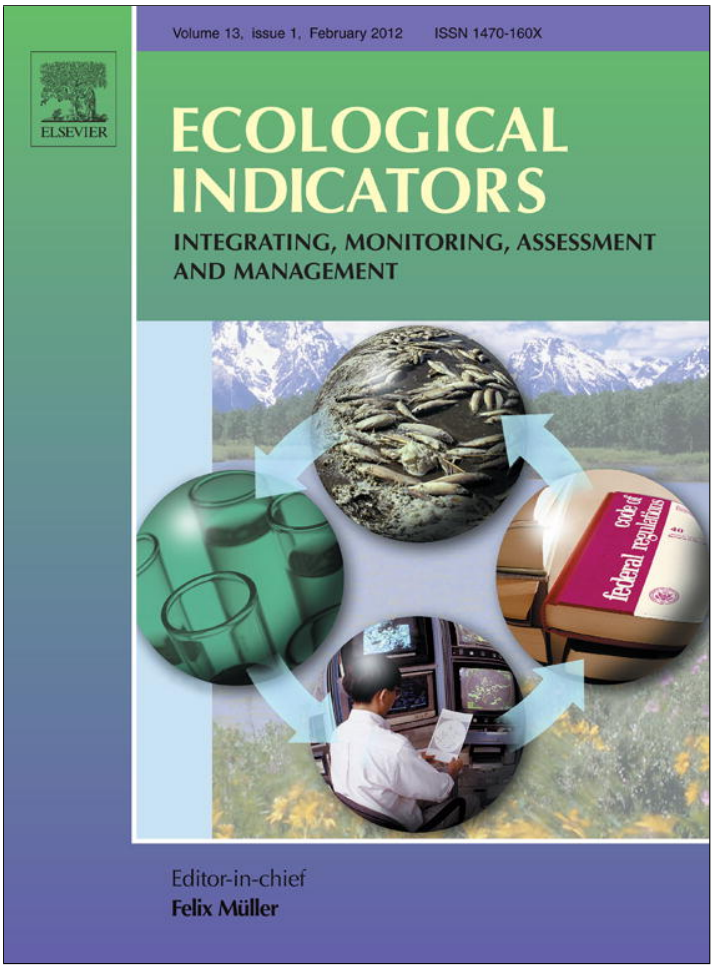

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Application of a versatile aquatic macrophyte integrity index for Minnesota lakes

Paul Radomski*, Donna Perleberg

Minnesota Department of Natural Resources, 1601 Minnesota Drive, Brainerd, MN 56401, United States

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ABSTRACT

The objective was to develop a Minnesota aquatic macrophyte integrity index that can use plant checklist data from existing and ongoing lake plant survey programs without alteration. Using the extensive lake survey data collected by numerous state programs, we created a suite of predictive models for macrophyte richness and floristic quality and identified aquatic macrophyte community outliers to set potential impairment thresholds. The highest-ranked predictive models included total phosphorus, disturbance indices, and ecoregion variables. Models with all in-lake macrophyte taxa generally performed better than those based on just submerged aquatic macrophyte or those based on submerged and floating-leaf taxa. The best generalized linear mixed model for aquatic macrophyte richness was a model containing total phosphorus, alkalinity, lake size, maximum depth, ecoregion, survey type, and several interactions. The best linear mixed effects model for floristic quality also included these predictive variables. Richness and floristic quality thresholds were calculated using these models with associated disturbance–response breakpoints. The approach took sampling protocol into account by providing different thresholds based on sample design. These thresholds then identify potentially biologically impaired lakes. There appeared to be no disturbance–response breakpoints between aquatic macrophyte richness and floristic quality for the Northern Lakes and Forest ecoregion of northeastern Minnesota.

1. Introduction

The Clean Water Act requires state governments to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters" (Water Pollution Control Act 101[a]). Herein, lake aquatic plant or macrophyte integrity means an assemblage of vascular plants and macroalgae having a species composition, richness, and functional organization comparable to that of an undisturbed or marginally disturbed lake of the region. The flora of lakes often defines the ecological character of lakes. Aquatic macrophyte communities provide many environmental services, such as absorbing nutrients that reduce water quality, reducing erosion from waves, and providing food and habitat for fish and wildlife. Determining the biological integrity of these near-shore and shallow water biological communities is consistent with the Clean Water Act.

Indexing biological integrity for lakes has proceeded similar to methods used for stream bio-assessment, with most efforts focused on fish-based indices (Beck and Hatch, 2009). These indices of biological integrity (IBI) often use several variables combined into a multimetric index, with the combination based on individual professional judgment. IBIs are developed by measuring attributes of biological communities that change in quantifiable and predictable ways in response to human disturbance. Several plant multimetric indices have been developed for palustrine ecosystems (Wilcox et al., 2002; DeKeyser et al., 2003; Miller et al., 2006; Clayton and Edwards, 2006; Rothrock et al., 2008). Nichols (1999a) proposed floristic quality index (FQI), which has been used alone or as a variable in a multimetric lake or wetland plant IBIs. Many of these IBIs use taxa richness and their development followed a traditional multimetric approach (Karr, 1981). There are also aquatic macrophyte indices that are based on a single variable (e.g., diversity, maximum depth of plant growth, plant coverage).

Nichols et al. (2000) proposed a multimetric index for Wisconsin aquatic macrophyte communities (AMCI). Individual indices included maximum depth of plant growth, percent littoral area vegetated, diversity, taxa richness, and relative frequencies of submersed, exotic, and sensitive species. The strength of the AMCI is that it is based on over 300 lake surveys from across the state and reference conditions, i.e., highest quality communities were determined for each ecoregion. One shortcoming may be that some of the individual indices may be correlated (e.g., maximum depth and percent of littoral area vegetated, and richness and diversity index). Another shortcoming appeared to be that in some lakes the index may be insensitive to water quality status, for example, nutrient loading to oligotrophic lakes may increase the AMCI score. Since macrophyte richness and abundance in oligotrophic lakes may benefit from additional nutrient availability, this

* Corresponding author. Tel.: +1 218 833 8643.
E-mail addresses: paul.radomski@state.mn.us (P. Radomski), donna.perleberg@state.mn.us (D. Perleberg).

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shortcoming is a common problem with aquatic macrophyte integrity indices.

Bourdaghs et al. (2006) studied the performance of the FQI for Great Lake coastal wetlands. They found that FQI detected differences between sites better than species richness alone, and it was an acceptable index of environmental condition. FQI had higher power to detect differences between predicted reference and degraded values than species richness indices. In addition, they found that the performance of FQI was not enhanced with weighting by abundance.

Two aquatic macrophyte IBIs have been developed for Minnesota waterbodies; however, each requires extensive survey work. Beck et al. (2010) developed a multimetric IBI that requires lake-wide macrophyte taxa frequency data and the 95th percentile of maximum depth of plant occurrence. The purpose of their research was not to propose a final IBI, but rather to identify issues for further index development. The metrics used included maximum depth of macrophyte growth, percentage of littoral area vegetated, number of plant taxa with relative abundance greater than 10%, number of native taxa, and relative frequencies of submersed, sensitive, and tolerant taxa. The index used point-intercept survey data from 97 lakes, and sample effort appeared to have a large effect on the estimate of plant richness and a lower, but significant, effect on the IBI score. Sensitivity to environmental stress varied by ecoregion, and the authors suggested development of metrics for each ecoregion rather than statewide relative frequency metrics. It was noted that lakes with low multimetric scores often had low species richness with tolerant species common or highly abundant. To us, this observation suggested that the use of aquatic macrophyte richness or floristic quality may have merit within a statistical model framework that included the use of ecoregions. Moore et al. (2012) developed a submersed macrophyte index to assess the condition of the impounded portion of the Upper Mississippi River where submersed macrophytes have historically occurred. They noted that the individual metrics revealed the importance of light transmission in the water and hydrologic conditions created by navigation dams. Both IBIs were also found to correlate to water quality indicators and development-related stressors.

Given the shortcomings of the various aquatic macrophyte integrity indices, there is value in developing a statistical model to estimate integrity of aquatic macrophyte communities. First, there are numerous existing lake plant surveys conducted by various monitoring programs in Minnesota. The objectives of these programs, the sampling effort and protocols, and the data collected vary considerably between them. The common element of these programs is the collection of a taxa list for the sampled lakes. Model response variables could include simply plant richness and its derivatives, such as floristic quality. Second, statistical models can incorporate stressor or disturbance variables without the need to a priori subjectively identify reference or healthy communities, which is complicated for aquatic macrophyte communities given their natural variability due to background water chemistry gradients across the state. Third, statistical model construction is less dependent on the professional opinion about the merits of inclusion of a variable, as there are standard methods to determine whether to include or remove a variable in a predictive model. A suite of predictive models can be judged by Akaike's information criteria (AIC). Under traditional IBI approaches, there is uncertainty on the validity of a simple additive combination. It has been debated elsewhere that combining IBI metrics alters variability and decreases interpretation (e.g., there is a large number of ways to get the same score, which limits understanding and interpretation). Use of a statistical model approach can reduce these problems and increase interpretability. Finally, since model development is based on objective and quantifiable methods, statistical model development may advance indices that are more defensible and, specifically in this case, could advance a versatile biological integrity index that would use a variety of lake plant surveys from ongoing programs.

