes have developed various anatomical or physiological adaptions to manage the osmotic stress associated with high ionic strength. Adaptation examples include concentrating ions or organic solutes within vacuoles (salt crystal formation) or maintaining stringent (conservative) control of ion exchange between root and shoots, which is a metabolic response and requires additional energy expenditure. Some plants adapted to high saline conditions have developed salt glands, which enable the plant to secrete excess salts, avoiding toxic concentrations of potentially harmful ions. The majority of plants possess some limited physical mechanisms such as vacuolization to potentially tolerate increased ionic strength; though, physiological tolerance to increased ionic strength is not present in all plants (Flowers et al 2010, Cushman 2001). Not surprisingly, plants more tolerant to salinity often also show tolerance to other environmental stressors such as nutrient loading, flooding or drought.

Several cations including K^+, Ca^{2+}, Fe^{2+} and Mg^{2+} are important to plant mineral nutrition. Ion transfer and sequestration are important in mediating plant osmotic balance, nutrient uptake, as well as photosynthetic, respiratory and related physiologic processes while also responding to increased ionic strength (Klosowski 2006, Cushman 2001). There may also be important differences between emergent plant species rooted in wetland sediments compared to free-floating or weak rooted aquatic species suspended in the water column (Hammer and Heseltine 1988, Hinneri 1976). There is limited research reporting on responses of large segments of freshwater plant growth forms or guilds to increased ionic strength, particularly due to pollutant loading and especially in wetlands (Borgnis and Boyer 2015, Miklovic and Galatowitsch 2005).

The impetus of this current investigation was to contribute wetland plant salinity response information toward development and revision of conductivity and chloride water quality standards in Minnesota primarily applicable to industrial and agricultural uses of surface waters including lakes, streams and wetlands.

Current state water-quality standards in Minnesota (Minn. Rules, Ch. 7050) include standards for conductivity, chloride and sulfate. Minnesota's Class 3A chloride standard of 50 mg/L is set to maintain quality surface water for industrial consumption uses, except food processing, with only minimal treatment and the Class 3B chloride standard for general industrial uses, including cooling and material transport with only moderate amounts of treatment is 100 mg/L. Minnesota's current Class 3C surface water chloride standard is 250 mg/L and is applicable to surface waters used for industrial cooling or material transport with minimal treatment. These industrial use standards are mostly intended to protect equipment from corrosion, scaling and from process fouling. Most Minnesota surface waters are classed as 3C except for wetlands. In wetland waters, the current 3D industrial use chloride standard is a narrative "maintain background", to protect and maintain suitability for industrial uses. Implementation of background narrative standards is more challenging than numeric standards due to the need to characterize "background" conditions often in case by case applications. In current rulemaking the Class 3 conductivity and chloride standards are proposed to be consolidated into a single narrative industrial use class.

Ensuring water is able to be used for agriculture, the State of Minnesota currently has a conductivity standard of 1000 µS/cm applied as a Class 4A standard to protect the suitability of surface waters for use as agricultural irrigation sources primarily for terrestrially grown crops (Minn. Rules 7050.0224 Subp. 2).

Similarly, the water quality standard of 10 mg/L for sulfate is a Class 4A standard applicable in surface waters used for the production of wild rice, during periods when the rice may be susceptible to damage by high sulfate levels. Related Class 4B standards protect surface waters for use by livestock and wildlife at a total salinity of 1,000 mg/L.

Minnesota has not adopted an aquatic life and recreation beneficial use (Class 2) standard for conductivity or sulfate. Minnesota's current aquatic life and recreation standard for chloride is 230 mg/L. This conforms to U.S. EPA national chloride criteria guidance (U.S. EPA 1988) based on literature at that time and principally on invertebrate and vertebrate test organism assay endpoints. In their guidance U.S. EPA reported that a final guideline based on plants was not available since methods for testing plant responses did not conform to testing guidelines used in the U.S at that time.

Recent work by U.S EPA scientists examined the effect of increased specific conductance (conductivity) on macroinvertebrates in Appalachian streams (U.S. EPA 2011, 2016 and Cormier et al 2013). In their research, they developed an approach to use biological and water chemistry field data to estimate a biological response benchmark for conductivity based on thresholds of extirpation. This approach applies the distribution and probability of observing individual macroinvertebrate genera across a conductivity gradient, assuming that each observed genus exhibits an optimal response range to conductivity. At some point beyond this optimal range, invertebrates experience stress due to continued increases in conductivity, up to a point of extirpation where some genera may no longer be observed (extirpated) from field samples. Where "extirpation" means "depletion of a genus within a population to the point where it is no longer a viable resource or it is unlikely to fulfill its function within the ecosystem". The extirpation methods utilized a cumulative distribution function (CDF) model biological response to stress and provide a means to estimate extirpation values of conductivity individually for each observed genera. The extirpation response benchmark or XC95 (extirpation concentration at 95%) represents the concentration below which 95% of observations of that genus occur. Griffith et al (2018) used the same extirpation probability approach to derive a conductivity benchmark for stream fish community data.

The hazardous concentration (HC) is a field benchmark, equivalent to the criterion continuous concentration (CCC) value typically derived from laboratory toxicity tests and applied as water quality criteria for the protection of aquatic organisms. Benchmarks differ from water quality criteria or standards in they are not defined in regulation, but provide scientific basis to potentially support resource management decisions.

In the approach used here and adapted from U.S. EPA 2011, 2016 and Cormier et al 2013 the hazardous concentration is based on the potential for extirpation of one or more species in wetland plant communities. A hazardous concentration can be derived for wetland plants by combining XC95 values from all qualifying observed species in response to the same stressor variable (e.g. conductivity). Setting the hazardous concentration at the 5th percentile represents the estimated value or concentration where there is a 5% or less chance that all observed species could be expected to persist. So at the HC05 there is a 95% chance that at least one observed plant species (or genera whichever taxon is analyzed) could be expected to become extirpated, at least in-part, due to exceeding the HC05 benchmark.

Work presented here provides parallel analysis and discussion of the extirpation probability approach to estimate aquatic life salinity benchmarks for specific conductance, chloride and sulfate for 114 aquatic plant species principally observed in depressional wetlands (1995 – 2012). These wetland plant and water chemistry data were collected by Minnesota Pollution Control Agency scientists.

In this investigation, an initial analysis of wetland data proceeded using plant data at the genus level and analyzed at a statewide extent. In that initial phase of the investigation, which is not reported on here, the data were split into two sets to provide both development and validation of wetland plant genera results. That design was insightful, though in 2019 an MPCA internal review team suggested it would be more informative to analyze the plant data: 1) as species; and 2) given the relatively small size of the available paired chemistry and plant data it would be better to pool the data into a single data set and examine benchmarks at a state and level II ecoregion (Omernick and Griffith 2014, Omernick 1987) scale. Examining benchmarks at an ecoregion scale was viewed as particularly important given the known natural background salinity gradient across MN. Results following this geographically revised design and species level taxonomy are presented and discussed below.

Revising state water quality standards or proposing expressed criteria as potential water quality standards was not a goal for this investigation. However, results presented and discussed here may provide support and contribute to future data acquisition efforts and to future revisions of state water quality standards. Findings discussed here intend to provide justification for the need to consider plant responses and wetland communities when developing and revising future water quality standards regarding salinity related variables.

Methods

MPCA wetland investigators collected wetland plant and water chemistry data between 1995 and 2012 from 587 discrete wetland stations (usually basins) to develop and implement biological condition indicators including indices of biological integrity (IBIs) focused on depressional wetlands (Gernes and Helgen 2002, 1999, Genet and Bourdaghs 2007, 2006, Genet 2015, 2012). Biological community and water chemistry data were collected from the nearshore wadeable extent, of primarily depressional wetland habitats. Field investigators collected plant data via 100-m^2 field plots (releve's) targeted to represent the wetland emergent community. Plots were typically 10 X 10 m and less frequently, 5 X 20 m. Rectangular plots allowed data collection when water depth was too deep to wade for establishing the preferred 10 X 10 m square plots. In the north central-northeastern region of the state, four clustered 5 x 5 m plots whose total area summed to 100-m^2 constituted a single sample. Multiple small plots were intended to improve sample representation of diverse wetland plant community structure and composition. In all cases, regardless of sample type, plots were located within the emergent community in the near-shore area often including a portion of adjacent shallow open water community when that community was present.

Water chemistry variables were measured or collected using accepted field and laboratory measurement and analysis techniques (MPCA 2015, 2008) from the same location within the wetland where biological sampling occurred. Specific conductance and water temperature were measured in situ using commercial multi-parameter water quality instruments following manufacturer's calibration recommendations at least weekly. Water chemistry grab samples were collected from the top 10-20 cm of the water surface near where biological sampling occurred. These samples were analyzed by the MN Department of Health, Environmental Laboratory, typically for alkalinity, sulfate, total chloride, total Kjeldahl nitrogen, total phosphorus, and total suspended solids though the analyte suite changed over time. For example, sulfate analysis began in 2001. Water chemistry sampling usually occurred in June or July during the most active plant community growth and development period. In accord with MPCA wetland monitoring protocols (MPCA 2015, 2008). Invertebrate community sampling typically occurred in June along with corresponding water chemistry sampling, followed by plant community sampling in July. Water chemistry samples were collected a second time, during roughly one third of the plant community visits. Depending on monitoring program goals there were also numerous occasions where water chemistry sample collections occurred without any concurrent biological sampling either plant or invertebrates. These supplemental water chemistry visits frequently occurred during months other than June and July. Thus, nearly twice the number of wetland chemistry samples were collected by MPCA investigators compared with the number of plant community visits. This additional sampling allowed an assessment of a broader temporal range of wetland chemistry variability.

To maximize the pairs of plant community sample visit paired with chemistry sampling visits it was often necessary to link plant visit data with corresponding chemistry data collected during the invertebrate sampling visit from the same year and same wetland sample station. This was recognized as not ideal, since the U.S. EPA extirpation benchmark methods recommend chemistry and biology data be synchronously collected on the same date and station visit. When available, chemistry sample visits collected concurrently with plant community sampling visits were used preferentially instead of the chemistry samples collected during the invertebrate visit from the same year and station. Sample number influences the confidence in the extirpation results as described below. Cormier et al (2018) and U.S. EPA (2016) recommended the total number of paired biology and chemistry samples exceed 500, though valid results may be obtained with smaller data sets (~200) if certain background conditions are met.

MPCA wetland monitoring protocols (MPCA 2015, 2008) specified collection of replicate data from 10% of wetland sampling stations within each specific wetland project to determine method variability. In a small number of project years MPCA wetland monitoring designs assessed the sample variability within sites by sampling multiple locations within a single wetland basin. As a result of both replicate and spatial variability replicate sampling; and repeated visits over multiple years to detect station trends, many wetland stations were sampled multiple times.

Water chemistry data came from geographically well distributed wetland stations that represented depressional wetland geochemical conditions dominant throughout Minnesota where bicarbonates, carbonates or in the west sulfates are the matrix ions (Gorham 1983). MPCA wetland sampling designs across several indicator development projects included natural wetlands as well as restored depressional wetlands and ponds constructed for livestock watering or stormwater treatment. Plant community data from these diverse depressional wetland stations represented the range of species expected to occur in shallow marsh, deep marsh, shallow open water and fringing fresh meadow and shrub-carr communities typical of Minnesota depressional wetlands. Even given the statewide distribution of depressional wetland stations, pH at these stations typically ranged within the circumneutral range between 6 and 9.

Only plant species with a minimum of 25 observations of paired water chemistry samples and biological survey results were included in the calculation of extirpation concentration (XC95) and hazardous concentration (HC05) benchmark values for each of the three salinity stressors. Species were included in the analysis only if they occurred at least once in high quality reference sites, thus assuring that they were characteristic of natural depressional wetlands. Reference quality designated wetland stations required they minimally met the following criteria: a) no significant presence of invasive plants; b) no history of drainage, filling or excavation associated with the wetland, c) the wetland must be well buffered with natural vegetation and not have direct discharges from municipal or industrial facilities, or receive direct agricultural runoff. Observations of nonnative invasive species observed in limited areal extent in at least at least one reference site were retained in the data set and analyzed for their XC95 benchmarks. Such invasive species included: *Cirsium arvense*, *Lythrum salicaria*, *Phalaris arundinacea*, *Typha angustifolia* and *Typha* X *glauca*.

Cumulative Distribution Function (CDF) plots distribute probability values from 0 to 1 (0% to 100%) as observations of a given plant species response to a range of observed concentrations of stressor. Using two-point interpolation from the x and y axis on CDF plots estimated the potential for extirpation of plant species across an empirical range of salinity variable concentrations. The CDF equation used in this study (1) weighted the results to control for uneven observation frequency across environmental gradients (Cormier and Suter 2013, U.S. EPA 2011). It is reasonable to assume that plant species will have an optimal salinity variable response range. Inclusion of data from a range of high quality reference sites and impaired sites assured a range of salinity variable exposures to assess species response.

$$F(x) = \frac{\sum_{i=1}^{Nb} Wi \sum_{j=1}^{Mi} I(Xij < x \text{ and } Gij)}{\sum_{i=1}^{Nb} Wi \sum_{j=1}^{Mi} I(Gij)} \qquad (1)$$

Where: X_{ij} is the conductivity value in the j^{th} sample of bin i,
N_b is the total number of bins,
M_i is the number of samples in the i^{th} bin,
G_{ij} is true if the genus of interest was observed in the jth sample of the bin i,
I is an indicator function that equals 1 if the indicated conditions are

Cumulative proportion for each species (2)

$$(P) = R \div (N+1)$$

Where, R is the cumulative proportion of each species' XC95 value and N is the number of genera (Cormier and Suter 2013).

An aquatic plant community hazardous concentration benchmark at the 0.05 percentile (HC05) was derived from analysis of combined and ranked plant species XC95 values. The HC05 criterion is analogous to U.S. EPA standardized toxicity expressions of criterion continuous concentration (CCC). U.S. EPA defined the CCC as the national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent that organisms could be exposed indefinitely and be expected to endure, without causing an unacceptable effect (U.S. EPA 1991). At the hazardous concentration value (HC05) there is a 95% chance of extirpating up to 5% of the species that could otherwise be expected to occur in the community. The cumulative proportion of each species calculated and summed using formula (2) yielded an estimate of the hazardous concentration (HC05).

Plant community and water chemistry data were stored, managed and assessed using MS Access 2016. MS Excel 2016 with the optional data analysis package loaded, permitted basic data summaries, analysis and some charting. Salinity extirpation concentration (XC95) derived by cumulative distribution function (CDF) analysis, including plotting, and the 0.05 Hazardous Continuous Concentration (HC05) estimates were generated in R (R Core Team 2019) version 3.2 Dark and Stormy Night, using XC95 extirpation analysis code from https://github(leppott/XC95) with 'tidyverse'; 'lubridate'; 'ggplot2'; "devtools' and 'XC95' packages loaded and active. Only genera with a minimum of 25 observations were included in XC95 and HC05 calculations. Additional statistical analyses, including Kruskal-Wallis median difference test were similarly analyzed in R with 'FSA' and 'Psych' packages loaded and active.

Plant taxonomic treatment used here followed federal plant taxonomic authority sources https://www.itis.gov/ and USDA Plants https://plants.sc.egov.usda.gov/java/.