Species richness, or the estimated number of species in a community, is the oldest, most fundamental, and perhaps least ambiguous concept of "diversity" (Peet, 1974) or relative "wealth" (May, 1988) of species in a community. This metric can be a useful tool to describe and compare aquatic macrophyte communities and may also reflect and detect changes in water quality conditions. Any estimate of number of species is dependent on search area (Peet, 1974; Bunge and Fitzpatrick, 1993), sampling method, site specific conditions, size of plant patch, plant architecture and growth form (Chen et al., 2009; Kery et al., 2006). Advantages of collecting plant checklist data (or presence/absence data) include that data collection can be done relatively quickly and does not require elaborate monitoring design (Elzinga et al., 2001). In contrast, quantitative aquatic plant data collection can be labor intensive, and, because these data are often collected with a specific method/protocol for a very specific objective, data from two different surveys may not be comparable (Nichols, 1984).

A second response variable that could be used with these Minnesota lake survey data is floristic quality index (FQI) which attempts to distinguish between plant communities that may have similar species richness but differ in species composition. FQI has been proposed as a tool to assess anthropogenic effects on plant communities. The theory behind FQI is that plant species differ in their tolerance to disturbance and exhibit a varying degree of fidelity to remnant natural habitats (Swink and Wilhelm, 1994; Wilhelm and Masters, 1995; Taft et al., 1997; Northern Great Plains Floristic Quality Assessment Panel, 2001). "Species conservatism" is the term used to describe the estimated probability that a species is likely to occur in a landscape that is relatively unaltered from what is believed to be pre-European settlement condition. Botanists subjectively assign a value, called the coefficient of conservation (C), to plant species based on their perception of the species conservatism. C values range from 0 (low conservatism) to 10 (high conservatism). FQI is calculated as: $\bar{C}\sqrt{S}$ where $\bar{C}$ is the mean C for all species and S is the number of species, or species richness. FQI incorporates species richness but uses a square root transformation on the species count (S) to reduce the influence of sampling area (Swink and Wilhelm, 1994).

There are several problems associated with C values used in FQI. Because C values are subjectively assigned, they are not precise measures of conservatism and do not support statistical testing (Bowles and Jones, 2006). Additionally, C values can be biased toward rare species and by personal preference and small variations in how botanists assign C values may result in large differences in the final FQI calculation. Despite these issues, Bowles and Jones (2006) concluded that FQI may be most applicable when comparing extremely low versus high quality vegetation or for expressing qualitative differences to lay audiences.

The amount and types of aquatic vegetation found in Minnesota lakes may be influenced by numerous physicochemical factors including light availability, water chemistry, wave exposure and substrate slope and type as well as by biological factors such as predation (Wetzel, 2001). Water transparency is one of the strongest influences on Minnesota's lake plant communities. Submersed macrophyte abundance, growth, and distribution are regulated by light availability (Wetzel, 2001). Light absorption, shading and competition with algae alter aquatic plant communities, and these interactions are confounded with turbidity, water clarity, and nutrient levels. The number of submerged aquatic macrophyte species often increases with increasing clarity as often measured by Secchi disk depth (Vestergaard and Sand-Jensen, 2000a; Strand and Weisner, 2001). Capers et al. (2009) specifically found that native

aquatic macrophyte species richness increased with water clarity. Productivity, or trophic status, is typically measured as total phosphorus. Species richness generally decreases with increasing nutrients (Capers et al., 2009; Beck et al., 2010).

Moyle (1945) described the influence of water chemistry on Minnesota lake macrophyte communities. Lakes of northeast Minnesota are derived from scouring of pre-Cambrian rock, contrasted with the lakes formed within glacial deposits outside of this region. Northeastern Minnesota lakes are soft water lakes, with alkalinity values typically less than 50 ppm and many waters have a total alkalinity between 10 and 20 ppm. Lakes of central and northern Minnesota are considerably harder with alkalinity ranging from 75 to 200 ppm. Lake alkalinity in southwestern and extreme western counties ranges from 100 to 250 ppm. Moyle (1945) suggests the natural separation between hard and soft waters seems to be at a total alkalinity of 40 ppm (30 ppm is the lower limit of toleration of more typical hard-water species and 50 ppm is the upper limit of toleration of characteristically soft-water species). The number of submerged species recorded in lakes generally increases with increased alkalinity. More taxa are adapted to alkaline, neutral pH waters and fewer taxa have the ability to live in softwater lakes of low pH (Moyle, 1945; Hellquist, 1980; Catling et al., 1986; Jackson and Charles, 1988; Rorslett, 1991; Weiher and Boylen, 1994; Srivastava et al., 1995; Vestergaard and Sand-Jensen, 2000a,b; Bornette et al., 2001; Lougheed et al., 2001; Capers et al., 2009). Conductivity is closely associated with alkalinity and bicarbonate availability. Borman et al. (2009) studied the occurrence of three groups of submerged macrophytes (soft-water isoetids, harder-water elodeid and characean species) and found that conductivity was closely associated with the proportion of isoetid communities that had been colonized by elodeids or Chara.

Other factors affect aquatic macrophyte richness. Morphological features such as lake basin slope (Duarte and Kalff, 1986) and the degree of exposure to wind (Chambers, 1987; Hudon et al., 2000) directly influence the abundance of submerged macrophytes and may indirectly influence species richness. There is a general conception that aquatic macrophyte species richness would increase with increased lake area, perhaps because large and deep lakes are more likely to have a range of habitats compared to small and shallow lakes. However no significant relationship has been found with submerged macrophyte species richness and lake surface area (Rorslett, 1991; Vestergaard and Sand-Jensen, 2000a). Gasith and Hoyer (1998) note that the changing influence of macrophytes along lake size and depth gradients is currently mostly speculative. Scheffer et al. (2006) suggests that submerged vegetation may be more diverse in small, isolated lakes but Newman (1998) suggests that, although counter-intuitive, lake size is unrelated to macrophyte species richness. Vestergaard and Sand-Jensen (2000a) suggest that the size of "colonized area" may be a better predictor of species richness. In addition, lakes connected with rivers or lakes in floodplains that are occasionally connected with rivers often have higher species richness (Amoros and Bornette, 2002). Animals may directly feed on aquatic plants or may have indirect impacts by increasing turbidity and uprooting vegetation. Lougheed et al. (1998) found a significant difference in submerged macrophyte richness in waterbodies containing carp (average of five or fewer species) compared to systems that did not support carp (10 or more species). Crayfish herbivory may select against perennial macrophytes and promote growth of pioneering plants like Chara (Rosenthal et al., 2006), and moose may selectively feed on broad-leaf macrophytes. Herbivory by snails has been associated with a decline in submerged macrophyte richness (Sheldon, 1987). Predicting and describing the specific plant community that may occur in a lake may be complicated because there may be complex interactions among these multiple abiotic and biotic factors (Thomaz et al., 2003) and because plant development can be variable even in lakes of similar type (Sculthorpe, 1967; Hutchinson, 1975; Gasith and Hoyer, 1998).

The objective of this study was to develop a lake macrophyte integrity index that can use a variety of data from existing and ongoing lake plant survey programs without alteration. Existing data were analyzed and a statistical method framework was used to construct various lake macrophyte integrity indices. An important component of index development is the need to test and validate to see if it accurately detects the effects of human disturbances on the biological assemblage. Rather than validate an index after its construction, we incorporate disturbance variables within the predictive model from the onset. In addition to the simplicity of this approach, the inclusion of disturbance variables within a statistical framework may provide a more robust indicator of biological integrity. Based on several ecological principles, we developed statistical models by including such variables as the alkalinity, total phosphorus, ecoregion, and lake size. The principles we used in threshold development included the following: aquatic macrophyte communities vary by regional and local factors; competition for light reduces plant species richness following eutrophication (e.g., grasslands, Hautier et al., 2009); and plant community temporal stability is a function of plant richness (e.g., grasslands, Tilman, 1996; Lehman and Tilman, 2000). A suite of predictive models was then judged by AIC and prediction errors. Finally, in areas with low disturbance and no substantial number of impairments, we reviewed case study lakes and compared aquatic macrophyte integrity index results with nutrient impairment.

2. Methods

2.1. Study lakes

Lake plant survey data were available from 3254 lakes, with a total of 4941 surveys available (37% of the lakes had more than one survey). Study lake distribution corresponds with the natural distribution of lakes in Minnesota. Using the Omernik Ecoregion Classification (Omernik, 1987), 55% of lakes occur in the Northern Lakes and Forest ecoregion, 31% in the North Central Hardwoods ecoregion, 7% in the Western Corn Belt Plains ecoregion and 7% in the other four ecoregions. A similar distribution occurs using the Minnesota DNR (MNDNR) Ecological Classification System (ECS): most lakes occurred in the Laurentian Mixed Forest (58%), Eastern Broadleaf Forest (25%) and Prairie Parkland (17%) sections. Few study lakes are in the northwestern and southeastern corners of the state, where occurrence of lakes is low.

Lake surface area ranged from 0.6 to 128,224 acres with a median area of 133 acres and mean area of 471 acres. Most lakes (73%) were between 10 and 350 acres in area. For lakes where shoreline mile length was available, shoreline length ranged from 0.6 to 341.5 miles with a mean of 4.7 miles. Deep and shallow lakes are included in this analysis. Littoral area was unknown for 15% of the lakes. For lakes with depth information, the majority (71%) were primarily shallow, with at least 51% of the lake area less than 15′ in depth.

2.2. Plant surveys

Seven different MNDNR Programs collected lake plant data using on-site (as opposed to remote sensing), watercraft-based (as opposed to sub-surface sampling with SCUBA or dredge) sampling methods. Each Program has different objectives, and the lakes selected for surveys and survey methods vary by the purpose of the Program. Four main methods were used: MCBS, Transect, NLAP and PI. The search/survey area covered by each method was different and it also varied between lake and by individual surveyor.

The common information collected by each survey was a list of all macrophyte taxa detected. The Minnesota County Biological Survey (MCBS) Program conducted rare plant searches by subjectively selecting lake areas of various sizes and conducting qualitative vegetation assessments. The other Programs conducted quantitative "sample-based" assessments (Gotelli and Colwell, 2001) with sample sites placed at regular intervals throughout the littoral zone; sample size and number varied by Program and method. Transect surveys were conducted to assess the general distribution, diversity and abundance of the aquatic plant communities. Sample sites were "belt transects" placed perpendicular to shore at regular intervals around the lakeshore. Number of transects ranged from 10 to 40 on most lakes. Sample area varied based on the length and width of each transect. NLAP surveys were conducted on a set of randomly selected lakes within the state to assess the diversity and abundance of the near-shore aquatic plant communities as part of the National Lakes Assessment Program (NLAP; U.S. EPA, 2007). Sample sites were plots that measured 15 meters along the shoreline and 10 m lakeward (Neuman, 2008). Most lakes had 10 plots and a few lakes had 11–14 plots; plots were spaced at regular intervals around the lakeshore. Lastly, point-intercept surveys (PI) were conducted on important fish, wildlife, and water recreation lakes to estimate the distribution and frequency of aquatic plant species. General methodology is described in Madsen (1999) and individual Programs have modified the PI method to meet their specific objectives. Sample sites were points that were spaced in a grid pattern across the littoral area of the lake. In theory, this was a dimensionless sample area but in reality, each point measured about 1 m^2 in area. The number of sample sites surveyed during a PI Survey varied by lake and by Program. For those surveys included in this analysis, the mean number of sample points was 116 with individual Program sample number means ranging from 54 to 447.

With the exception of the PI surveys, search area could only be estimated. In general, the MCBS method generally covered a greater search area because they targeted one site on a lake and conduct a detailed search within that area. The Transect method also has a large search area but the transect length varied greatly between lakes and the transect width varied among surveyors. The NLAP method identified a discrete sample area (10 m × 15 m) but the actual search area within that site varied among surveyors. The PI survey is the only method that has a discrete search area with each site approximating 1 m^2. While the PI individual search area is small, the total search area (1 m^2 times the number of sample sites) increased as more sites were surveyed.

Plant survey data collected between 1993 and 2010 were assembled. Only surveys conducted during peak plant growth season (June 1–September 30) were included, with most conducted from mid-July through August. About 45% of the surveys were Transect surveys, 33% were MCBS surveys and 22% were PI surveys. The majority of PI surveys were conducted by MNDNR Shallow Lakes Program on lakes where maximum depth was typically 15′ or less. PI data collected on deep lakes by other Programs accounted for only 5% of all surveys. NLAP surveys accounted for less than 1% of the surveys. Most survey types were spatial distributed such that they corresponded with the natural distribution of lakes in Minnesota.

2.3. Plant taxonomy and nomenclature

All surveys recorded each detected vascular plant taxa to the highest taxonomic rank possible (often species level) based on individual surveyor plant identification knowledge and the condition of the plant sample. For certain species, field identification to the species level was not possible if diagnostic features, such as fruits, were not present and the specimen would only be identified to the genus level. For MCBS data, which were collected by a botanist, we retained the original taxonomic identification. For all other surveys, some plant taxa were combined to the genus level or grouped as a species complex due to uncertainty in original species identification. MCBS surveys did not record non-vascular plants but all other surveys recorded macroalgae to the genus level (*Chara* or *Nitella*), liverworts to the species level (*Riccia fluitans*) and aquatic mosses to the division level (Bryophyta).

Nomenclature followed MNTaxa (2011) and taxa were assigned to one of four life forms: emergent, floating-leaved, free-floating and submerged. Only in-lake macrophytes were included in this analysis because surveyors did not consistently record wetland emergent plants during surveys.

2.4. Environmental data

Three statewide lake water chemistry datasets were reviewed for water chemistry data. For the 3254 lakes where we have plant data, total phosphorus and alkalinity data existed for about 70% and 53% of the lakes, respectively. Two disturbance indices were used – measures of watershed and shoreland alteration. Watershed disturbance was estimated by summing all the disturbed land uses within the catchment area of the lake divided by the catchment area of the lake. Shoreland disturbance was estimated by summing up all the developed land use classes within 75 m of the lake divided by the total area within 75 m of the lake. The 2001 National Land Cover Data were used to estimate these disturbance indices. These data were available for 2012 of the 3254 aquatic plant surveyed lakes.

2.5. Aquatic macrophyte response variables

Two response variables were used to formulate a macrophyte integrity index. The first was aquatic macrophyte richness, which simply was the number of all aquatic macrophyte taxa found in the lake for an individual survey. Second, FQI was calculated for each aquatic plant survey by multiplying the mean *C* of the plants observed in the survey times the square root of the number of taxa in the survey. *C* values ranged from 1 to 10 and were primarily those from Nichols (1999a); values from WisFlora (2011) and Milburn et al. (2007) were used for several species. For macrophytes recorded to the genus level only where a *C*-value does not exist in the literature, a mean value was calculated from species in that genus that occur in Minnesota (e.g., for plants recorded as "*Elodea* sp." the mean *C* value of "5" was assigned by calculating the mean values for *Elodea canadensis* [3] and *E. nuttallii* [7]). While standard floristic analyses assign a default *C* value of "0" to any introduced species (Rothrock and Homoya, 2005), we assigned *C* values to introduced species based on their ability to tolerate turbidity and other forms of disturbance. Two submerged introduced species (*Potamogeton crispus* and *Myriophyllum spicatum*) were assigned a *C* value of 3; the introduced floating-leaved pink waterlily (hybrid waterlily, *Nymphaea* X sp.) was assigned a value of 6, and the introduced emergent species (*Butomus umbellatus*) was assigned a value of 5. Mean *C* value for the 140 taxa observed was 7.3 and 71% of the taxa had *C* values of 7 or higher. By comparison, mean *C* values for Wisconsin and Michigan wetland flora are 6.0 and 5.4, respectively (Bourdaghs et al., 2006), and 2.8 for Mississippi wetland flora (Herman et al., 2006). Distribution in *C* values across plant communities varies. Minnesota lake macrophyte flora likely have a higher average *C* value due to a smaller pool of taxa, a higher number of habitat-specific taxa, fewer non-native taxa, and fewer taxa that occur in highly disturbed sites.

2.6. Statistical analysis

The analytical methods used followed a structured approach that included: exploration of the data; use of correlation to test for

collinearity and to identify important variables related to aquatic plant richness; model development that incorporated natural and human-impact gradient variables; model selection based on Akaike information criteria (AIC); and use of reference conditions and disturbance breakpoints to set aquatic plant thresholds.

First to assess aquatic macrophyte relationships, ordinations of aquatic macrophyte communities were created using non-metric multidimensional scaling (NMDS) and typical species groups for aquatic plant surveys were created using cluster analysis. Data used in the ordination was taxa presence by lake. Lakes with multiple surveys were summarized to provide a single taxa list per lake (this resulted in a data set of 140 taxa in 3241 lakes with one or more macrophyte taxa present). Aquatic macrophyte community dissimilarities were determined by the Jaccard distance measure. The ordination was performed using three dimensions in the statistical programming language R (The R Foundation for Statistical Computing, 2011) with the Vegan package. The ordination or map of the aquatic plant community included lake coordinates and weighted centroids of species locations based on all lakes containing a particular taxon. Environmental variables were also overlaid as vectors on the ordination to provide some interpretation of community composition. In the cluster analysis used to identify typical species assemblages in Minnesota lakes surveyed, we used the Jaccard distance measure with the flexible beta linkage method at a beta equal to −0.25 for the similarity in the distribution of species present in a minimum of 5% of the lakes surveyed.