Results

Figure 1 presents conductivity at 587 depressional wetlands across Minnesota's three level II ecoregions (Omernick and Griffith 2014, Omernick 1987). Cumulatively these stations represented an increasing gradient of conductivity from NE Minnesota to the west and SW. These stations represent a natural background conductivity gradient which has likely been exacerbated by pollutant loadings or anthropomorphic physical alterations. Surficial geology accounts for significant geographic differences in surface water ionic composition across the state. In particular, bicarbonates and carbonates are important ionic constituents throughout Minnesota. In the northcentral and southcentral to southeast region of the state Ca^{2+} and Mg^{2+} carbonates are the dominant ion, derived from weathered glacial till particularly under circumneutral to slightly alkaline pH typical in most of Minnesota surface waters, including wetlands (Gorham et al. 1983). Sulfate, derived from Cretaceous shale, tends to increase in the western region of the state. Lower average precipitation contributes to the dominance of sodium and sulfate ionic composition in surface waters near the border of Minnesota with North and South Dakota especially when conductivity levels exceed 900 µS/cm. The relatively low conductivity values observed in the Mixed Wood Shield ecoregion in NE Minnesota are characteristic for this region (Gorham et al. 1983, Moyle and Hotchkiss 1945). The majority of this ecoregion features intact native vegetation and naturally low total alkalinity resulting in naturally low ionic strength. In contrast the Temperate Prairies ecoregion in the west and southern regions of the state in agricultural land-use are characterized by high conductivity. Figure 1 stations symbolized as greater than 820 µS/cm may represent sulfate dominated chemistry (Gorham 1983). Many of the sampled wetlands with low to moderate conductivity (<25.1 – 485) in the Temperate Prairies ecoregion primarily have isolated or precipitation driven hydrology, reducing surface discharge loading influence from the surrounding landscape. Genet and Bourdaghs (2007) reported an average conductivity below 20 µS/cm for depressional wetland reference sites in the

Temperate Prairies ecoregion. Many of those reference sites were isolated basins and well buffered by adjacent perennial vegetation.

Figure 2 illustrates frequency distributions of log10 transformed conductivity, chloride and sulfate sample observations from all available samples, i.e. not just samples able to be paired with biol. data. Bin width varied by variable. For conductivity bins were 1.232 units (0.0906 log10); chloride bins were 1.171 (0.0821 log10) wide and for sulfate the bins were 1.208 (0.0821 log10) wide. Conductivity (A) observations were nearly normally distributed with a slight skew toward higher concentrations. Distributions of both chloride (B) and sulfate observations were strongly influenced by non-detect samples. If the non-detects had been excluded the chloride histogram would have had a closer to normal distribution. If the non-detects, would have been removed in the sulfate histogram the distribution would be have been nearly uniform.

Table 1 present's corresponding descriptive statistics for conductivity, chloride and sulfate statewide and across Minnesota's three level II ecoregions. These results are derived from the entire available wetland chemistry sample set. Many of the observations in the full data set were trend or replicate samples collected independent of biological sampling. Thus the total number of wetland chemistry samples was notably greater than the number of chemistry biology samples presented in the plant species extirpation analysis discussed below.

In parallel with the descriptive statistics in Table 1, Figure 3 presents boxplots of conductivity, chloride and sulfate statewide and across the three level two ecoregions. Data for these boxplots were log10 transformed to reduce the large number of outliers above the upper bounds. Conductivity boxplot (A) results from the Mixed Wood Shield (MWS) clearly showed the first and third quartiles of conductivity to be notably lower compared to conductivity distributions statewide and from the Temperate Prairies and Mixed Wood Plains. Chloride boxplot group (B) showed the chloride distribution in the MWS is similarly lower compared to the other two ecoregions. These differences for the MWS ecoregion could be expected based on differences in natural background chemistry and geology. Sulfate boxplot (C) showed the median distribution of sulfate in the Temperate Prairies ecoregion to be notably elevated compared to statewide results and also the MWP and MWS ecoregions. Considering these boxplot results it was not surprising that a Kruskal-Wallis analysis found significant differences geographically for all three salinity variables. For conductivity, Kruskal-Wallis chi-squared = 581.15, df = 3 and p-value 2.2 e-16 ($p < 0$). Chloride Kruskal-Wallis chi-squared results were 219.74, df =3, p-value < 2.2 e-16 ($p < 0$). Kruskal-Wallis sulfate analysis was similarly significantly different across geography, chi-squared 592.76, df =3 and p-value < 2.2 e-16. Post hoc Dunns tests were performed using the Benjamini-Hochberg stepwise discrimination method to further examine significance in the salinity variables against geography. Findings from the Dunn’s test are presented in Table 2 for each salinity variable where different geographic cases are paired stepwise. All ecoregions and statewide pairing combinations for conductivity and sulfate were found to be significantly different. Chloride pairings were also found to be significantly different in all combinations except chloride data distributions in the TP compared to the MWP which were not significantly different.

Figure 4 illustrates boxplots of wetland salinity variables by ecoregion and month for conductivity (A-C), chloride (D-F) and sulfate (G-I). Plots were derived from the entire MPCA wetland sample set from 1995 – 2016. All data were log10 transformed to remove the majority of outliers. In both the Temperate Prairies and the Mixed Wood Plains the median salinity variable concentrations tended to be lower in the summer and higher in spring and fall periods. Chloride medians tended to present a more erratic pattern. These results represent the full range of conditions present in Minnesota depressional wetlands as opposed to only representing seasonal natural background conditions present in reference only data.

Table 3 presents descriptive statistics by salinity variable for paired chemistry and plant samples statewide and by ecoregion. Ranges of variable values were noticeably different In terms of paired sample numbers statewide for each salty parameter. U.S. EPA researchers have recommended 500 to

200 paired samples to obtain reliable extirpation analysis. Chloride (n = 158) and sulfate (n = 111) paired samples in the MWS ecoregion were below these recommendations and thus the extirpation results in these cases should be considered to be potentially less reliable.

Extirpation analysis output for each species within each analysis region included: a) a general affects model (GAM) probability plot, a cumulative distribution function (CDF) plot, XC95 estimate, and the number of sample observations. The XC95 results for each included species provides a threshold, defined as the probability of occurrence where 95% of the observations of this species occur, across the value range of each measured stressor. One hundred and fourteen plant species (114) were included in the extirpation analysis, across four geographic extents: statewide, Temperate Prairies (TP), Mixed Wood Plains (MWP) and Mixed Wood Shield (MWS) ecoregions; where each species with sufficient observations associated with each of the three salinity stressors resulted in nearly 1,380 results specific to chemical variable by plant species by region. Regions with insufficient observations of a given species (e.g. < 25) were not included in analysis and therefore the total number of species analyzed by region varied.

General affects model probability plots usually followed three general patterns: a) increasing; b) optimal or c) decreasing. These three patterns are illustrated in Figure 5 against statewide conductivity analysis for three common wetland plant species; *Calamagrostis canadensis, Asclepias incarnata ssp. incarnata* and *Stucknia pectinatus*. Figure 6 provides cumulative distribution function plot results for these same three plant species. Species XC95 estimates from the CDF analysis was output in R and reported here.

Calamagrostis canadensis (Canada bluejoint) is an emergent grass that occurs throughout Minnesota and is common in many wetland communities, particularly shallow marshes, fresh meadows and shrub-carrs. This species shows a decreasing probability distribution in response to increasing conductivity

(Figure 5A). Decreasing probability response distribution could also be recognized as a response curve where toxicity response of a species shows the test species is unable to remain viable as the stressor increases.

The XC95 for *C. canadensis* related to conductivity statewide was 362 µS/cm (Figures 3A & 4A). Appendices A, B and C provide; conductivity, chloride and sulfate XC95 estimates respectively for individual species statewide and regionally. Conductivity XC95 results for *C. canadensis* ranged from 295 in the MWS to 596 in the MWP. An XC95 estimate of conductivity response for *C. canadensis* was not able to be analyzed in the TP due to insufficient observations (Appendix A). Chloride extirpation XC95 results for *Calamagrostis canadensis* statewide was 85 mg/L and ranged from 14 mg/L in the TP; 89 mg/L in the MWP and 26 mg/L in the MWS (Appendix B). Sulfate related XC95 for *C. canadensis* statewide was 530 mg/L. Regionally *C. canadensis* XC95 sulfate estimates ranged widely; from 530 mg/L in the TP; to 11,400 mg/L in the MWP ecoregion the XC95 value was estimated to be 158 mg/L (Appendix C). These results corroborate a contention that some species may have a large amplitude response to sulfate in-part due to differences in regionally and locally different buffering factors such as associated iron and organic carbon in the habitat as reported for sulfide by Marbo et al. (2017) for wild rice (*Zizannia palustris)*. Unfortunately in this investigation sample observations for wild rice and sulfate were insufficient statewide, as well as regionally to provide sulfate related XC95 results.

Similar to *C. canadensis*, *Asclepias incarnata* ssp. *incarnata (*swamp milkweed*)* is an emergent forb locally common throughout Minnesota, typically occurring in shallow marshes, wet meadows and shrub-carrs. In contrast, however, *A. incarnata ssp. incarnata* exhibits an optimized data distribution in the statewide response to conductivity (Figure 5B). Optimized distributions have an initial low probability, which increases with increased stress influence up to an optimal point and then decreases back down toward zero. An optimized distribution pattern was the most common response pattern to conductivity among the 114 wetland plant species results presented in Appendix A. Some species showed an inverted optimized probability response with a high probability of occurrence at low stressor, with the probability decreasing as the stressor increased up to point where the probability curve rebounded near the highest observed stressor. The statewide conductivity XC95 for *Asclepias incarnata ssp. incarnata* was estimated to be 1491 which is distributed at the top of the upper quartile of all species XC95 values (Figure 7 boxplots). The MWP ecoregion conductivity XC95 of 485 was significantly lower and about the lowest quartile range of tested species in this region. Unfortunately there were not sufficient observations of swamp milkweed in either the TP or the MWS to confidently estimate an XC95. Examining the other two salinity stressors with *A. incarnata* ssp. *incarnata*, found the statewide chloride XC95 to be 96 mg/L and slightly lower in the MWP where the XC95 as calculated to be 89 mg/L. These results were near the statewide chloride median for tested species and just below the lower quartile of all species in the MWP (Figure 7). Analysis of *A. incarnata* ssp. *incarnata* to sulfate resulted in a statewide XC95 of 814 mg/L which was just above the upper quartile for all tested species statewide in response to sulfate (Figure 7). Similar to this species response to conductivity and chloride the regional finding for sulfate was significantly lower, where the XC95 was 24 mg/L in the MWP which was near the first (25%) quartile of 22.25 mg/L.

Like *Calamagrostis canadensis* and *Asclepias incarnata* ssp. *incarnata*, *Stuckenia pectinatus* (sago pondweed) is common throughout MN, however it is a submergent plant. As shown in Figure 5 *Stuckenia pectinatus* extirpation cumulative distribution function response to statewide conductivity showed an increasing pattern with an XC95 of 2340. This species similarly had an increasing response pattern to conductivity in the TP and MWP ecoregions. Cormier and Suter (2013) have suggested XC95 results may not be as reliable for species exhibiting an increasing response since the full response may not be able to be examined as the true extirpation may occur beyond the range of the test data. Regionally, the XC95 estimates for *Stuckenia pectinatus* ranged from 2163 µS/cm in the TP down to 885 µS/cm in the MWP. Within their regional test areas these XC95 results were either at or above the respective third quartile for conductivity response (Figure 7). Considering the ion specific salinity stressors, chloride and sulfate the response of *Stuckenia pectinatus* was similarly near or above the respective upper quartile. For chloride statewide the XC95 for *Stuckenia pectinatus* was estimated as 150 mg/L and ranged from 211 in the TP to 110 mg/L in the MWP. Insufficient observations of this species prevented a commensurate XC95 estimate in the MWS. The response of *Stuckenia pectinatus* to sulfate statewide found an XC95 of 902 mg/L and ranged from 902 mg/l in the TP to 182 in the MWP, without a response estimate available in the MWS. The response pattern for *Stuckenia pectinatus* to sulfate was an increasing curve for all geographies with sufficient observations. Responses of this species to chloride was a bell shaped optimal curve statewide as well as in the TP and the MWP.

Similar estimates for all observed species in each of the four investigated regions are provided in three appendices, one for each salty variable (A) conductivity; (B) chloride; and (C) sulfate. Each appendix presents individual species results, including XC95 estimates, number of observations of each respective species and response curve type. Response curve pattern were not always obvious, occasionally requiring judgment. Curves were preferentially designated to be decreasing or increasing and were only judged as optimal when several observation points distributed in two defined groups along the X-axis. Curves with an upper decline on the y-axis, then a short curve up, but then arcing down and continuing to decrease often toward a group of points along the X-axis distributed distant from the axis origin were judged to be decreasing.

Cattails *(Typha sp.)* are common, often dominant emergent plants especially in shallow marshes. Three *Typha* taxa, occur in Minnesota. Two of these taxa (*T. latifolia* and *T. angustifolia*) are recognized as species. *Typha latifolia* (broad-leaved cattail) is native and *T. angustifolia* (narrow-leaved) is a nonnative invasive species originating from the mid-Atlantic coast (Bansal et al. 2019). Minnesota's third *Typha* taxa (*T*. X *glauca*) is a hybrid of the two species. MPCA sample observations are nearly equally divided among the three *Typha* taxa. Table 4 presents XC95 results for *Typha* taxa for each region. Many wetland investigators consider *Typha,* though not uniformly across the genus, to be tolerant to many stressors, including salts i.e. high conductivity (Bansal et al. 2019, Timoney 2015, Milburn et al 2007, Miklovic and Galatowitsch 2005).

In examining the XC95 results by *Typha* taxa across the test areas by stressor, *Typha latifolia* consistently showed a lower XC95 result than *T. angustifolia* and *T*. X *glauca* with one notable exception. In the MWS where the sulfate XC95 result for *T. latifolia* 158 m/L was compared to the very low sulfate XC95 value of 14 mg/L for *T*. X *glauca* in the MWS (Table 4). In the three regions where XC95 results were available for all three *Typh*a taxa *T. angustifolia* had a higher XC95 result in three salinity stressors than both *T*. X *glauca* and *T. latifolia*. Combined, these results suggest the hybrid *T*. X *glauca* is less tolerant to salinity stressors than *T. angustifolia*. Figure 8 illustrates GAM probability plots for the three *Typha* taxa in three ecoregions and statewide in response to conductivity. *Typha latifolia* consistently showed an optimal response curve whereas the other two *Typha* taxa consistently, though in a couple cases somewhat aberrantly, resulted in increasing probability plot responses. Recognizing increasing GAM probability plot patterns may demonstrate a less reliable XC95 result since the upper end of the curve does not flatten within the range of observed conductivities, which implies a potentially higher conductivity would be needed to result in extirpation of that taxa. These results support a contention that the native cattail *T. latifolia* is most sensitive to salinity variables among the three *Typha* taxa present in MN.

Tables 5, 6 and 7 list plant species based on extirpation analysis are potentially most sensitive or tolerant with respect to the salinity variables conductivity, chloride and sulfate as derived from species XC95 results. Ranked XC95 estimates for each species analyzed statewide and in the three ecoregions were used to derive lists of sensitive or tolerant species for each salinity stressor. The threshold for sensitive species was set at less or equal to the 10th percentile. Tolerant species were those that occurred at or above the 90th percentile XC95 results in each respective region. Several species occurred in either the < 10th percentile or > the 90th percentile ranking in only one region. To improve confidence of a species having a reliable response as sensitive or tolerant, only species ranked in the respective percentiles in at least two regions are included in Tables 5, 6 and 7. Complete tabular listing of XC95 results for all 114 species analyzed statewide and by the three ecoregions are provided in the Appendix A (conductivity), B (chloride) and C (sulfate). The majority of species presented here as sensitive or tolerant based on XC95 extirpation values were emergent perennials mostly typical of shallow marsh or fresh meadow wetland communities. Though a few submergent and floating leaved species typical of deep marshes or shallow open water wetlands also are included in these lists.