Second, models were developed to predict aquatic macrophyte richness and floristic quality index (FQI) using a data set of 2406 surveys and 1344 lakes (54% of the lakes had more than one survey). As taxa richness is a count (S; number of different taxa per lake) that is often non-normal distributed, a generalized linear mixed model (GLMM) with a Poisson distribution with a logarithmic link was used. GLMMs combine the linear mixed-effects model approach, which incorporates random effects (i.e., lake effect), and generalized linear models, which handle non-normal data (i.e., count data). Linear mixed-effects models were used to test for significant fixed effects on FQI and develop predictive models for potential impairment thresholds. The general form, in the notation of Laird and Ware (1982), is

$$y = X_i\beta + Z_i b_i + \varepsilon_i$$

where y is FQI, $X_i\beta$ are the fixed effects, $Z_i b_i$ are the random effects, and ε_i are the residual errors. Fixed effects are parameters associated with an entire population or from observations taken on all treatments of interest, and random effects are associated with individual experimental units drawn at random from a population. The analysis was conducted using the package nlme (Pinheiro and Bates, 2000) in the statistical programming language R. Models were fit using restricted maximum likelihood, except when comparing models of different fixed effect structure with likelihood ratio tests, then models were fit using maximum likelihood. Lakes were modeled as random-effects. The analysis assumed that data from different lakes are statistically independent. Mixed-effects models have benefits over other frequentist procedures since they use likelihood-based estimation and they recognize that there is some dependency between observations from the same lake (Pinheiro and Bates, 2000).

The model development strategy followed the suggestions of Wolfinger and Chang (1995) and Zuur et al. (2009), where fixed-effects are selected, exploration of residual patterns, variance or correlation structures are selected and tested, fixed-effects are tested, and finally inferences for fixed-effects are made. The influence of survey type, alkalinity, total phosphorus, lake size, water depth, ecoregion class, watershed disturbance, and shoreland disturbance were analyzed as fixed-effects. The four different survey types used to sample aquatic plant communities included: Minnesota County Biological Survey (MCBS), National Lakes Assessment Project aquatic plant surveys (NLAP), point-intercept surveys (PI), and transect surveys (T). Several different ecoregion classes were used; they included MNDNR Province (Cleland et al., 1997), U.S. EPA Levels 2 and 3 (Omernik, 1987), and Level 3M, where U.S. EPA Ecoregions 1A and 1B were combined and 3B was split (Boundary Lakes and Hills [50n], Toimi Drumlins [50p], and North Shore Highlands [50t] made up an eastern Northern Lakes and Forest ecoregion (3Be) and the remaining Level 4 ecoregions constituted the western Northern Lakes and Forest ecoregion (3Bw)). The Level 3M ecoregion classification was constructed after exploration of the aquatic plant richness and FQI distributions in U.S. EPA Level 4 classes.

After initial testing to determine significant fixed-effects, a suite of 12 candidate models was developed that incorporated fixed-effects for the taxa richness and FQI response variables. AIC score was used to select preferred models for FQI and taxa richness, and Akaike weights were used to quantify the strength of evidence for alternative models (Burnham and Anderson, 2002). The basic idea behind AIC is penalizing the likelihood for the model complexity – the number of explanatory variables used in the model. The approach has considerable merit, and it has become the cornerstone of judging predictive models. We evaluated the performance of the models based primarily on the distribution of relative errors. Relative error was defined as the percentage $r = (\text{estimated} - \text{observed})/\text{observed} \times 100$ for a given model. We also summarized overall performance by the mean and median relative error and by the mean and median of the absolute values of the relative errors. We used median relative error to indicate relative bias, the tendency to consistently underestimate or overestimate. We used median absolute relative error to summarize the uncertainty or imprecision in the fitted estimates. The R code for the preferred models is provided (Appendix A).

Three variables were identified as important predictors of aquatic macrophyte impairment: total phosphorus, watershed disturbance, and shoreland disturbance. We quantified the relationship between the aquatic plant richness and floristic quality response variables and each of the three-predictor variables. Recursive partitioning was used to estimate breakpoints in these relationships. This technique identifies the most significant split in a response variable determined by the largest likelihood-ratio chi-square statistic based on a predictor variable (Brenden et al., 2008). For each ecoregion, the first split identified by the partitioning was used as the breakpoint for each variable. All available data were used in the partitioning to identify breakpoints for watershed and shoreland disturbance, and only lakes that exceeded the reference conditions noted by Heiskary and Wilson (2005) were used to identify the total phosphorus breakpoints.

To provide potential thresholds for impairment, FQI and species richness for each ecoregion class were then predicted for shallow lakes (maximum depth < 15′) and deeper water lakes (≥15′) based on the estimated breakpoints and the preferred models (Zar, 1999). For ecoregions where there were no clear breakpoints for all three predictor variables, FQI and aquatic plant richness thresholds were set based on a review of case histories.

3. Results

3.1. Aquatic macrophyte species

A total of 140 taxa were recorded in MNDNR's lake aquatic plant surveys, and the taxa include 83 submerged, 8 free-floating, 16 floating-leaved and 33 emergent plants. Thirty-eight percent of all taxa and 67% of the emergent taxa were unique to MCBS surveys.

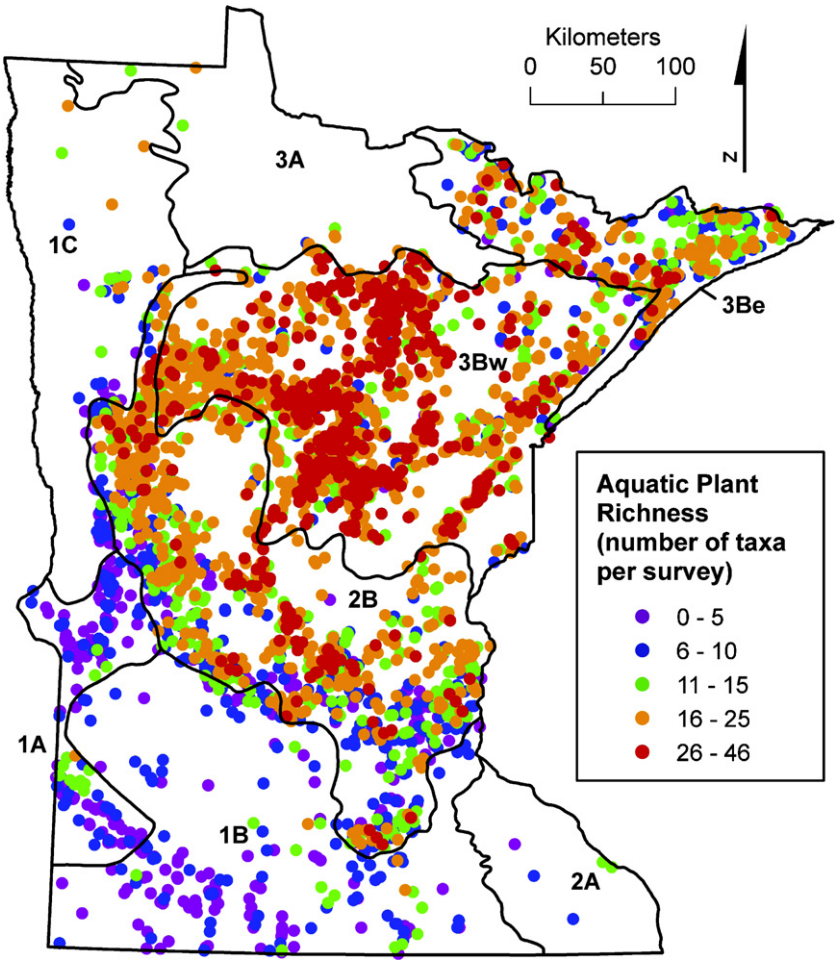

Fig. 1. Aquatic macrophyte richness in Minnesota lakes by Omernik ecoregions.

Fifteen taxa were exclusively recorded by non-MCBS surveys and include non-vascular taxa that were not recorded by MCBS and genera that were identified to the species level by MCBS.

Aquatic macrophyte richness ranged from 0 to 46 unique taxa per survey, with a mean of 16 taxa per survey. A high percentage (38%) of the taxa were uncommon, occurring in less than 3% of all surveys. Only 21% of the taxa were commonly occurring in surveys, occurring in at least 20% of all surveys. The mean floristic quality index (FQI) for all surveys was 23.7 (standard deviation of 8.8). The median FQI was 25.2, and the range was from 0 to 46.4.

3.2. Ordination

The strongest correlations between the dimensions from the ordination and environmental variables were total phosphorus ($r = 0.58$) and alkalinity ($r = 0.54$). Species on the margins of this ordination included species present in high alkalinity (150–300 ppm), such as prairie bulrush (*Bolboschoenus maritimus*), sea naiad (*Najas marina*), horned pondweed (*Zannichellia palustris*), and American lotus (*Nelumbo lutea*), and species present in low alkalinity (<100 ppm) and low total

Table 1
Typical aquatic macrophyte taxa list groupings in Minnesota lake surveys as delineated by cluster analysis.