Coefficients of conservativeness (Milburn et al 2007) for species are also provided in the sensitive and tolerant tables. These coefficients represent a species fidelity to varying degrees of competition, stress or disturbance were developed through an objective review process by expert botanists familiar with MN wetland flora to consider all wetland or aquatic plants recognized as occurring in MN. Coefficients of conservativeness (*C*-values) range from 10 to 1 where species with high fidelity to undisturbed habitats, low stress would be assigned a value of or near 10 and more opportunistic stress tolerant species would be assigned a value close to 1. Nonnative species are assigned a 0 or an "*" to indicate no contribution to natural community integrity. Generally species with coefficients $\geq$ 6 could be considered sensitive and species with C-values $\leq$ 4 could be considered tolerant to stress. Though an objective panel of professional judgement review process was used to assign species *C*-values there remained some degree of subjectivity in the assignments. In contrast, data presented in Tables 5, 6 and 7 are entirely quantitative and empirical in response only to salinity related stressors. Both approaches are provided to afford readers easy comparison between the two assessments. Generally there was good concordance between the assigned *C*-values and extirpation based sensitive or tolerant species responses to the three salinity stressors, with one exception. Milburn et al. (2007) assigned *Potamogeton pusillus* a *C* of 7 suggesting it is more of a sensitive species whereas in this work it is the only species to repeatedly occur in the list of tolerant species in several regions for all three salinity stressors. Some investigators (Harguinteguy et al. 2016 and Monferran et al. 2012) report *Potamogeton pussilus* as able to grow in habitats polluted with heavy metals, particularly Cr, Cu and Zn.

Table 8 presents a summary of two forms of overall plant community environmental benchmark thresholds for each of the three salinity stressors within the four regions considered within this investigation. Part A presents thresholds based on the 95th percentile of least impaired depressional wetland reference sites along with the number of reference sites in each region for each of the three stressor variables. This approach was adapted from methods and results presented by Genet and Bourdaghs (2007, 2006). Table 8 part B provides extirpation based probability derived hazardous concentration (HC05) estimates for wetland plant community responses to each of the three stressors across regions.

Results for specific conductance criteria generally finds the 95th percentile reference site method results in more conservative (higher) values compared to the extirpation based method. This pattern did not hold in the Mixed Wood Plains (MWP) ecoregion where the 330 mg/L HC05 estimate for conductivity was higher, compared to the 232 mg/L criteria derived using the 95th percentile of reference site conductivity results. HC05 extirpation results for chloride and sulfate were consistently higher than the 95th percentile of reference site results for all respective regional comparisons of these two anions. Statewide and ecoregion based results from both criteria derivation methods are notably lower than the Class 3 industrial use standard (1000 µS/cm) for specific conductance and similarly the Class 2 aquatic life and recreational use standard for chloride of 230 mg/L is much higher than the wetland chloride criteria presented in Table 8 using the two different threshold derivation methods. Most of the statewide and regional sulfate benchmarks based on the extirpation HC05 criteria and the 95th percentile of the reference site observations are similar to or slightly above the 10 mg/L Class 4 standard to protect waters for propagation of wild rice. One notable exception was evident from the results presented in Table 8.

The sulfate extirpation HC05 concentration benchmark of 69.6 mg/L for the Temperate Prairies ecoregion of western and southwestern Minnesota is nearly seven times the Class 4 sulfate standard of 10 mg/L.

Discussion

Specific conductance is a specific ionic strength water quality response variable recognized to be biologically relevant (Cormier et al. 2013, U.S. EPA 2011). There are no previous known extrapolations or models of specific conductance effect on Minnesota depressional wetland condition. Genet (2012) considered reporting on specific conductance as a wetland stress criterion, but instead chose to report on the specific ion-chloride. Extensive scientific work has considered chloride gradients and corresponding plant community structure from hypersaline to freshwater environments. An early Minnesota specific examination of aquatic plants to ion specific gradients, particularly sulfate was reported by Moyle and Hotchkiss (1945). Many investigators have since recognized the adverse effects of increased salinity on freshwater wetland plant communities (Borgnis and Boyer 2015, Timoney 2015, Miklovic and Galatowitsch 2005, Hinneri 1976). These more recent investigations have reported on primarily specific anions such as chloride and sulfate rather than the non-specific indicator conductivity.

Minnesota waters, including depressional wetlands occur across a gradient of natural background salinity conditions including gradients of conductivity, chloride and sulfate due to surficial geology and surface to groundwater interactions (Gorham et al 1983). Genet (2015) reported sulfate and chloride concentrations in the Mixed Wood Plains and Temperate Prairie ecoregions exhibited significant differences with higher concentrations in the Temperate Prairies ecoregion. Water level fluctuation and desiccation within wetlands additionally accentuate ambient natural variation of these parameters. Hydrologic and landscape alterations along with pollutant loading have the potential to additionally affect ionic strength including concentrations of chloride and sulfate in Minnesota wetland waters. Natural and anthropogenically derived regional differences are further substantiated by the results presented here and underscore the importance of examining biological responses within geographic strata that exhibit similar ionic composition. Applying this design element reduces the confounding influence of variant background conditions. Researchers who developed the extirpation benchmark approach (Cormier and Suter 2013, U.S EPA 2016, 2011) that was used here, recognized and asserted the importance of analyses datasets should represent similar conductivity variable background conditions. Recognizing the importance of examining extirpation response within similarly composed ionic backgrounds an initial design of this investigation was abandoned. In that first design the available dataset was split roughly into 2/3 – 1/3 respectively as a development set and a validation set. This design was dropped in favor of analyzing the biological response at an individual ecoregion and statewide scale as discussed in the current study. In their Appalachian stream dataset (Cormier and Suter 2013, U.S EPA 2016, 2011) the data used are constrained to the same level III ecoregion scale to avoid influence of variant regional conditions. In the current investigation, a level II ecoregion stratification was used which is a higher geographic scale than the level III ecoregion scale. Due to the limited size of the available paired chemistry and corresponding plant dataset in this investigation it was judged to be inadvisable to stratify analysis at a level III ecoregion scale. Recognizing the wide ranged salinity gradient present in MN it is acknowledged results from the statewide scale may be less useful as reliable benchmark criterial, however results at this scale were presented to compare to results at the level II ecoregion scale.

Not surprisingly, analysis of statewide chemistry sampling results and HC05 benchmarks are intermediate to ecoregion findings for all three salinity stressors. Potential future development of either ion specific or general salinity water quality standards should consider regionally based development approaches. However, based on findings reported here if resource or other constraints prevent utilizing regionally based ionic stressor standards development a statewide development path should be expected to result in an intermediate result that will be too high in some regions of the state and too low in others. Thus exceedances in some parts of the state could entirely be due to natural background conditions. It is clear from the results presented here that wetland and aquatic plants have a wide range of sensitivity or tolerance to salinity based stressors. It is also clear that potential future water quality standards meant to be protective of aquatic life and recreation, i.e. Class 2 standards should consider responses from wetland and aquatic plants.

Groundwater-surface water interaction and evapotranspiration significantly affects conductivity values and likely also surface water concentrations of chloride and sulfate. Groundwater discharge conveys solutes and increases conductivity in surface water wetlands. Under natural conditions as is present in much of the Mixed Wood Shield ecoregion, conductivity is typically highest in mid-summer as ground water flow tends to decrease, evapotranspiration reaches maximum levels and wetland water levels frequently decrease resulting in dissolved ion concentration increases. Precipitation events add water to the system resulting in temporary dilution effects. This general pattern is common especially in the MWS, though, as demonstrated in Figure 4 it is not uniform across all wetland settings, regions, salinity variables and seasonal progression. There is a large amount of chloride concentration temporal variability across all three Minnesota level II ecoregions, with results plotted on a natural log scale used in Figure 3B showed a greater median and larger quartile amplitude of chloride concentration occurring in the Mixed Wood Plains compared particularly to the MWS ecoregion, though not as great a difference in the TP. It is reasonable to suspect part of this result is reflective of Minnesota population demographics and associated infrastructure as 81 of the 100 most populous cities in Minnesota https://www.minnesota-demographics.com/cities_by_population occur in this region, including the Minneapolis, St. Paul and the surrounding suburban metropolitan area occur in the Mixed Wood Plains. These local and regional population centers could be expected to have increased anthropogenically derived sources of chloride loading from roadway deicer’s and water softener elution discharges and in both the MWP and Temperate Prairies application of agricultural fertilizers (Dugan et al 2017).

Additionally, wetland salinity chemistry in wetlands often varies spatially within the same basin (Winter 2003). Conductivity is frequently higher near shore compared to central deeper water column areas in wetlands. Pollutant loadings are frequently received at the near shore margin and likely influence conductivity values as well as concentrations of chloride and sulfate. For the data sets used in this analysis wetland water chemistry and plant community data used in the extirpation analysis were mostly collected near the wetland shore margin during the early to mid-summer period.

Some readers may be interested in individual species potential extirpation (XC95) results. Appendices A, B and C list species XC95 estimates based in response to conductivity, chloride and sulfate stress respectively along with best fit GAM plot response type, either decreasing, optimal or decreasing. Some wetland plant species appeared to be clearly more sensitive or tolerant to salinity variables as examined here. Evaluations of extirpation sensitivity or tolerance were presented in Table 5 (conductance), Table 6 (chloride) and Table 7 (sulfate). Somewhat surprisingly few species showed sensitivity to more than one salinity variable. *Dulichium arundinaceum* showed sensitivity to conductivity and chloride. While *Potamogeton natans* showed sensitivity to conductivity and sulfate. For a given variable most species were sensitive in only a single region, including statewide analysis. Only *Eupatorium perfoliatum* var. *perfoliatum* was recognized as sensitive to conductivity statewide and in the MWP. *Carex lasiocarpa* var. *americana* was listed as chloride sensitive statewide, in all four geographic regions: statewide, TP, MWP and MWS. *Carex utricularia* appeared as sensitive to chloride statewide, and in the TP and the MWP. Lysimachia thrysiflora was listed as sensitive to chloride in the TP and MWP ecoregions. For all three variables the list of sensitive species was relatively short compared to the list of tolerant species. With three exceptions, given the larger number of species presented as tolerant to conductivity, chloride and sulfate species by species comparison is not explicitly discussed here. Readers are referred to Tables 5, 6 and 7. Three species *Cirsium arvense*, *Potamogeton pusillus* and *Typha angustifolia* were recognized as tolerant to all three salinity variables. Potamogeton pusillus was unique among the tolerant taxa in that it appeared as tolerant in the statewide tolerant listing and each of the ecoregions not only for chloride, but also for sulfate.

As has been discussed, stratification to geographic extents which are similar in ionic composition and strength is an important consideration for developing ion based criteria. However the interaction between ions and their potentially related biological response may also be critical. In this study measurements of individual anion constituents of wetland waters were determined from identical samples collected in space and time. In examining the biological response of respective ionic constituents the influence of one ion constituent over response to another cannot be effectively separated in field data. For example, if a plant species is particularly responsive to sulfate, possibly even extirpated from sample then the response of that species, in-fact because of the missing species the entire plant community response to chloride may be masked by the sulfate influence. This potential confounding influence of mixed chemistry constituents present in field data is difficult, if not impossible to control is acknowledged as a concern with field data investigations. Further, since the extirpation approach is affected by number of observations and the HC05 estimate increases as number of included taxa decreases (Cormier and Suter 2013, Cormier et al 2013, U.S. EPA 2011) in the sulfate example above the HC05 for chloride response could be higher as a result of the influence of sulfate.

Cormier et al. (2013) assert the importance of protective benchmarks (i.e. HC05) not occurring within (below) the natural background range. When natural gradient ranges differ greatly across different geographies as they do in Minnesota, benchmarks applicable in one ecoregion may not be applicable in adjacent ecoregions and in some instances possibly not even within the same ecoregion if that ecoregion includes areas of greatly dissimilar surface geology. For example the Temperate Prairies ecoregion which includes glacial lacustrine plains in the NW as well as areas of relatively recent and older glacial moraine and till plains in the S. Reference site thresholds derived at the 95th percentile (Table 8) represent the best available estimates of natural background levels in depressional wetlands of the three salty parameters discussed here. Extirpation derived hazardous concentration (HC05) benchmarks for Minnesota’s three level II ecoregions and statewide are also presented in Table 8. Comparing these two benchmarks in respective geographic extents finds the conductivity HC05 to be lower than the 95th reference centile in all regions except the Mixed Wood Plains. This might suggest the plant based HC05 may not be a reliable benchmark approach as a conductivity threshold in most regional extents of the state. Regardless these results still support aquatic plants as being a relatively sensitive assemblage that are responsive to the effects of conductivity.

In contrast the plant extirpation-based HC05 benchmarks for chloride and for sulfate were higher or similar to the 95th centile reference site natural background results in statewide results and for each of the three ecoregions. This meets one important principle in support of the HC05 biological response indicator.

The Hazardous Concentration (HC05) results for chloride ranged from 38.3 down to 8.9 mg/L representing a range of measurable biological response that is minimally 6 times lower and maximally nearly 26 times lower than the current Class 2 aquatic life based chloride standard of 230 mg/L. These HC05 chloride results suggest significant chloride sensitivity in some aquatic plant species and wetland plant communities that are well below the state aquatic life and recreation standard. There is growing public awareness and concerns about increasing ambient, surface and groundwater, chloride concentration and salinization resulting from widespread use of deicing agents, water softener related wastewater discharges, agricultural mineral fertilizer applications and soil erosion (Dugan et al. 2017, Herbert et al. 2015).

Sulfur is a secondary plant nutrient typically available to most plants in the oxidized sulfate form. The biologically-reduced form, sulfide, occurs in anoxic wetland substrates and can be toxic to aquatic plants when present in elevated concentrations. The geochemistry of sulfide is very complex. The MPCA wetland-monitoring program has not routinely collected sulfide data, which requires controlled sample extraction methods from sediment pore water. Extensive research of sulfide toxicity affecting wild rice

Zizannia palustris by Myrbo et al. (2017) has demonstrated complex sulfide biogeochemistry related to presence of carbon and iron compounds within sediments. The HC05 results presented here for sulfate range from 9.4 mg/L in the MWP to 69.6 mg/L in the TP ecoregion which was significantly higher than statewide and in the other two ecoregions. However the sulfate 69.6 mg/L HC05 result in the TP was not surprising in the context of that region typically exhibiting the highest concentrations of sulfate. The 69.6 mg/L is just below the median sulfate concentration from the entire data set in this region and less than one half the mean concentration of sulfate in the TP. Sulfate HC05 results in the MWP (9.4) and MWS (14.2) ecoregions were fairly similar to the existing 10 mg/L sulfate standard applicable to waters that support populations of wild rice (*Zizania palustris*). Though *Zizania palustris* was often observed in the wetland samples there were not sufficient (≥ 25) wild rice observations associated with sulfate samples to permit an XC95 estimate statewide or in any of the three ecoregions.