Group	Associated species
1	*Chara* sp., *Schoenoplectus* sp., *Potamogeton* sp., *Elodea* sp., *Nuphar* sp., *Sagittaria* sp., *Najas flexilis*, *Potamogeton zosteriformis*, *Ceratophyllum demersum*, *Stuckenia pectinata*, *Potamogeton richardsonii*, *Myriophyllum sibiricum*, *Vallisneria americana*, *Potamogeton gramineus*, *Potamogeton amplifolius*, *Nymphaea odorata*, *Utricularia vulgaris*, *Potamogeton natans*
2	*Heteranthera dubia*, *Potamogeton friesii*, *Potamogeton illinoensis*, *Potamogeton praelongus*, *Ranunculus aquatilis*, *Lemna trisulca*, *Spirodela polyrhiza*, *Zizania palustris*, *Sparganium* sp., *Persicaria amphibia*, *Phragmites australis*
3	*Elodea canadensis*, *Schoenoplectus acutus*, *Lemna* sp., *Potamogeton pusillus*, *Nuphar variegata*, *Sagittaria latifolia*, *Eleocharis palustris*
4	*Nitella* sp., *Isoetes* sp., *Potamogeton robbinsii*, *Bidens beckii*, *Sparganium* sp., *Eleocharis* sp., *Equisetum fluviatile*
5	*Water moss*, *Eleocharis* sp., *Utricularia* sp., *Myriophyllum* sp., *Schoenoplectus pungens*, *Potamogeton crispus*, *Lemna* sp., *Bolboschoenus fluviatilis*
6	*Najas gracillima*, *Isoetes echinospora*, *Potamogeton spirillus*, *Potamogeton epihydrus*, *Sparganium fluctuans*, *Eriocaulon aquaticum*, *Myriophyllum tenellum*, *Sparganium angustifolium*, *Eleocharis acicularis*, *Sagittaria cristata*, *Juncus pelocarpus*
7	*Najas guadalupensis*, *Potamogeton foliosus*, *Potamogeton strictifolius*, *Stuckenia filiformis*, *Sparganium eurycarpum*, *Myriophyllum verticillatum*, *Sparganium emersum*, *Eleocharis erythropoda*, *Schoenoplectus tabernaemontani*, *Sagittaria rigida*
8	*Schoenoplectus subterminalis*, *Utricularia gibba*, *Utricularia intermedia*, *Utricularia minor*, *Brasenia schreberi*

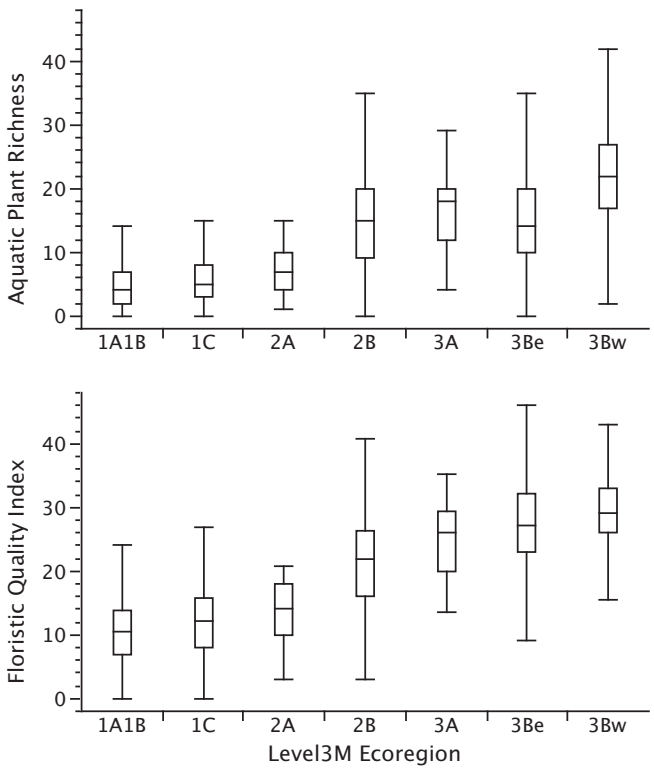

Fig. 2. Aquatic macrophyte richness and floristic quality by Omernik ecoregions. The box is the interquartile range. The vertical endpoints are not longer than 1.5 times the interquartile range, and the line within the box is the median.

phosphorus, which include such species as water lobelia (*Lobelia dortmanna*), alternative-flower water milfoil (*Myriophyllum alterniflorum*), lavender bladderwort (*Utricularia resupinata*), horned bladderwort (*Utricularia cornuta*), lake quillwort (*Isoetes lacustris*), and hidden-fruit bladderwort (*Utricularia geminiscapa*).

Cluster analysis of species or taxa lists provided eight groups of ecological associations or surveyor affinities (Table 1). Group 1 consisted of many commonly occurring taxa in Minnesota lakes, such as muskgrass (*Chara*), bulrush (*Schoenoplectus*), arrowhead (Sagittaria), coontail (*Ceratophyllum demersum*), yellow water lily (*Nuphar*), white water lily (*Nymphaea odorata*), and various species of pondweeds (*Potamogeton*). These are the only 18 taxa that occurred with a frequency of greater than 30%. Group 2 included several species of pondweed, water stargrass (*Heteranthera dubia*), wild rice (*Zizania palustris*), and several other species of macrophytes. Most of the taxa in the group occurred in 20–30% of the surveys. Groups 3, 6 and 7 included numerous species, with most of the species only recorded by MCBS surveys. Groups 4, 5, and 8 consisted of less taxa. Groups 6 and 8 represent low alkalinity and low productivity lake assemblages.

3.3. Aquatic macrophyte richness and floristic quality

Aquatic macrophyte richness and floristic quality generally increased from the southwest (Western Corn Belt Plains and the Northern Glaciated Plains) to the north and east (Figs. 1 and 2). The western part of the Northern Lakes and Forest ecoregion had the highest average richness and floristic quality, and lakes in these ecoregions also had the highest variability in aquatic macrophyte richness. Floristic quality in Minnesota lakes was most variable in the North Central Hardwoods and eastern part of the Northern Lakes and Forest ecoregions.

Cumulative distribution functions for selected aquatic macrophytes by lake plant richness curves show that some species were found across a wide of range of lake plant richness, whereas, some species are good indicators of high richness (Fig. 3). Sago pondweed (*Stuckenia pectinata*), curly leaf pondweed (*Potamogeton crispus*), and coontail (*Ceratophyllum demersum*) were found in lakes with both low and high aquatic plant richness. Sago and curly leaf pondweed were also found across a wide range of total phosphorus concentrations, but commonly in lakes with high phosphorus concentrations (Fig. 4). Whitestem pondweed (*Potamogeton praelongus*) and water marigold (*Bidens beckii*) generally occurred in high aquatic plant diversity lakes. In at least 90% of the surveys in which these species were detected, there were at least 13 other macrophyte taxa detected. Other widespread species that, on a statewide basis, appeared to be good indicators of diverse aquatic plant lakes include Illinois pondweed (*Potamogeton illinoensis*) and water celery (*Vallisneria americana*).

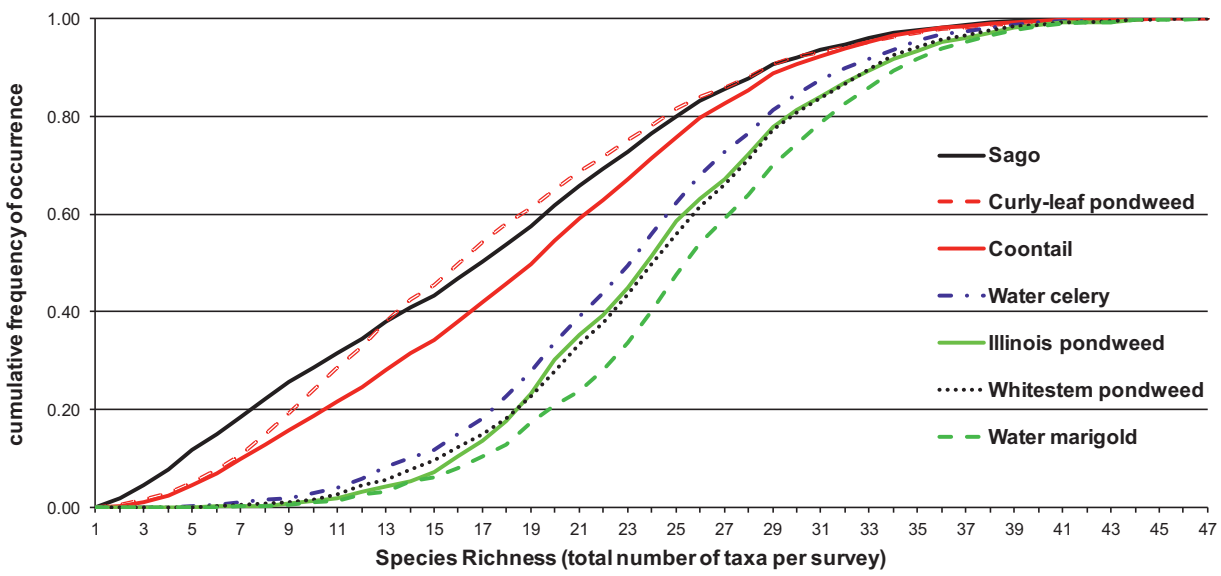

Fig. 3. Cumulative distribution functions for selected aquatic macrophyte taxa by lake macrophyte richness.

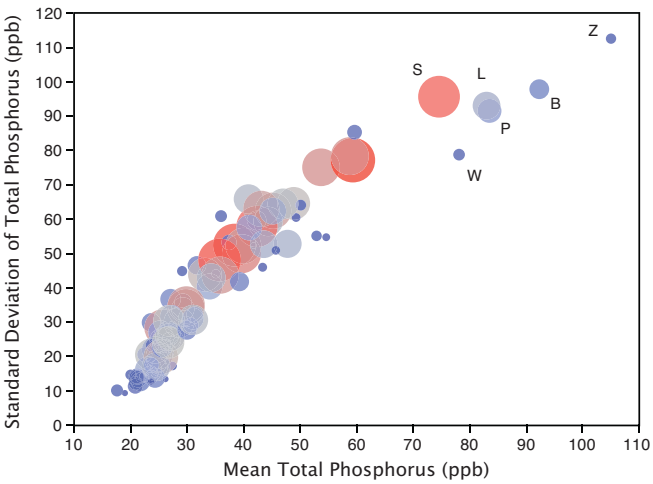

Fig. 4. The mean lake total phosphorus concentration plotted against the standard deviation of the lake total phosphorus concentration for each aquatic macrophyte taxa surveyed. The size and shading of the points is based on the percent occurrence of the taxa in all lakes surveyed, and only taxa with one percent or greater occurrence are presented. Taxa with high mean and standard deviation include: *Zannichella palustris*, Z; *Bolboschoenus fluviatilis*, B; *Potamogeton crispus*, P; *Lemna* sp., L; *Wolffia* sp., W; and *Stuckenia pectinata*, S.

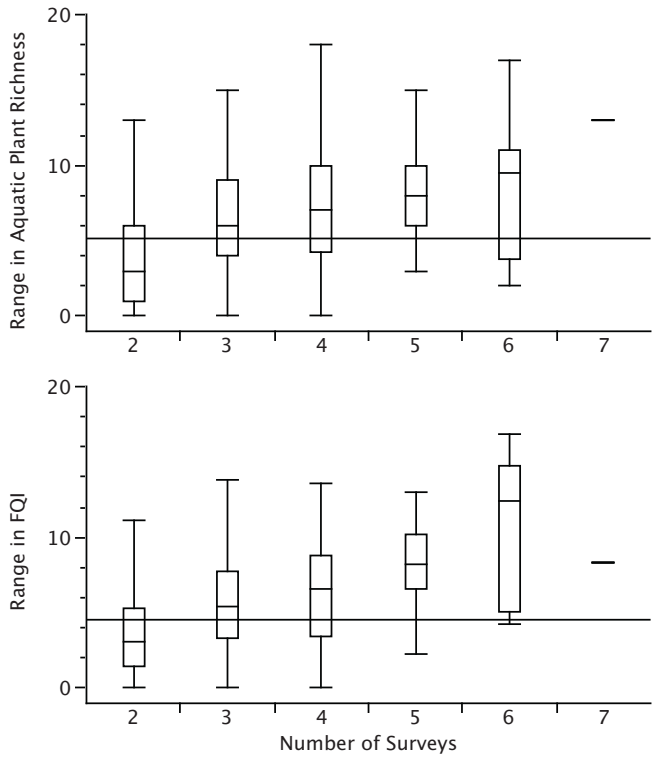

Fig. 5. Box plots of aquatic macrophyte richness and floristic quality index (FQI) range distributions from lakes with multiple surveys grouped by the number of surveys conducted. The box is the interquartile range. The vertical endpoints are not longer than 1.5 times the interquartile range, and the line within the box is the median. The horizontal line is the mean – 5 for aquatic macrophyte taxa (standard deviation = 4.1) and 4.5 for FQI (standard deviation = 3.5).

3.4. Repeatability of surveys

For lakes with multiple aquatic plant surveys, the mean range in aquatic macrophyte richness was 5 and the mean range in FQI was 4.5 ($N = 1204$; Fig. 5). As the number of surveys increased, the range of richness and FQI increased. Variation in the total number of plants recorded may reflect seasonal or longer-term changes in plant communities but may also reflect differences in survey methods including differences in surveyor ability in plant identification and differences in search effort (size of area surveyed and/or types of habitat surveyed).

3.5. Modeling

Initial testing of generalized linear mixed models and linear mixed models suggested that total phosphorus, alkalinity,

Table 2
Suite of candidate models used to understand the relative influence of variables on aquatic macrophyte richness and floristic quality in Minnesota lakes. Each model included the variables checked. Selected fixed effects included: Ecoregion (either EPA Level 3, EPA Level 3M, EPA Level 2, or MNDNR Province), total phosphorus (TP), and disturbance variables (DV; watershed and shoreland disturbance). Other fixed variables used in these models included: alkalinity, lake size, maximum lake depth, survey type, and interaction terms. Akaike information criteria (AIC) were estimated by maximum likelihood. Models are ranked by increasing AIC.

Rank	EPA Level 3	EPA Level 3M	EPA Level 2	MNDNR Province	TP	DV	AIC	ΔAIC
Aquatic macrophyte richness								
1		×			×	×	3942	0
2		×			×		4018	76
3	×				×	×	4075	133
4		×				×	4084	142
5			×		×	×	4087	145
6	×				×		4181	239
7			×		×		4182	240
8	×					×	4184	242
9				×	×	×	4194	252
10			×			×	4200	258
11				×	×		4254	312
12				×		×	4377	435
Floristic quality index								
1		×			×	×	14,389	0
2	×				×	×	14,424	35
3			×		×	×	14,428	38
4		×			×		14,455	66
5	×				×		14,478	89
6			×		×		14,482	93
7				×	×	×	14,505	116
8		×				×	14,512	123
9	×					×	14,532	142
10			×			×	14,538	149
11				×	×		14,558	169
12				×		×	14,645	256

Table 3
Comparison of the best models with aquatic plant richness and floristic quality index response variables for all aquatic plant taxa, submerged plant taxa, and submerged and floating-leaf plant taxa. Coefficients of determination (r^2) for observed and predicted richness or floristic quality for each model are presented.

Model	r^2 for observed and fitted	Mean relative error (%)	Median relative error (%)	Mean absolute relative error (%)	Median absolute relative error (%)
Aquatic macrophyte richness					
All macrophyte taxa	0.86	12.25	0.54	24.37	12.11
Submerged plant taxa	0.82	14.13	−0.86	28.87	14.25
Submerged and floating-leaf taxa	0.85	12.91	−0.15	26.54	13.27
Floristic quality index					
All macrophyte taxa	0.89	3.53	0.01	11.32	6.95
Submerged plant taxa	0.87	4.27	−0.71	14.09	8.51
Submerged and floating-leaf taxa	0.88	3.42	−0.32	12.42	7.73

ecoregion class, lake size, maximum depth, survey type, and watershed and shoreland disturbance indices were important predictors of aquatic macrophyte richness and floristic quality. The highest-ranked models for aquatic macrophyte richness and floristic quality included total phosphorus, the disturbance indices, and the Level 3M ecoregion factor (Table 2). The other ecoregion factors, in particular the MNDNR Province ecoregions, had lower predictive capacity for these response variables. Although not presented, similar patterns to those in Table 2 were found for richness and FQI response variables that included only submerged macrophyte taxa and submerged and floating-leaf taxa.

Models with response variables with all aquatic macrophyte taxa generally performed better (median relative error close to zero, and median absolute relative error smaller) than those based on just submerged macrophytes or those based on submerged and floating-leaf taxa (Table 3). The best generalized linear mixed model for aquatic macrophyte richness, indicated by the lowest AIC score, was a model containing total phosphorus, alkalinity, lake size, maximum depth, ecoregion, survey type, and several interactions (Table 4). The best linear mixed effects model for floristic quality also included these predictive variables (Tables 5 and 6).