On a relative risk basis, analysis of stressors in a baseline assessment of Minnesota wetland condition, Genet (2012) reported chloride as the leading depressional wetland stressor to plant communities for statewide extent. In the second cycle of the same wetland survey, Genet (2015) found chloride remained a leading relative risk stressor in depressional wetland plant communities. He found relatively high chloride concentrations occurred in nearly 50% of the depressional wetlands sampled as part of the second cycle. In the same study, Genet reported sulfate being a moderate risk pollutant but still likely adversely affecting depressional wetland plant communities. Further, report Genet derived a reference site only 5th percentile (i.e. 5% or less of reference sites) for chloride of 8.6 mg/L in the TP; 7.9 mg/L in the MWP and 3.3 mg/L in the MWS. In this investigation the HC05 ecoregion chloride results were 8.9 mg/L in the TP, 38.3 mg/L in the MWP and 8.9 mg/L in the MWS. These chloride extirpation benchmarks are comparable to the reference site ecoregion thresholds for “Poor” condition, except in the MWP where the chloride HC05 result was notably higher than the reference site result. Similarly comparing Genet’s (2015) 5th centile reference site threshold for sulfate with sulfate HC05 results finds the following. The TP reference site criterion for sulfate is 127.4 mg/L, in the MWP the sulfate Poor threshold is 12.5 mg/L. Genet (2015) does not provide a sulfate criterion for the MWS. In this study the HC05 benchmark for sulfate was 69.9 mg/L in the TP and 9.4 in the MWP. Findings by Genet (2015 & 2012) are reasonably comparable to the HC05 results reported here by ecoregion and support the significance of sulfate and chloride as important stressors in Minnesota wetlands.

Results presented here could have clearly benefitted from additional paired data observations. As discussed earlier, this is particularly the case for specific conductance and sulfate biological response in the Mixed Wood Shield Ecoregion. The number of paired field chemistry and biology samples are fewer then the number of samples recommended in Cormier et al (2018) and U.S. EPA (2016). They recommended a minimum of 200-and preferably at least 500 paired samples to obtain stable HC05 values in their macroinvertebrate work. This current study included more than 800-paired water chemistry and biological samples in statewide analysis. At the ecoregion scale paired sample numbers were considerably below the preferred 500 sample number in the TP and MWP, but above the minimum 200 sample count recommendation. In the MWS ecoregion the number of paired samples (157) was considerably below the recommended 200 sample minimum. Consequently results in the MWS may not be reliable without further sample extrapolation testing or additional data. As additional data become available it may be appropriate to repeat the extirpation and hazardous concentration analysis. Ideally, larger data sets would increase the number of wetland plant species and expand the number of wetland communities evaluated for potential stress due to increases in specific conductance, chloride concentrations and/or sulfate concentrations. Regardless, the results and findings presented and discussed here provide justification to consider the protection of wetland aquatic plants when evaluating aquatic life standards for specific conductance, chloride and sulfate.

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Attachment 2

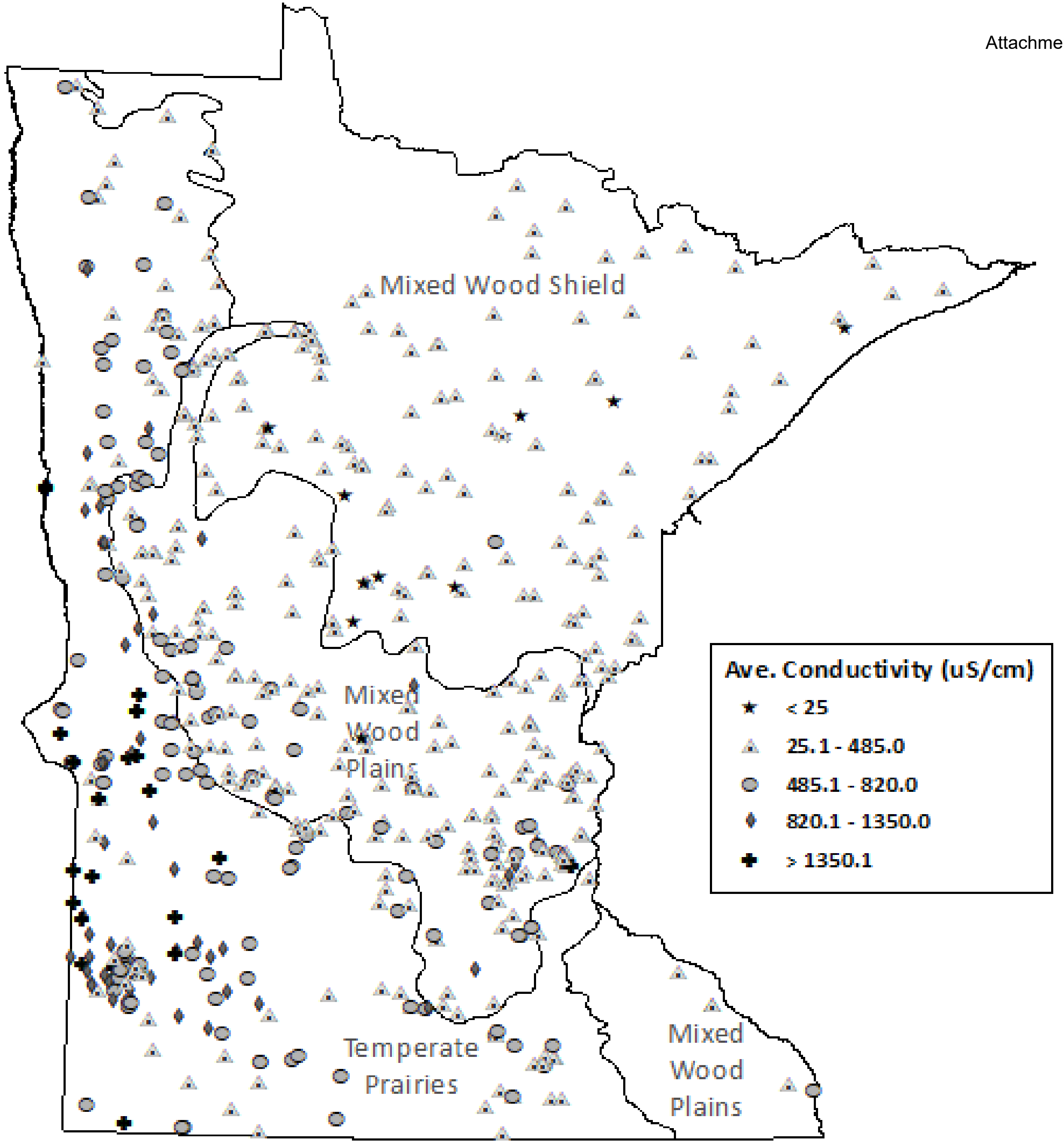

Figure 1. Wetland sampling locations (n= 587) showing the range of specific conductance (µS/cm) at each sampling location. Stations with more than one conductivity sample are presented as averages for that station.

Figure 2. Histograms of data depressional wetland data distribution for: A) Conductivity; B) chloride (Cl) and C) sulfate (SO-24). Data were parsed into 40 equal bins. Bin ranges were 0.0906 log10 units wide for conductivity (n = 1376), 0.0687 log10 units wide for chloride (n = 1432 and 0.0821 log10 units wide for sulfate (n = 1300).

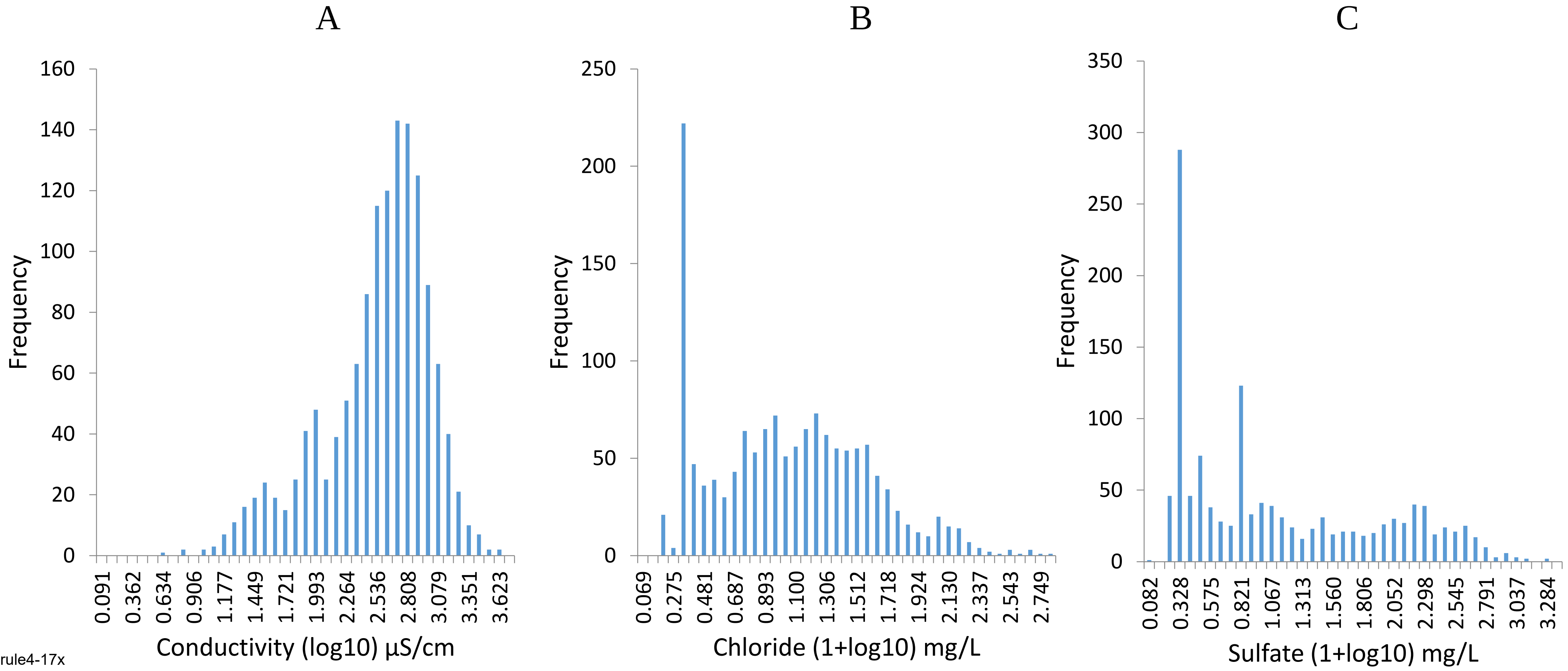

Table 1. Table 1. Descriptive statistics for laboratory derived chemistry variables (Sulfate and Chloride) and field derived Conductivity results. Minimum and maximum wetland chemistry sample results include those paired with plant data provided for each variable along with data collection date range. Plant sample counts with corresponding chemistry sample results also provided. Laboratory detection limits = "detect limit".

Parameter	Statistic	Statewide	Temperate Prairies	Mixed Wood Plains	Mixed Wood Shield
Conductivity (µS/cm)	n =	1376	547	602	227
	Mean	486.1	756.9	377.9	120.8
	Median	394.5	645.0	329.5	73.0
	Minimum	3.6	5.83	3.6	8.4
	Maximum	4195	4195	1836	597
Chloride (mg/L)	n =	1432	546	651	235
	Mean	22.4	16.1	34.1	4.6
	Median	8.7	11.8	11.1	1.1
	Minimum	0.5	1	0.5	0.5
	Maximum	560	211	560	49.2
Sulfate (mg/L)	n =	1300	523	589	188
	Mean	67.1	152.9	11.0	4.0
	Median	5.0	82.1	2.1	1
	Minimum	0.5	1	0.5	0.5
	Maximum	1920	1920	291	158

Figure 3. Box plots of salinity variables; conductivity, chloride and sulfate statewide and across the three level II ecoregions present in Minnesota. Variables are plotted at log10 scale. Chloride and sulfate were transformed by adding 1 to data values prior to applying log10 transformations to avoid negative log values. Temperate Prairies = TP; Mixed Wood Plains = MWP; and MWS = MWS.

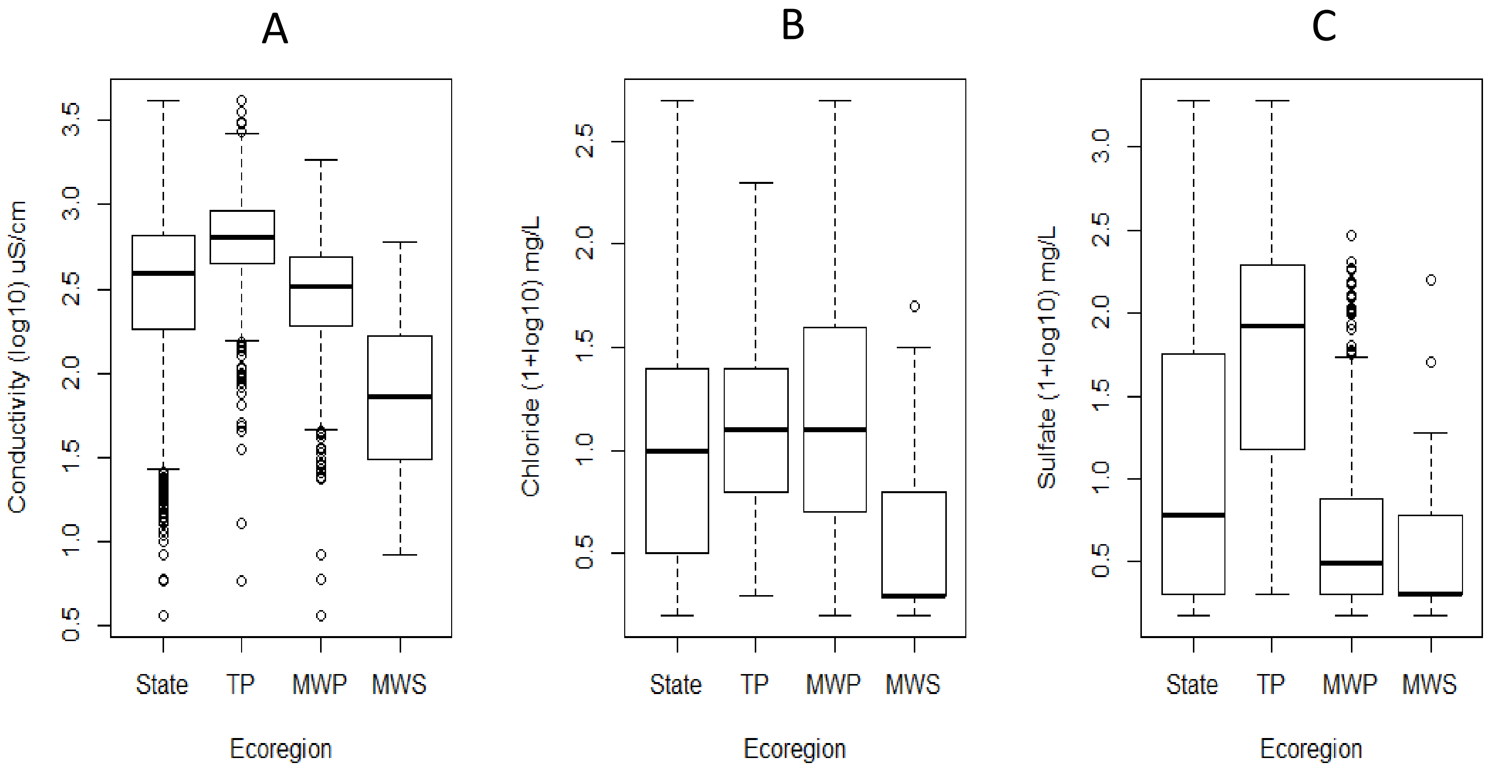

Table 2. Dunns post hoc test multiple comparison p-value results, after Kruskal-Wallis test showed differences in medians across ecoregion and statewide data for conductivity, chloride and sulfate. Dunns test applied the Benjamini-Hochberg stepped pairwise comparison method.