3.6. Disturbance–response breakpoints and thresholds for impairment

Aquatic macrophyte richness and floristic quality were lower in lakes with high phosphorus concentrations and landscape disturbance (Fig. 6). Total phosphorus breakpoints ranged from 55 to 169 ppb depending on ecoregion. Disturbance–response breakpoints for the watershed disturbance predictive variable ranged from 60% to 78%, and those for the shoreland disturbance ranged from 24% to 51% (Figs. 7 and 8). The potential aquatic macrophyte richness and FQI thresholds for biological impairment for shallow lakes (maximum depth < 15′) and deeper water lakes (≥15′) in the

Table 4
A summary of the best generalized linear mixed model for aquatic macrophyte richness. The explanatory variables included ecoregion (EPA Level 3), survey type (SURV_TYPE; Minnesota County Biological Survey (MCBS), National Lakes Assessment Project aquatic plant surveys (NLAP), point-intercept surveys (PI), and MNDNR aquatic plant transect surveys (Transect)), total phosphorus (TP), alkalinity (ALK), lake size (acres square root transformed; ACRESSQ), maximum lake depth (square root transformed; MAXDEPTHSQ), watershed disturbance (WDist), shoreland disturbance (Shoredist) as fixed effects. Interactions are included (*). Lakes were modeled as random effects.

Source of variation	Coefficient	SE	*z*	*P*
Intercept	2.340e+00	1.195e−01	19.5878	<0.0001
TP	−3.275e−03	6.321e−04	−5.1806	<0.0001
ALK	−2.776e−04	1.416e−04	−1.9606	0.0499
ACRESSQ	5.183e−03	1.621e−03	3.1984	0.0014
MAXDEPTHSQ	2.693e−02	6.739e−03	3.9959	<0.0001
Wdist	−7.474e−01	1.507e−01	−4.9584	<0.0001
Shoredist	−1.451e−01	1.118e−01	−1.2973	0.1950
Ecoregion – 1A and 1B	0			
Ecoregion – 1C	5.363e−01	3.252e−01	1.6493	0.0991
Ecoregion – 2A	−1.761e+00	6.755e−01	−2.6071	0.0091
Ecoregion – 2B	6.071e−01	1.149e−01	5.2839	<0.0001
Ecoregion – 3A	3.246e−01	7.133e−01	0.4550	0.6490
Ecoregion – 3B (East)	2.561e−01	1.187e−01	2.1569	0.0031
Ecoregion – 3B (West)	6.683e−01	1.147e−01	5.8266	<0.0001
SURV_TYPE – MCBS	0			
SURV_TYPE – NLAP	−2.412e−01	5.872e−02	−4.1074	<0.0001
SURV_TYPE – PI	−1.069e−01	1.959e−02	−5.4557	<0.0001
SURV_TYPE – Transect	−7.325e−02	1.274e−02	−5.7506	<0.0001
TP*ACRESSQ	4.914e−05	1.517e−05	3.2404	0.0012
TP*MAXDEPTHSQ	2.422e−04	1.158e−04	2.0912	0.0365
TP*Shoredist	−2.794e−03	1.420e−03	−1.9683	0.0490
TP*Ecoregion – 1C	−3.338e−03	2.140e−03	−1.5594	0.1190
TP*Ecoregion – 2A	4.931e−02	1.734e−02	2.8435	0.0045
TP*Ecoregion – 2B	−8.223e−04	4.980e−04	−1.6511	0.0987
TP*Ecoregion – 3A	7.336e−03	4.921e−02	0.1491	0.8810
TP*Ecoregion – 3B (East)	1.268e−03	1.593e−03	0.7961	0.4260
TP*Ecoregion – 3B (West)	−6.507e−04	9.071e−04	−0.7173	0.4730
ACRESSQ*MAXDEPTHSQ	−4.386e−04	1.631e−04	−2.6888	<0.0001
Wdist*Ecoregion – 1C	9.114e−02	6.133e−01	0.1486	0.8820
Wdist*Ecoregion – 2A	−6.321e+00	2.658e+00	−2.3780	0.0174
Wdist*Ecoregion – 2B	4.290e−01	1.646e−01	2.6053	0.0092
Wdist*Ecoregion – 3A	2.625e+00	1.919e+00	1.3676	0.1710
Wdist*Ecoregion – 3B (East)	6.262e+00	1.608e+00	3.8936	<0.0001
Wdist*Ecoregion – 3B (West)	1.001e+00	2.223e−01	4.5041	<0.0001

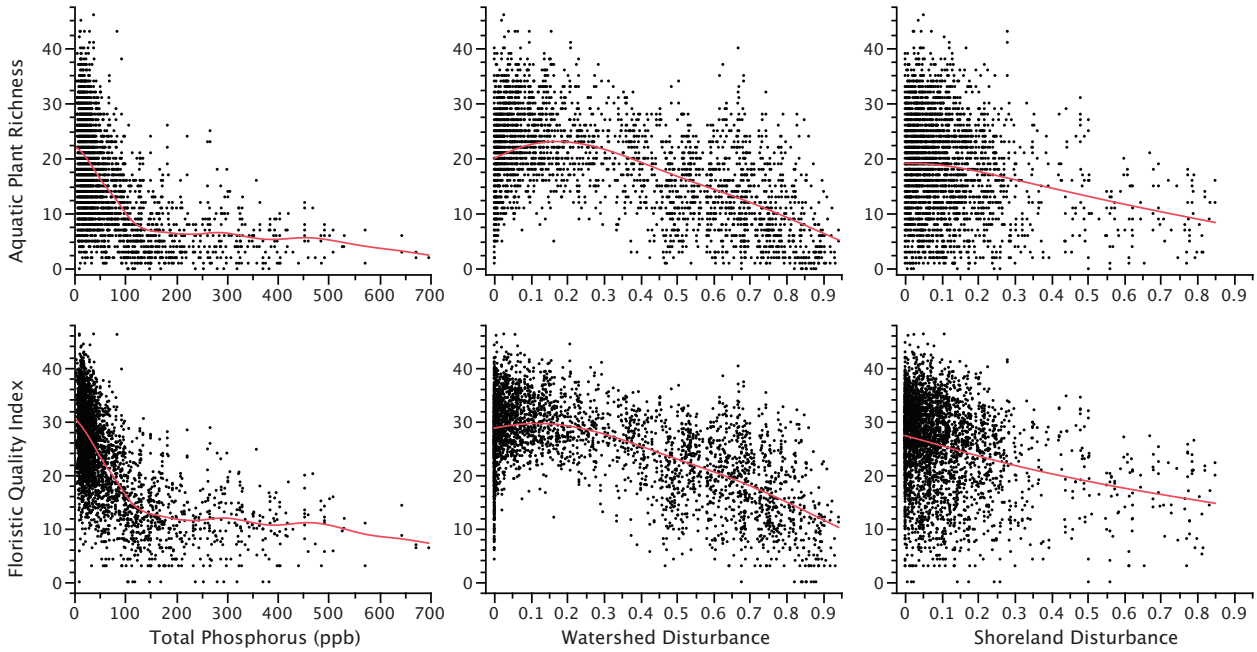

Fig. 6. Aquatic macrophyte richness and floristic quality in relation to total phosphorus, watershed disturbance, and shoreland disturbance. Smoothing spline lines are shown (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Northern Glaciated Plains, Western Corn Belt Plains, Lake Agassiz Plain, and North Central Hardwoods ecoregions were predicted based on breakpoints for predictor variables in the best models identified, and those threshold values are in Table 7.

There appeared to be no disturbance–response breakpoint pattern between aquatic macrophyte richness and floristic quality and the predictive variables for the Northern Lakes and Forest ecoregion. In this ecoregion a review of case histories was used to select the lower 2.5th percentile of aquatic macrophyte richness and floristic quality. This definition of an outlier would represent disparate aquatic macrophyte communities for the ecoregion and identify potentially impaired aquatic macrophyte communities (Table 7).

Applications of the potential thresholds to lakes with existing transect and point-intercept surveys provided an estimate of aquatic macrophyte impairments and repeatability of their designation. Using aquatic macrophyte richness thresholds, a maximum of 27% of the lakes surveyed ($n = 555$) would be classed impaired.

Table 5
Analysis of variance summary of the linear mixed effects model for floristic quality. The explanatory variables included total phosphorus (TP), alkalinity (ALK), lake size (acres square root transformed; ACRESSQ), maximum lake depth (square root transformed; MAXDEPTHSQ), watershed disturbance (WDist), shoreland disturbance (Shoredist), ecoregion class, and survey type (SURV_TYPE) as fixed effects. Interactions are included (*). Lakes were modeled as random effects.

Source of variation	*F*-value	*P*
Intercept	154.065	<0.0001
TP	17.640	<0.0001
ALK	17.488	<0.0001
ACRESSQ	5.249	0.0221
MAXDEPTHSQ	16.611	<0.0001
Wdist	21.033	<0.0001
Shoredist	16.001	0.0001
Ecoregion	15.739	<0.0001
SURV_TYPE	3.250	0.0212
TP*ACRESSQ	10.659	0.0011
TP*Ecoregion	4.019	0.0005
Wdist*Ecoregion	3.072	0.0054
ALK*Wdist	9.076	0.0026

Table 6
A summary of the best linear mixed effects model for floristic quality. The explanatory variables included total phosphorus (TP), alkalinity (ALK), lake size (acres square root transformed; ACRESSQ), maximum lake depth (square root transformed; MAXDEPTHSQ), watershed disturbance (WDist), shoreland disturbance (Shoredist), ecoregion class, and survey type (SURV_TYPE; Minnesota County Biological Survey (MCBS), National Lakes Assessment Project aquatic plant surveys (NLAP), point-intercept surveys (PI), and MNDNR aquatic plant transect surveys (Transect)) as fixed effects. Interactions are included (*). Lakes were modeled as random effects.