	Conductivity		Chloride		Sulfate	
Pairings	Z-statistic	Adjusted p-value	Z-statistic	Adjusted p-value	Z-statistic	Adjusted p-value
State - TP	13.63	< 0	-2.85	0.005	-15.63	< 0
State – MWP	-3.96	< 0	4.2	< 0	-9.15	< 0
State - MWS	-16.02	< 0	-12.54	< 0	-10.6	< 0
TP – MWP	-14.94	< 0	0.95	0.03 - ns	-21.03	< 0
TP – MWS	-23.26	< 0	-13.14	< 0	-19.25	< 0
MWP - MWS	12.25	< 0	14.2	< 0	4.45	< 0

Figure 4. Boxplots of conductivity, chloride and sulfate concentrations by month (April – Oct.) in Minnesota's three ecoregions: Temperate Prairies = TP; Mixed Wood Plains = MWP; and Mixed Wood Shield = MWS

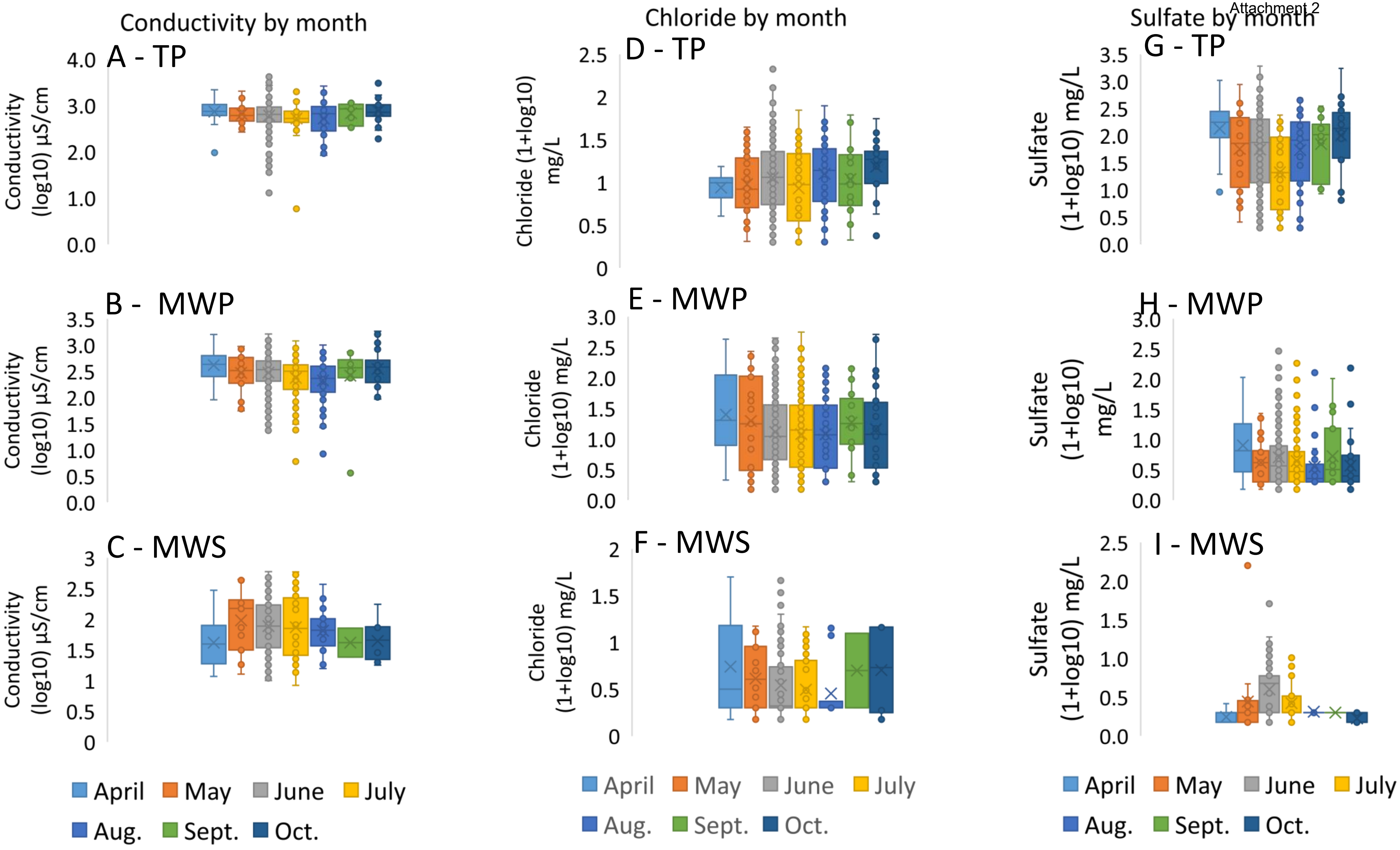

Table 3. Descriptive statistics for paired biology and chemistry data sets.

Parameter	Statistic	Statewide	Temperate Prairies	Mixed Wood Plains	Mixed Wood Shield
Conductivity (µS/cm)	n =	811	304	350	157
	Mean	452.7	728.8	353.5	139.5
	Median	372	635.5	325.5	10.2
	Minimum	5.8	5.8	23.4	10
	Maximum	3141	3141	1628	531
Plant species	n =	115	38	73	53
Chloride (mg/L)	n =	834	296	380	158
	Mean	20.0	16.7	29.1	4.1
	Median	7.4	9.4	12	1.2
	Minimum	0.5	1	0.5	1
	Maximum	560	211	560	48
Plant species	n =	114	37	72	56
Sulfate (mg/L)	n =	723	285	327	111
	Mean	62.3	141.6	12.5	5.8
	Median	5	50.4	2.5	2.3
	Minimum	1	1	1	1
	Maximum	1920	1920	291	158
Plant species	n =	107	36	66	43

Figure 5. Generalized additive model (GAM) probability plots for three common wetland plant species analyzed against statewide conductivity data to illustrate the three typical responses to a range of specific conductance values. Each plot includes a trend line fitted to the probability plot along with 95% confidence interval lines to illustrate the three main data distribution patterns:. (A). *Calamagrosti*s canadensis illustrates a decreasing response in plot A., plot (B) *Asclepias incarnata ssp. incarnata* responds in an optimized curve suggesting an optimal conductivity range; and (C) *Stucknia pectinatus* in Plot C illustrates an increasing or positive response to increased conductivity. Vertical dashed lines in each plot represents the 95th percentile extirpation concentration (XC95) from the weighted cumulative distribution function.

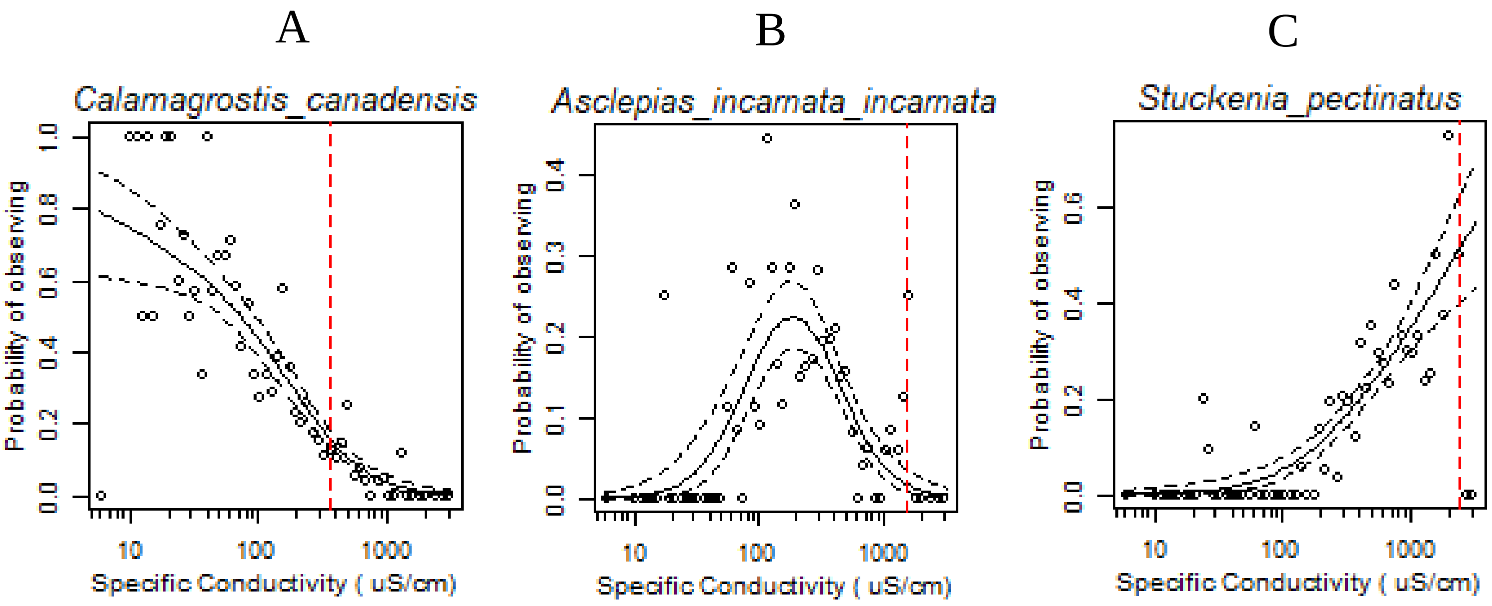

Figure 6. CDF plot output from wt.cdf from XC95 package in R. Analysis illustrates different responses to a range of specific conductance values for three common wetland plant species (A) *Calamagrostis canadensis*, (B) *Asclepias incarnata incarnata* and (C) *Stuckenia pectinatus*. Vertical dashed line represents the 95th percentile extirpation value (XC95) from the weighted cumulative distribution function.

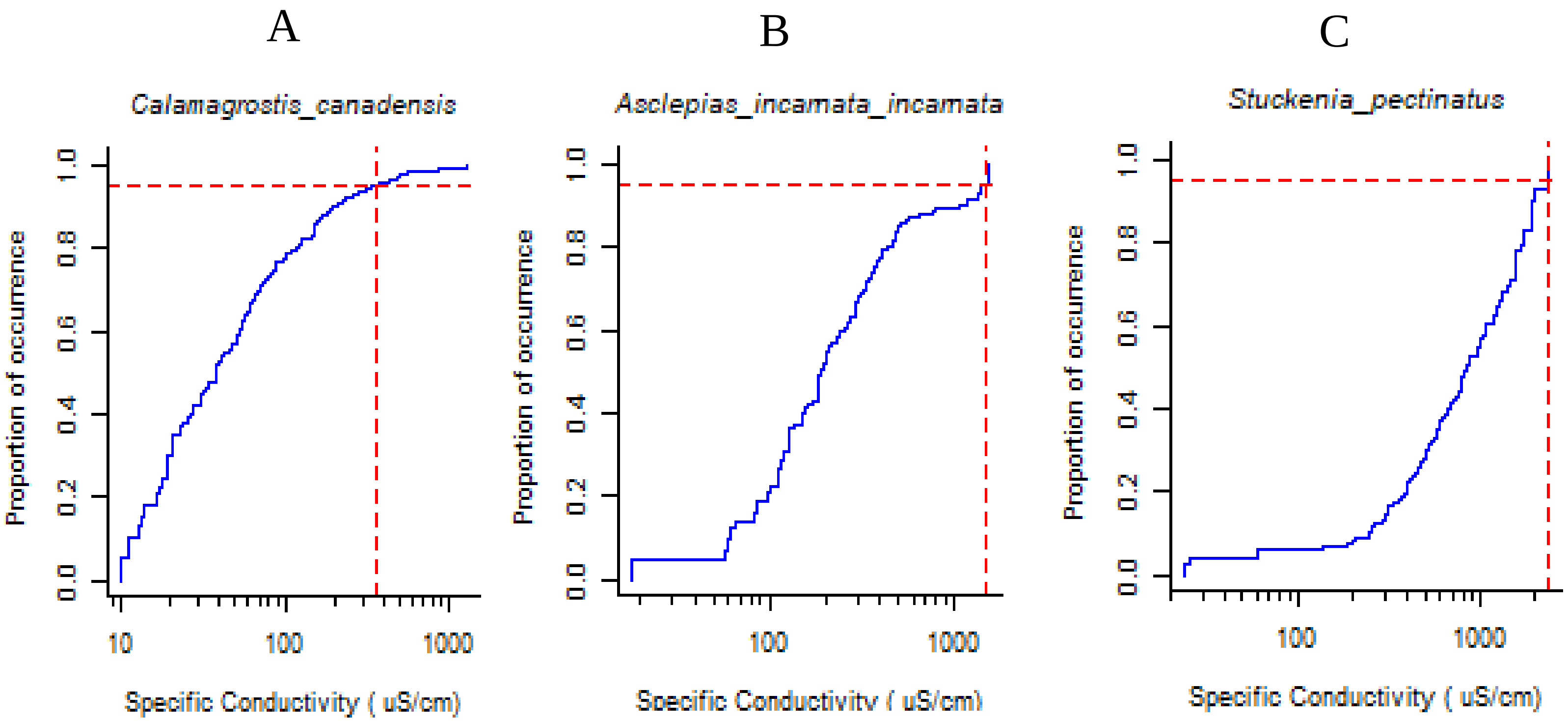

Figure 7. Boxplots of XC95 results regionally for three salinity stressor variables: conductivity, chloride and sulfate

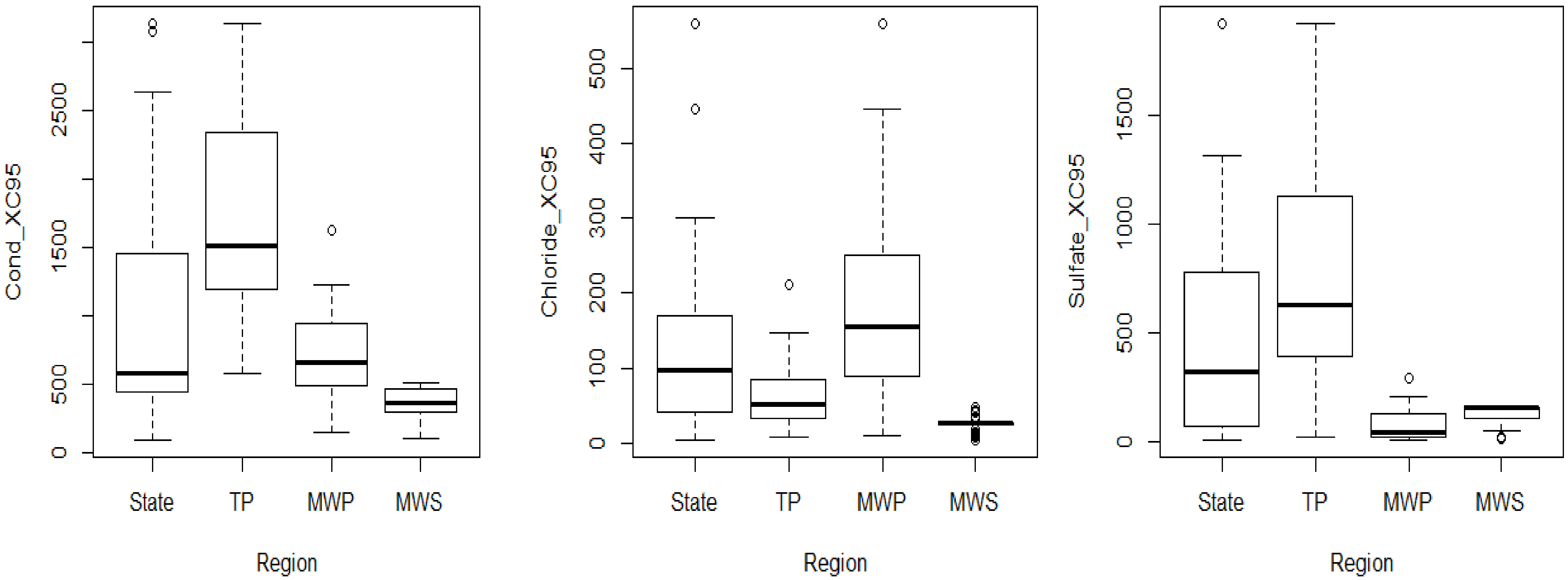

Table 4. XC95 estimates for the three cattail (Typha) taxa occurring in MN against conductivity, chloride and sulfate statewide and by ecoregion. Ecoregions are Temperate Prairies (TP); Mixed Wood Plains (MWP) and Mixed Wood Shield (MWS). Following each respective XC95 value and separated by a hyphen are the probability plot response patterns: optimum (op), increasing (in) and decreasing (de).

Stressor	Typha Taxa	Statewide	TP	MWP	MWS
Conductivity	*Typha latifolia*	1010 - op	1482 - op	544 - op	432 - in
	Typha angustifolia	3141 - in	3141 - in	1628 - in	
	Typha X *glauca*	2507 – in	2347 – in	1200 – in	497 - in
Chloride	*Typha latifolia*	48 – de	46 – de	45 - de	34 - in
	Typha angustifolia	560 – op	146 – in	560 - in	
	Typha X *glauca*	446 – in	211 – in	352 – in	33 - in
Sulfate	*Typha latifolia*	902 – de	899 – de	30 – de	158 - in
	Typha angustifolia	1310 – in	1310 – in	291 - op	
	Typha X *glauca*	910 – in	1060 – in	203 – in	14 - op

Figure 8. Illustrations of generalized additive model (GAM) probability plots for the three cattail (Typha) taxa that occur in Minnesota in response to conductivity across four geographic regions: statewide (A); the Temperate Prairies (B. TP); Mixed Wood Plains (C. MWP); and Mixed Wood Shield (D. MWS).

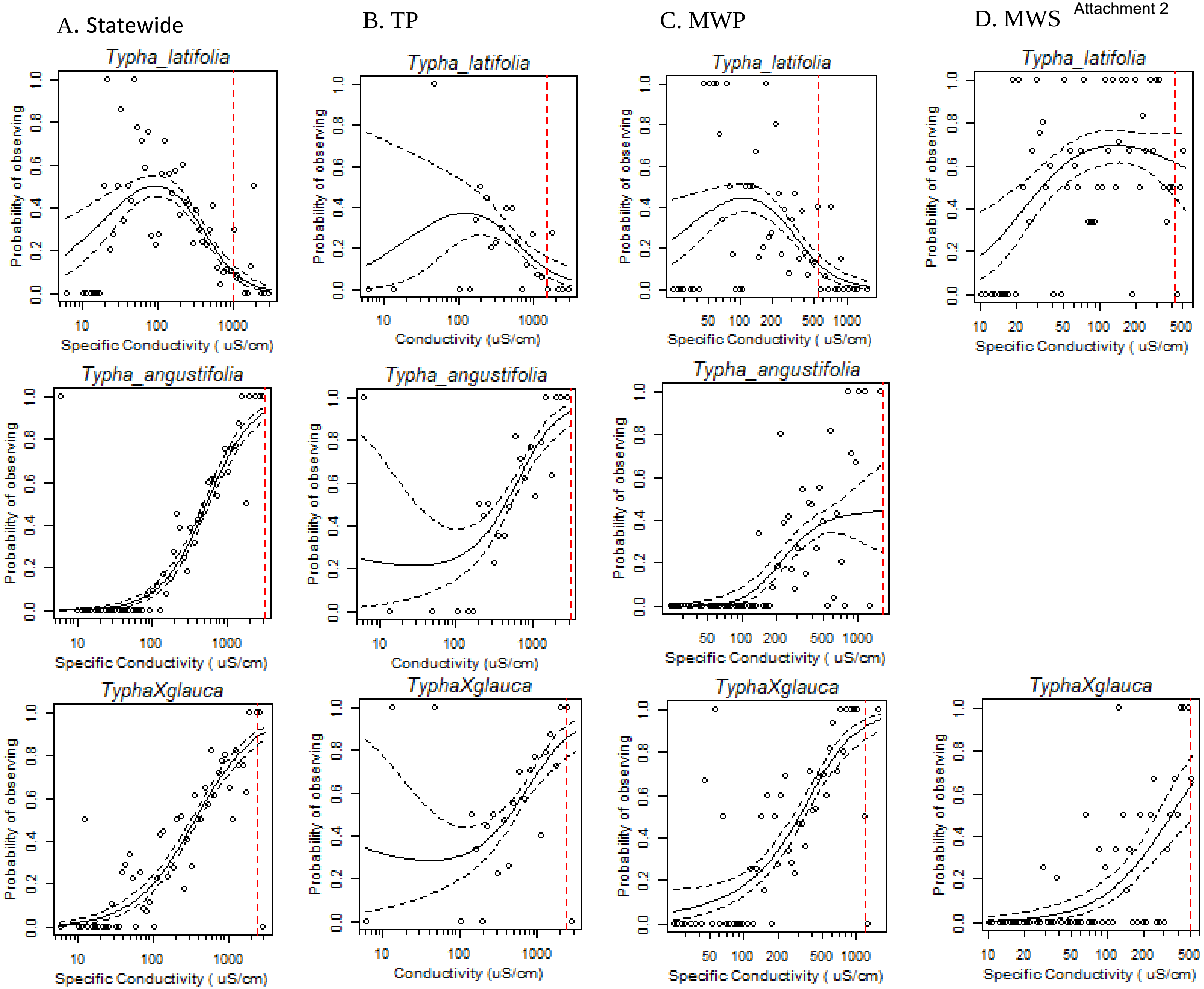

Table 5. XC95 estimates and number of observations (n =) for species demonstrating the greatest sensitivity (A) and greatest tolerance (B) to specific conductance across the four analysis regions. Selections determined by XC95 estimates within the 10th percentile for of all species specific conductance XC95 results within each respective region or the 90th percentile of species XC95 results for tolerant selections. Percentile XC95 percentile thresholds provided for each respective region. Final selections for either sensitive or tolerant species required estimates of a minimum of two regions.

A. Species sensitive to specific conductance		Cond - state 10th percentile XC95 < 216.8		Cond - TP 10th percentile XC95 < 997.5		Cond - MWP 10th percentile XC95 < 408.4		Cond - MWS 10th percentile XC95 < 181.6	
Species	Species *C* value[1]	XC95	n =	XC95	n =	XC95	n =	XC95	n =
Brasenia schreberi	7	**98**	42					**126**	156
Calla palustris	8	**167**	50					**164**	47
Dulichium arundinaceum	8	**111**	69					**110**	54
Eupatorium perfoliatum var. *perfoliatum*	4	**186**	51			**357**	44		
Glyceria canadensis	7	**132**	61					**176**	47
Lysimachia terrestris	7	**170**	29					**136**	26
Potamogeton natans	5	**203**	68						
Spiraea alba	5	**181**	30						
Triadenum fraseri	6	**181**	87					**167**	63

B. Species tolerant to specific conductance		Cond - state 90th percentile XC95 ≥ 2340		Cond - TP 90th percentile XC95 ≥ 3141		Cond - MWP 90th percentile XC95 ≥ 1200		Cond - MWS 90th percentile XC95 ≥ 494	
Species	Species *C* value[1]	XC95	n =	XC95	n =	XC95	n =	XC95	n =
Alisma trivale	4	**3141**	195	**3141**	48				
Ceratophyllum demersum	2	**2638**	465	**3141**	171				
Cirsium arvense	*	**3141**	83	**3141**	38				
Lemna minor	5	**2638**	672	**3141**	253	**1200**	344		
Lemna trisulca	5	**3087**	423	**3141**	199				
Phalaris arundinacea	*	**2340**	501	**3141**	155				
Potamogeton pussilus	7	**3141**	198	**3141**	62				
Schoenoplectus tabermaemontani	4					**1230**	84	**513**	30
Scutellaria lateriflora	5	**2340**	130			**1230**	98	**494**	
Typha angustifolia	*	**3141**	329	**3141**	198	**1628**	108		
Typha X glauca	*	**2507**	410			**1200**	204	**513**	30
Urtica dioica ssp. *gracilis*	1	**2340**	84			**1628**	50		

[1] Milburn, Bourdaghs and Husveth 2007

Table 6. XC95 estimates and number of observations (n =) for species demonstrating the greatest sensitivity (A) and greatest tolerance (B) to chloride across the four analysis regions. Selections determined by XC95 estimates within the 10th percentile for species chloride XC95 sensitivity responses within each respective region or the 90th percentile of species XC95 results for tolerant selections. Percentile XC95 percentile thresholds provided for each respective region. Final selections for either sensitive or tolerant species required estimates of a minimum of two regions.

A. Species chloride sensitivity		Chloride - state 10th percentile XC95 <19.2		Chloride - TP 10th percentile XC95 < 17.6		Chloride - MWP 10th percentile XC95 < 45.4		Chloride - MWS 10th percentile XC95 ≤ 13	
Species	Species *C* value[1]	XC95	n =	XC95	n =	XC95	n =	XC95	n =
Carex lasiocarpa var. *americana*	7	**16**	130	**8**	25	**24**	42	**13**	63
Carex utricularia	7	**13**	157	**13**	28	**10**	55		
Coumarum palustre	7	**11**	65					**12**	53
Dulichium arundinaceum	8	**11**	77					**13**	59
Lysimachia thyrsiflora	6			**9**	28	**39**	89		
Torreychloa pallida	8	**4**	25					**4**	25

B. Species chloride tolerance		Chloride - state 90th percentile XC95 ≥ 446		Chloride - TP 90th percentile XC95 ≥ 172		Chloride - MWP 90th percentile XC95 ≥ 560		Chloride - MWS 90th percentile XC95 ≥ 33	
Species	Species *C* value[1]	XC95	n =	XC95	n =	XC95	n =	XC95	n =
Cirsium arvense	*	**560**	74			**560**	25		
Galium trifidum ssp. *trifidum*	6	**560**	231			**560**	100		
Lythrum salicaria	*	**560**	88			**560**	80		
Lemna minor	5	**446**	706					**44**	106
Mentha arvensis	3	**560**	70			**560**	36		
Pilea pumila var. *pumila*	3	**560**	556			**560**	52		
Potamogeton pusillus	7	**560**	216	**211**	59	**560**	111	**48**	45
Riccia fluitans	-	**560**	240			**560**	99		
Rumex britannica	6	**560**	95			**560**	53		
Salix interior	2	**446**	56						
Typha angustifolia	*	**560**	315			**560**	119		
Typha X *glauca*	*	**446**	446	**211**	158			**33**	34
Urtica dioica spp. *gracilis*	1	**560**	69			**560**	41		

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[1] Milburn, Bourdaghs, and Husveth 2007

Table 7. XC95 estimates and number of observations (n =) for species demonstrating the greatest sensitivity (A) and greatest tolerance (B) to sulfate across the four analysis regions. Selections determined by XC95 estimates within the 10th percentile for all species sulfate XC95 results within each respective region or the 90th percentile of species XC95 results for tolerant selections. Percentile XC95 percentile thresholds provided for each respective region. Final selections for either sensitive or tolerant species required estimates of a minimum of two regions.

A. Species sulfate sensitivity		Sulfate - state 10th percentile XC95 < 45.5		Sulfate - TP 10th percentile XC95 < 255		Sulfate - MWP 10th percentile XC95 < 18.2		Sulfate - MWS 10th percentile XC95 < 17.2	
Species	Species *C* value[1]	XC95	n =	XC95	n =	XC95	n =	XC95	n =
Nymphaea odorata	6	**25**	74			**17**	36		
Potamogeton natans	5	**21**	55					**17**	33
Sagittaria rigida	7	**13**	56			**11**	37		
Wolffia columbiana	5	**20**	85			**17**	64		

B. Species sulfate tolerance		Sulfate - state 90th pctl XC95 $\geq$ 1310		Sulfate - TP 90th pctl XC95 $\geq$ 1627.5		Sulfate - MWP 90th pctl XC95 $\geq$ 560		Sulfate - MWS 90th pctl XC95 $\geq$ 158	
Species	Species *C* value[1]	XC95	n =	XC95	n =	XC95	n =	XC95	n =
Alisma trivale	4	**1920**	173	**1920**	47			**158**	43
Ceratophyllum demersum	2	**1310**	426	**1920**	138			**158**	39
Cirsium arvense	*	**1920**	73	**1920**	34	**203**	25		
Lemna minor	5					**203**	334	**158**	74
Lemna trisulca	5	**1310**	398					**158**	29
Lycopus uniflorus	5	**1310**	207					**158**	81
Phalaris arundinacea	*	**1920**	432			**203**	231	**158**	61
Potamogeton pusillus	7	**1920**	198	**1920**	58	**203**	104	**158**	36
Rumex britannica	6					**291**	71	**158**	25
Typha angustifolia	*	**1310**	294			**291**	111		

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[1] Milburn, Bourdaghs, and Husveth 2007

Table 8. MPCA wetland stressor assessment thresholds derived at the 95th percentile from all samples at least disturbed reference sites. Where 95% of the reference site observations would be less than (<) this value. Compared to the 0.05 percentile estimated hazardous concentration (HC05) for analyzed wetland plant species.

Reference Site based (95th percentile)

Applicable Region	Conductance (µS/cm)	Chloride (mg/L)	Sulfate (mg/L)
Statewide (Reference Sites	461 (n = 66)	7.8 (n = 58)	17.6 (n = 54)
Temperate Prairies	634 (n = 13)	6.6 (n = 7)	27.7 (n = 7)
Mixed Wood Plains	232 (n = 14)	7.6 (n = 13)	9.3 (n = 17)
Mixed Wood Shield	189 (n = 41)	4.4 (n = 38)	5 (n = 18)

B. Extirpation based Hazardous Concentration (HC05)

Applicable Region	Conductance (µS/cm)	Chloride (mg/L)	Sulfate (mg/L)
Statewide (HC05)	166 (n = 811)	10.8 (n = 834)	22.6 (n = 723)
Temperate Prairies	590 (n = 303)	8.9 (n = 296)	69.6 (n = 285)
Mixed Wood Plains	330 (n = 366)	38.3 (n = 380)	9.4 (n = 327)
Mixed Wood Shield	131 (n = 156)	8.9 (n = 158)	14.2 (n = 111)

Appendix A. Wetland plant species individual extirpation estimates in response to conductivity (µS/cm) in fc geographic regions; statewide, Temperate Prairies ecoregion (TP), Mixed Wood Plains ecoregion (MWP) anc Wood Shield ecoregion (MWS). Number of paired plant and conductivity observations (n =) are provided as generalized affects model (GAM) probability plot curve "fit" (Pattern) where biol. response related to chemi as increasing (in); optimal (op); decreasing (de) or undefined (*).

	Statewide			TP			MWP			
SPECIES	**XC95**	n	Pattern	**XC95**	n	Pattern	**XC95**	n	Pattern	**XC95**
Acer negundo	**2340**	44	*op*				**784**	42	*op*	
Acorus americanus	**357**	50	*op*				**158**	37	*op*	
Alisma triviale	**3141**	195	*op*	**3141**	48	*de*	**608**	98	*de*	**492**
Alopecurus aequalis var. *aequalis*	**538**	44	*op*							
Asclepias incarnata ssp. *incarnata*	**1491**	109	*op*				**485**	74	*op*	
Bidens cernua	**643**	51	*op*				**534**	49	*de*	
Brasenia schreberi	**98**	42	*de*							**126**
Calamagrostis canadensis	**362**	188	*de*				**596**	84	*de*	**295**
Calla palustris	**167**	50	*op*							**164**
Campanula aparinoides	**464**	95	*op*				**550**	45	*op*	**241**
Carex atherodes	**1259**	72	*	**1233**	42	*de*				
Carex bebbii	**444**	33	*de*							
Carex comosa	**911**	85	*op*				**910**	66	*op*	
Carex diandra	**387**	46	*op*							**336**
Carex hystericina	**562**	90	*op*				**636**	48	*in*	**482**
Carex interior	**134**	26	*de*							
Carex lacustris	**452**	233	*de*	**1244**	27	*op*	**552**	110	*op*	**466**
Carex lasiocarpa var. *americana*	**475**	136	*de*	**1540**	25	*op*	**531**	48	*op*	**286**
Carex retrorsa	**501**	36	*op*							
Carex stipata var. *stipata*	**597**	30	*op*							
Carex stricta	**614**	92	*de*				**709**	40	*de*	**269**
Carex utriculata	**474**	161	*de*	**993**	29	*op*	**496**	61	*de*	**356**
Ceratophyllum demersum	**2638**	465	*in*	**3141**	171	*in*	**833**	265	*in*	**471**
Chara vulgaris	**1595**	57	*op*	**1595**	36	*op*				
Cicuta bulbifera	**497**	206	*de*				**945**	100	*de*	**472**
Cicuta maculata	**892**	57	*op*				**837**	45	*op*	
Cirsium arvense	**3141**	83	*in*	**3141**	38	*op*	**1157**	28	*op*	
Comarum palustre	**181**	67	*op*							**326**
Cornus sericea ssp. *sericea*	**578**	40	*op*							
Dulichium arundinaceum	**111**	69	*de*							**110**
Eleocharis aciculari s var. *acicularis*	**843**	55	*op*				**667**	32	*op*	
Eleocharis palustris	**1030**	322	*de*	**1540**	97	*op*	**497**	144	*op*	**304**
Elodea canadensis	**480**	40	*op*				**487**	34	*op*	
Epilobium coloratum	**1540**	51	*op*							
Epilobium eptophyllum	**506**	72	*op*				**454**	31	*op*	
Equisetum fluviatile	**506**	49	*op*							
Eupatorium perfoliatum var. *perfoliatum*	**186**	51	*de*				**357**	44	*de*	
Galium tinctorium	**503**	43	*op*				**545**	26	*op*	
Galium trifidum ssp. *trifidum*	**499**	219	*de*	**1132**	26	*de*	**652**	97	*de*	**466**

Glyceria borealis	**301**	147	*de*				**410**	42	*de*	**298**
Glyceria canadensis	**132**	61	*de*							**176**
Glyceria grandis var. grandis	**501**	201	*op*	**1056**	37	*de*	**468**	97	*de*	**473**
Impatiens capensis	**814**	134	*op*				**973**	92	*in*	**494**
Iris versicolor	**419**	64	*de*				**498**	34	*de*	
Leersia oryzoides	**554**	216	*op*	**1362**	28	*op*	**722**	138	*op*	**468**
Lemna minor	**2638**	672	*in*	**3141**	253	*in*	**1200**	344	*in*	**479**
Lemna trisulca	**3087**	423	*in*	**3141**	199	*in*	**713**	211	*in*	**382**
Lycopus americanus	**1213**	130	*de*	**1646**	25	*	**786**	71	*de*	**493**
Lycopus uniflorus	**1157**	228	*de*				**529**	109	*de*	**466**
Lysimachia terrestris	**170**	29	*de*							**136**
Lysimachia thyrsiflora	**451**	203	*de*	**584**	28	*op*	**541**	94	*de*	**300**
Lythrum salicaria	**1628**	53	*op*				**1628**	48	*in*	
Mentha arvensis	**1628**	70	*op*				**1628**	35	*op*	
Mimulus ringens var. *ringens*	**589**	32	*op*							
Myriophyllum sibiricum	**1540**	64	*op*	**1540**	34	*op*				
Myriophyllum verticillatum	**534**	58	*op*				**498**	27	*de*	
Najas flexilis	**451**	71	*op*				**463**	35	*op*	**339**
Najas guadalupensis	**457**	29	*op*							
Nuphar lutea ssp. *variegata*	**334**	57	*op*							**297**
Nymphaea odorata	**232**	93	*de*				**497**	47	*op*	**204**
Onoclea sensibilis	**349**	25	*op*							
Phalaris arundinacea	**2340**	501	*op*	**3141**	155	*de*	**945**	40	*de*	**478**
Phragmites australis	**995**	26	*op*							
Pilea fontana	**910**	47	*				**935**	40	*op*	
Pilea pumila var. *pumila*	**1628**	56	*op*				**1628**	50	*in*	
Poa_ alustris	**520**	61	*op*				**973**	25	*	**492**
Polygonum amphibium	**901**	228	*op*	**1287**	79	*de*	**903**	101	*op*	**328**
Polygonum hydropiperoides	**770**	25	*op*							
Polygonum lapathifolium	**1044**	79	*op*				**1020**	49	*de*	
Polygonum sagittatum	**303**	66	*op*				**339**	46	*de*	
Potamogeton foliosus ssp. *foliosus*	**1493**	75	*op*	**1556**	30	*in*	**680**	40	*op*	
Potamogeton gramineus	**484**	38	*de*							
Potamogeton natans	**203**	68	*de*							**347**
Potamogeton pusillus	**3141**	198	*de*	**3141**	62	*op*	**1157**	90	*in*	**396**
Potamogeton strictifolius	**2638**	148	*in*	**2638**	56	*in*	**552**	74	*op*	
Potamogeton zosteriformis	**497**	145	*op*				**497**	112	*op*	
Ranunculus flabellaris	**702**	41	*op*							
Ranunculus pensylvanicus	**748**	41	*op*							
Ranunculus sceleratus	**2340**	39	*in*							
Riccia fluitans	**996**	251	*op*	**1002**	95	*op*	**729**	109	*de*	**501**
Ricciocarpos natans	**677**	47	*							
Rorippa palustris	**1385**	76	*op*	**757**	44	*de*				
Rumex maritimus	**1421**	112	*in*	**1217**	62	*de*	**1020**	57	*in*	
Rumex britannica	**500**	101	*op*				**1157**	61	*op*	**333**
Sagittaria latifolia	**478**	313	*op*	**1130**	48	*op*	**498**	180	*op*	**479**
Sagittaria rigida	**269**	51	*de*				**343**	39	*op*	

Salix discolor	**520**	39	*op*							
Salix interior	**1628**	58	*op*	**704**	31	*op*				
Salix petiolaris	**515**	39	*de*							
Schoenoplectus acutus var. *acutus*	**2340**	87	*op*	**2340**	67	*op*				
Schoenoplectus fluviatilis	**1921**	135	*in*	**1506**	95	*de*	**408**	34	*op*	
Schoenoplectus tabernaemontani	**1540**	181	*op*	**1460**	71	*de*	**1230**	84	*op*	**513**
Scirpus cyperinus	**221**	144	*de*				**272**	53	*de*	**238**
Scolochloa festucacea	**2340**	32	*op*							
Scutellaria galericulata	**487**	167	*op*				**719**	102	*op*	**443**
Scutellaria lateriflora	**2340**	130	*op*				**1230**	98	*op*	**494**
Sium suave	**1300**	177	*op*	**1254**	68	*op*	**697**	71	*op*	**373**
Solanum dulcamara var. *dulcamara*	**3141**	25	*							
Sparganium erectum ssp. *stoloniferum*	**214**	72	*op*							**304**
Sparganium eurycarpum	**1209**	159	*op*	**1203**	70	*de*	**1200**	78	*op*	
Spiraea alba	**181**	30	*op*							
Spirodela polyrrhiza	**554**	368	*op*	**590**	49	*op*	**782**	262	*de*	**494**
Stachys palustris	**1331**	43	*							
Stuckenia pectinatus	**2340**	166	*in*	**2163**	111	*in*	**785**	60	*in*	
Thelypteris palustri s var. *pubescens*	**277**	59	*op*				**360**	28	*op*	**347**
Triadenum fraseri	**181**	87	*op*							**167**
Typha angustifolia	**3141**	329	*in*	**3141**	198	*in*	**1628**	108	*in*	
Typha latifolia	**1010**	263	*op*	**1482**	70	*op*	**544**	108	*op*	**432**
Typha X glauca	**2507**	410	*in*	**2347**	1911	*in*	**1200**	204	*in*	**497**
Urtica dioica ssp. *gracilis*	**2340**	84	*in*				**1628**	50	*in*	
Utricularia intermedia	**282**	36	*de*							**383**
Utricularia macrorhiza	**987**	383	*de*	**1536**	139	*op*	**608**	151	*op*	**427**
Utricularia minor	**589**	97	*de*				**610**	42	*op*	**317**
Wolffia columbiana	**599**	85	*op*				**842**	70	*op*	
Zizania palustris	**407**	36	*op*				**398**	35	*op*	

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MWS	
n	Pattern
61	*in*
29	*de*
91	*de*
47	*op*
33	*op*
29	*op*
38	*in*
99	*de*
62	*de*
30	*de*
74	*op*
53	*in*
104	*op*
53	*op*
54	*de*
97	*de*
100	*op*

86	*de*
47	*de*
75	*in*
37	*in*
56	*in*
106	*in*
33	*op*
39	*in*
99	*
26	*op*
32	*op*
26	*op*
35	*op*
51	*de*
83	*in*
33	*in*
52	*de*
41	*de*
46	*in*
55	*in*
34	*op*
96	*op*

30	*in*
92	*de*
57	*op*
27	*in*
42	*op*
57	*op*
77	*in*
28	*op*
63	*op*
93	*in*
30	*in*
25	*de*
107	*op*
40	*de*

Appendix B. Wetland plant species individual extirpation estimates in response to chloride (mg/L) in four geographic regions; statewide, Temperate Prairies ecoregion (TP), Mixed Wood Plains ecoregion (MWP) and Mixed Wood Shield ecoregion (MWS). Number of paired plant and chloride observations (n =) are provided as is the generalized affects model (GAM) probability plot curve "fit" (Pattern) where biol. response related to chemistry shows as increasing (in); optimal (op); decreasing (de) or undefined (*).

	Statewide			TP			MWP			MWS		
SPECIES	**XC95**	n	Pattern	**XC95**	n	Pattern	**XC95**	n	Pattern	**XC95**	n	Pattern
Acer negundo	**89**	40	*in*				**89**	34	*in*			
Acorus americanus	**19**	46	*op*									
Alisma triviale	**140**	197	*de*	**33**	47	*de*	**140**	87	*de*	**33**	63	*op*
Alopecurus aequalis var. *aequalis*	**29**	53	*de*									
Asclepias incarnata ssp. *incarnata*	**96**	94	*op*				**89**	63	*de*			
Bidens cernua	**42**	57	*op*				**40**	46	*de*			
Brasenia schreberi	**23**	44	*de*							**26**	30	*
Calamagrostis canadensis	**85**	187	*op*	**14**	26	*de*	**89**	68	*op*	**26**	93	*de*
Calla palustris	**25**	53	*de*							**26**	50	*de*
Campanula aparinoides	**35**	84	*de*				**53**	38	*de*	**26**	30	*
Carex atherodes	**68**	82	*de*	**69**	45	*de*						
Carex bebbii	**79**	29	*de*									
Carex comosa	**86**	85	*de*				**76**	62	*de*			
Carex diandra	**20**	49	*de*							**24**	30	*de*
Carex hystericina	**170**	92	*de*				**170**	49	*de*	**24**	40	*op*
Carex interior	**10**	25	*de*									
Carex lacustris	**170**	236	*op*	**28**	28	*de*	**170**	107	*op*	**26**	101	*de*
Carex lasiocarpa var. *americana*	**16**	130	*de*	**8**	25	*de*	**24**	42	*de*	**13**	63	*de*
Carex retrorsa	**170**	27	*op*									
Carex stipata var. *stipata*	**110**	35	*de*									
Carex stricta	**73**	88	*de*				**100**	39	*de*	**26**	28	*
Carex utriculata	**13**	157	*de*	**13**	28	*de*	**10**	55	*op*	**26**	74	*de*
Ceratophyllum demersum	**170**	500	*op*	**211**	143	*op*	**170**	301	*op*	**26**	54	*
Chara vulgaris	**45**	57	*op*	**52**	35	*de*						
Cicuta bulbifera	**170**	219	*op*				**194**	104	*op*	**26**	104	*op*
Cicuta maculata	**200**	60	*in*				**200**	43	*in*			
Cirsium arvense	**560**	74	*in*	**130**	34	*in*	**560**	25	*op*			
Comarum palustre	**11**	65	*de*							**12**	53	*de*
Cornus sericea ssp. *sericea*	**93**	37	*in*									
Dulichium arundinaceum	**11**	77	*de*							**13**	59	*de*
Eleocharis acicularis var. *acicularis*	**93**	57	*op*				**89**	34	*op*			
Eleocharis palustris	**170**	323	*de*	**95**	91	*de*	**170**	130	*de*	**26**	102	*de*
Elodea canadensis	**300**	58	*in*				**300**	53	*in*			
Epilobium coloratum	**32**	42	*op*									
Epilobium leptophyllum	**200**	65	*de*				**200**	31	*op*			
Equisetum fluviatile	**50**	53	*op*									
Eupatorium perfoliatum var. *perfoliatum*	**25**	40	*de*									
Galium tinctorium	**61**	39	*de*									
Galium trifidum ssp. *trifidum*	**560**	231	*de*	**20**	25	*de*	**560**	100	*de*	**25**	106	*de*
Glyceria borealis	**29**	155	*de*				**37**	46	*de*	**24**	86	*de*

Glyceria canadensis	**9**	64	*de*							**26**	53	*de*
Glyceria grandis var. *grandis*	**170**	196	*de*	**50**	36	*de*	**170**	81	*de*	**33**	79	*op*
Impatiens capensis	**199**	134	*in*				**199**	97	*in*	**33**	33	*in*
Iris versicolor	**89**	68	*op*				**99**	31	*op*	**26**	28	*in*
Leersia oryzoides	**175**	220	*op*				**170**	139	*de*	**33**	57	*
Lemna minor	**446**	706	*in*	**84**	218	*op*	**446**	379	*in*	**44**	106	*in*
Lemna trisulca	**120**	445	*op*	**106**	176	*in*	**89**	235	*op*	**12**	33	*de*
Lycopus americanus	**89**	126	*op*	**84**	25	*op*	**87**	65	*op*	**9**	36	*op*
Lycopus uniflorus	**300**	235	*de*				**300**	106	*de*	**26**	105	*de*
Lysimachia terrestris	**32**	31	*de*							**13**	29	*op*
Lysimachia thyrsiflora	**35**	205	*de*	**9**	28	*de*	**39**	89	*de*	**26**	88	*de*
Lythrum salicaria	**560**	88	*in*				**560**	80	*in*			
Mentha arvensis	**560**	70	*in*				**560**	36	*in*			
Mimulus ringen s var. *ringens*	**42**	32	*de*									
Myriophyllum sibiricum	**79**	77	*	**52**	30	*de*	**80**	35	*			
Myriophyllum verticillatum	**28**	56	*op*									
Najas flexilis	**300**	94	*op*				**300**	54	*in*	**24**	29	*
Najas guadalupensis	**110**	32	*in*				**110**	26	*op*			
Nuphar lutea ssp. *variegata*	**12**	53	*de*							**24**	34	*de*
Nymphaea odorata	**106**	106	*op*				**117**	47	*op*	**26**	56	*de*
Phalaris arundinacea	**170**	504	*op*	**84**	142	*de*	**170**	273	*op*	**42**	88	*in*
Pilea fontana	**300**	55	*in*				**300**	48	*in*			
Pilea pumila var. pumila	**560**	56	*in*				**560**	52	*in*			
Poa palustris	**100**	60	*de*				**107**	27	*in*	**26**	29	*op*
Polygonum amphibium	**77**	222	*de*	**69**	74	*de*	**77**	98	*de*	**8**	50	*de*
Polygonum hydropiperoides	**105**	29	*in*									
Polygonum lapathifolium	**114**	93	*op*				**120**	57	*de*			
Polygonum sagittatum	**42**	61	*de*				**42**	41	*de*			
Potamogeton foliosus ssp. *foliosus*	**300**	73	*op*				**300**	47	*op*			
Potamogeton gramineus	**10**	42	*de*									
Potamogeton natans	**21**	72	*de*							**26**	46	*in*
Potamogeton pusillus	**560**	216	*in*	**211**	59	*op*	**560**	111	*in*	**48**	45	*in*
Potamogeton strictifolius	**140**	163	*in*	**84**	42	*op*	**140**	96	*op*	**19**	25	*
Potamogeton zosteriformis	**89**	158	*de*				**89**	119	*de*			
Potentilla norvegica ssp. *monspeliensis*	**99**	25	*de*									
Ranunculus flabellaris	**110**	41	*de*									
Ranunculus pensylvanicus	**37**	35	*de*									
Ranunculus sceleratus	**53**	41	*op*									
Riccia fluitans	**560**	240	*de*	**32**	82	*de*	**560**	99	*op*	**26**	59	*op*
Ricciocarpos natans	**110**	48	*de*									
Rorippa palustris	**54**	79	*de*	**69**	38	*de*	**40**	28	*op*			
Rumex maritimus	**69**	136	*op*	**84**	55	*in*	**49**	74	*op*			
Rumex britannica	**560**	95	*de*				**560**	53	*de*	**26**	32	*
Sagittaria latifolia	**170**	314	*de*	**69**	47	*de*	**170**	170	*de*	**48**	97	*
Sagittaria rigida	**300**	60	*de*				**300**	39	*op*			
Salix discolor	**77**	43	*op*									
Salix interior	**446**	56	*	**20**	28	*de*						

Salix petiolaris	**170**	43	*de*									
Schoenoplectus acutu s var. *acutus*	**35**	99	*op*	**28**	70	*de*	**49**	26	*de*			
Schoenoplectus fluviatilis	**58**	129	*op*	**49**	87	*in*	**110**	39	*op*			
Schoenoplectus tabernaemontani	**170**	171	*de*	**49**	65	*op*	**170**	72	*de*	**33**	34	*
Scirpus cyperinus	**170**	139	*de*				**170**	45	*op*	**24**	91	*de*
Scolochloa festucacea	**49**	38	*de*									
Scutellaria galericulata	**180**	166	*op*				**200**	96	*de*	**26**	58	*op*
Scutellaria lateriflora	**110**	145	*in*				**110**	105	*in*	**15**	25	*op*
Sium suave	**34**	168	*de*	**29**	67	*de*	**50**	61	*de*	**24**	40	*de*
Solanum dulcamara var. *dulcamara*	**200**	27	*in*									
Sparganium erectum ssp. *stoloniferum*	**71**	71	*de*							**24**	58	*de*
Sparganium eurycarpum	**199**	156	*op*	**34**	69	*op*	**199**	68	*op*			
Spirodela polyrrhiza	**199**	394	*op*	**42**	49	*op*	**191**	264	*op*	**33**	81	*de*
Stachys palustris	**110**	40	*op*									
Stuckenia pectinatus	**150**	195	*op*	**211**	107	*in*	**110**	84	*op*			
Thelypteris palustris var. *pubescens*	**61**	55	*de*				**89**	26	*de*	**13**	27	*
Torreyochloa pallida	**4**	25	*de*							**4**	25	*de*
Triadenum fraseri	**14**	92	*de*							**24**	69	*de*
Typha angustifolia	**560**	315	*op*	**146**	169	*in*	**560**	119	*in*			
Typha latifolia	**48**	273	*de*	**46**	68	*de*	**45**	105	*de*	**34**	99	*in*
Typha X *glauca*	**446**	446	*in*	**211**	179	*in*	**352**	253	*in*	**33**	34	*in*
Urtica dioica ssp. *gracilis*	**560**	69	*in*				**560**	41	*op*			
Utricularia intermedia	**13**	38	*de*							**24**	27	*
Utricularia macrorhiza	**170**	400	*de*	**38**	125	*de*	**170**	160	*de*	**26**	114	*de*
Utricularia minor	**110**	119	*de*				**110**	53	*de*	**26**	45	*de*
Wolffia columbiana	**140**	102	*in*				**140**	80	*in*			
Zizania palustris	**10**	26	*de*									

Appendix C. Wetland plant species individual extirpation estimates in response to sulfate (mg/L) in four geographic regions; statewide, Temperate Prairies ecoregion (TP), Mixed Wood Plains ecoregion (MWP) and Mixed Wood Shield ecoregion (MWS). Number of paired plant and sulfate observations (n =) are provided as is the generalized affects model (GAM) probability plot curve "fit" (Pattern) where biol. response related to chemistry shows as increasing (in); optimal (op); decreasing (de) or undefined (*).

	Statewide			TP			MWP			MWS		
SPECIES	**XC95**	n	Pattern	**XC95**	n	Pattern	**XC95**	n	Pattern	**XC95**	n	Pattern
Acer negundo	**1310**	27	*in*									
Acorus americanus	**180**	40	*de*									
Alisma triviale	**1920**	173	*de*	**1920**	47	*de*	**22**	83	*de*	**158**	43	*in*
Alopecurus aequalis var. *aequalis*	**163**	49	*de*									
Asclepias incarnata ssp. *incarnata*	**814**	82	*de*				**24**	55	*de*			
Bidens cernua	**350**	55	*de*				**26**	44	*de*			
Brasenia schreberi	**50**	31	*de*									
Calamagrostis canadensis	**530**	143	*de*	**530**	25	*de*	**24**	51	*de*	**158**	67	*in*
Calla palustris	**158**	40	*de*							**158**	37	*op*
Campanula aparinoides	**51**	69	*de*				**24**	33	*de*			
Carex atherodes	**350**	67	*	**309**	43	*de*						
Carex bebbii	**199**	27	*de*									
Carex comosa	**57**	68	*de*				**57**	55	*de*			
Carex diandra	**103**	44	*de*							**12**	26	*de*
Carex hystericina	**40**	86	*de*				**40**	48	*de*	**158**	35	*op*
Carex lacustris	**158**	181	*de*	**314**	28	*de*	**20**	86	*de*	**158**	67	*op*
Carex lasiocarpa var. *americana*	**814**	99	*de*	**392**	25	*de*	**7**	34	*de*	**158**	40	*in*
Carex stipata var. *stipata*	**29**	31	*de*									
Carex stricta	**814**	73	*de*				**24**	36	*de*			
Carex utriculata	**422**	123	*de*	**471**	28	*de*	**22**	47	*de*	**16**	48	*de*
Ceratophyllum demersum	**1310**	436	*op*	**1920**	138	*in*	**122**	259	*in*	**158**	39	*in*
Chara vulgaris	**814**	57	*op*	**811**	36	*de*						
Cicuta bulbifera	**58**	173	*de*				**56**	91	*de*	**158**	71	*de*
Cicuta maculata	**250**	53	*de*				**100**	39	*			
Cirsium arvense	**1920**	73	*in*	**1920**	34	*op*	**203**	25	*			
Comarum palustre	**158**	45	*de*							**158**	35	*in*
Cornus sericea ssp. *sericea*	**33**	27	*de*									
Dulichium arundinaceum	**50**	50	*de*							**50**	35	*in*
Eleocharis acicularis var. *acicularis*	**435**	51	*de*				**126**	32	*de*			
Eleocharis palustris	**724**	271	*de*	**602**	91	*de*	**21**	113	*de*	**50**	67	*de*
Elodea canadensis	**20**	51	*de*				**20**	46	*op*			
Epilobium coloratum	**814**	40	*de*									
Epilobium leptophyllum	**445**	57	*de*				**6**	30	*de*			
Equisetum fluviatile	**37**	48	*de*									
Eupatorium perfoliatum var. *perfoliatum*	**52**	29	*de*									
Galium tinctorium	**58**	28	*de*									
Galium trifidum ssp. *trifidum*	**528**	199	*de*	**530**	25	*op*	**40**	96	*de*	**158**	78	*in*
Glyceria borealis	**168**	121	*de*				**10**	43	*de*	**158**	55	*op*
Glyceria canadensis	**49**	48	*de*							**50**	37	*in*
Glyceria grandis var. *grandis*	**260**	173	*de*	**390**	35	*op*	**20**	77	*de*	**158**	61	*op*

Impatiens capensis	**183**	124	*de*				**203**	93	*op*	**15**	27	*de*
Iris versicolor	**45**	49	*de*									
Leersia oryzoides	**500**	193	*de*				**24**	125	*de*	**158**	44	*in*
Lemna minor	**923**	620	*	**1200**	212	*in*	**203**	334	*in*	**158**	74	*in*
Lemna trisulca	**1310**	398	*in*	**1310**	167	*op*	**128**	202	*in*	**158**	29	*in*
Lycopus americanus	**435**	102	*de*				**203**	54	*in*			
Lycopus uniflorus	**1310**	207	*de*				**23**	102	*de*	**158**	81	*in*
Lysimachia thyrsiflora	**46**	164	*de*	**22**	28	*de*	**40**	75	*de*	**158**	61	*in*
Lythrum salicaria	**281**	74	*de*				**33**	67	*op*			
Mentha arvensis	**814**	65	*				**23**	35	*op*			
Mimulus ringens var. *ringens*	**445**	29	*de*									
Myriophyllum sibiricum	**814**	53	*op*	**650**	28	*de*						
Myriophyllum verticillatum	**156**	50	*de*									
Najas flexilis	**76**	77	*de*				**20**	45	*de*			
Najas guadalupensis	**69**	32	*de*				**27**	26	*de*			
Nuphar lutea ssp. *variegata*	**50**	43	*de*							**158**	26	*in*
Nymphaea odorata	**25**	74	*de*				**17**	36	*de*	**50**	35	*op*
Phalaris arundinacea	**1920**	432	*de*	**1335**	140	*de*	**203**	231	*de*	**158**	61	*in*
Pilea fontana	**56**	53	*de*				**57**	48	*op*			
Pilea pumila var. *pumila*	**320**	53	*de*				**49**	49	*de*			
Poa palustris	**106**	48	*de*									
Polygonum amphibium	**400**	184	*de*	**358**	74	*de*	**203**	78	*de*	**18**	32	*de*
Polygonum hydropiperoides	**240**	29	*op*									
Polygonum lapathifolium	**724**	87	*de*				**291**	55	*op*			
Polygonum sagittatum	**50**	52	*de*				**24**	39	*de*			
Potamogeton foliosus ssp. *foliosus*	**724**	64	*op*				**126**	38	*op*			
Potamogeton gramineus	**7**	30	*de*									
Potamogeton natans	**21**	55	*de*							**17**	33	*de*
Potamogeton pusillus	**1920**	198	*de*	**1920**	58	*	**203**	104	*op*	**158**	36	*in*
Potamogeton strictifolius	**1200**	129	*de*	**1200**	38	*in*	**21**	77	*de*			
Potamogeton zosteriformis	**530**	123	*de*				**9**	90	*de*			
Ranunculus flabellaris	**445**	36	*									
Ranunculus pensylvanicus	**500**	31	*de*									
Ranunculus sceleratus	**1310**	40	*in*									
Riccia fluitans	**200**	203	*de*	**201**	80	*op*	**40**	85	*de*	**158**	38	*in*
Ricciocarpos natans	**180**	44	*de*									
Rorippa palustris	**453**	79	*	**457**	38	*op*	**126**	28	*de*			
Rumex maritimus	**528**	131	*op*	**528**	54	*op*	**291**	71	*in*			
Rumex britannica	**281**	84	*de*				**21**	49	*de*	**158**	25	*in*
Sagittaria latifolia	**327**	272	*de*	**393**	47	*de*	**126**	153	*de*	**158**	72	*in*
Sagittaria rigida	**13**	56	*de*				**11**	37	*de*			
Salix discolor	**29**	32	*de*									
Salix interior	**137**	45	*de*	**92**	28	*de*						
Salix petiolaris	**81**	39	*de*									
Schoenoplectus acutus var. *acutus*	**1310**	89	*in*	**954**	68	*de*						
Schoenoplectus fluviatilis	**902**	118	*in*	**891**	85	*op*	**24**	30	*de*			
Schoenoplectus tabernaemontani	**724**	151	*in*	**724**	64	*op*	**182**	61	*	**158**	26	*in*

Scirpus cyperinus	**430**	108	*de*				**20**	40	*de*	**158**	65	*in*
Scolochloa festucacea	**1310**	34	*									
Scutellaria galericulata	**59**	129	*de*				**40**	80	*de*	**158**	37	*in*
Scutellaria lateriflora	**1310**	136	*de*				**56**	101	*in*			
Sium suave	**500**	155	*de*	**437**	67	*de*	**24**	59	*op*	**158**	29	*in*
Sparganium erectum ssp. *stoloniferum*	**118**	49	*de*							**158**	37	*in*
Sparganium eurycarpum	**426**	142	*de*	**384**	68	*de*	**203**	60	*op*			
Spirodela polyrrhiza	**69**	335	*de*	**78**	49	*de*	**30**	222	*de*	**158**	64	*op*
Stachys palustris	**440**	36	*in*									
Stuckenia pectinatus	**902**	174	*in*	**902**	105	*op*	**182**	68	*in*			
Thelypteris palustris var. *pubescens*	**48**	42	*de*									
Triadenum fraseri	**106**	68	*de*							**158**	46	*in*
Typha angustifolia	**1310**	294	*in*	**1310**	166	*in*	**291**	111	*op*			
Typha latifolia	**902**	233	*de*	**899**	67	*de*	**30**	96	*de*	**158**	70	*in*
Typha X *glauca*	**910**	400	*in*	**1060**	150	*in*	**203**	222	*in*	**14**	28	*op*
Urtica dioica ssp. *gracilis*	**1310**	65	*				**182**	39	*in*			
Utricularia intermedia	**170**	29	*de*									
Utricularia macrorhiza	**740**	344	*de*	**740**	121	*op*	**39**	141	*in*	**158**	82	*de*
Utricularia minor	**170**	106	*de*				**39**	52	*in*	**50**	33	*in*
Wolffia columbiana	**20**	85	*de*				**17**	64	*de*			