Source of variation	Coefficient	SE	*t*-Value	*P*
Intercept	18.202	1.466	12.412	<0.0001
TP	−0.025	0.006	−4.200	<0.0001
ALK	−0.016	0.004	−4.181	<0.0001
ACRESSQ	0.020	0.009	2.291	0.0221
MAXDEPTHSQ	0.307	0.075	4.076	<0.0001
Wdist	−9.036	1.970	−4.586	<0.0001
Shoredist	−4.263	1.066	−4.000	0.0001
Ecoregion – 1A and 1B	0			
Ecoregion – 1C	9.862	4.684	2.105	0.0355
Ecoregion – 2A	−12.466	7.859	−1.586	0.1129
Ecoregion – 2B	9.156	1.429	6.408	<0.0001
Ecoregion – 3A	11.335	11.485	0.987	0.3239
Ecoregion – 3B (East)	8.189	1.537	5.328	<0.0001
Ecoregion – 3B (West)	11.982	1.456	8.231	<0.0001
SURV_TYPE – MCBS	0			
SURV_TYPE – NLAP	−2.417	0.838	−2.883	0.0040
SURV_TYPE – PI	−0.274	0.308	−0.891	0.3729
SURV_TYPE – Transect	0.034	0.206	0.163	0.8703
TP*ACRESSQ	0.001	0.0002	3.265	0.0011
TP*Ecoregion – 1C	−0.012	0.020	−0.580	0.5619
TP*Ecoregion – 2A	0.332	0.181	1.832	0.0673
TP*Ecoregion – 2B	−0.022	0.005	−4.102	<0.0001
TP*Ecoregion – 3A	−0.212	0.805	−0.264	0.7918
TP*Ecoregion – 3B(East)	0.019	0.023	0.842	0.4000
TP*Ecoregion – 3B(West)	−0.027	0.013	−2.177	0.0297
Wdist*Ecoregion – 1C	−8.459	8.333	−1.015	0.3103
Wdist*Ecoregion – 2A	−38.406	23.176	−1.657	0.0977
Wdist*Ecoregion – 2B	2.028	2.045	0.991	0.3216
Wdist*Ecoregion – 3A	33.687	31.374	1.074	0.2832
Wdist*Ecoregion – 3B (East)	67.911	25.532	2.660	0.0079
Wdist*Ecoregion – 3B (West)	8.079	3.222	2.507	0.0123
ALK*Wdist	0.020	0.007	3.013	0.0026

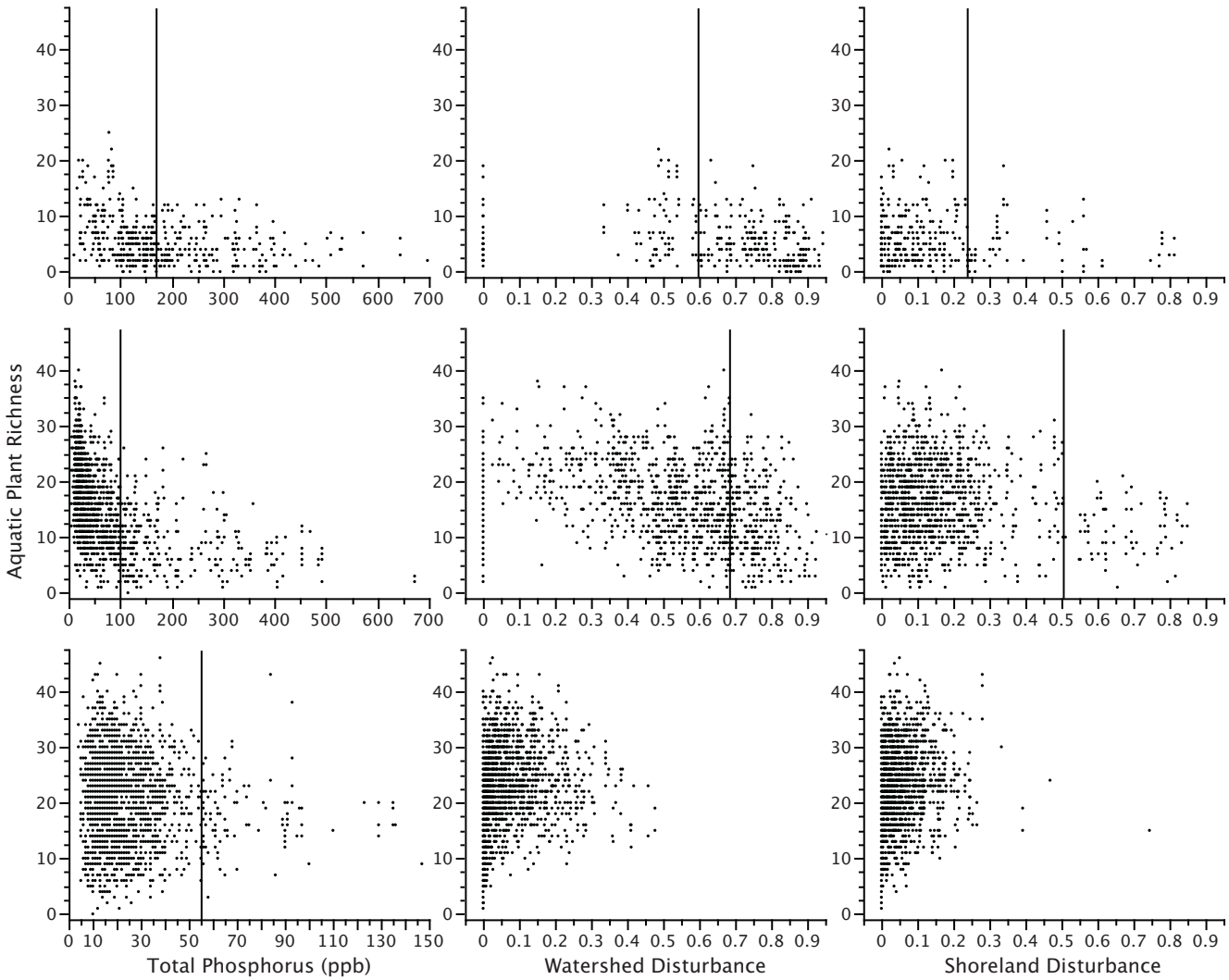

Fig. 7. Scatterplots of the aquatic macrophyte richness and predictor variables with breakpoints (vertical line) by Omernik Level I ecoregion (top, Great Plains; middle, Eastern Temperate Forest; and bottom, Northern Forests).

While there was considerable plant richness and floristic quality variability within lakes, the identification of lakes that were poor in these attributes was had high repeatability – only 5% of the lakes had a survey both above and below the determined plant richness thresholds (Table 8). Using floristic quality, a maximum of 20% of the lakes surveyed would be classed impaired ($n = 402$), with 4% had a survey above and below thresholds. Lakes in the Northern Glaciated Plains and Western Corn Belt Plains ecoregions were most

Table 7
Potential aquatic macrophyte richness and floristic quality thresholds for assessment of biological integrity (richness/floristic quality index). Four survey types: National Lakes Assessment Project aquatic plant surveys (NLAP), aquatic plant transect surveys (Transect), point-intercept surveys (PI), and Minnesota County Biological Survey (MCBS). Thresholds for 3B are based on percentiles, and thresholds for 1A–1C, and 2B are based on predictive models with breakpoints for total phosphorus, watershed disturbance, and shoreland disturbance values. Values less than or equal to the values in this table exceed threshold.

Ecoregion	NLAP	Transect and PI	MCBS
Northern Glaciated Plains and Western Corn Belt Plains – 1A and 1B			
Deeper water lakes (≥15′ max depth)	5/5.7	5/8.0	6/8.1
Shallow lakes (<15′ max depth)	4/5.4	4/7.7	5/7.8
Lake Agassiz Plain – 1C			
Deeper water lakes (≥15′ max depth)	4/5.4	5/9.1	6/9.2
Shallow lakes (<15′ max depth)	4/6.0	4/8.4	5/8.5
North Central Hardwoods – 2B			
Deeper water lakes (≥15′ max depth)	10/16.3	12/18.6	13/18.7
Shallow lakes (<15′ max depth)	9/15.5	11/17.8	12/17.9
Northern Lakes and Forest – 3B West			
Deeper water lakes (≥15′ max depth)	11/16.0	11/20.2	11/19.6
Shallow lakes (<15′ max depth)	6/12.9	7/16.6	6/14.0
Northern Lakes and Forest – 3B East			
Deeper water lakes (≥15′ max depth)	3/9.1	3/12.4	4/10.6
Shallow lakes (<15′ max depth)	6/13.5	6/15.8	7/15.9

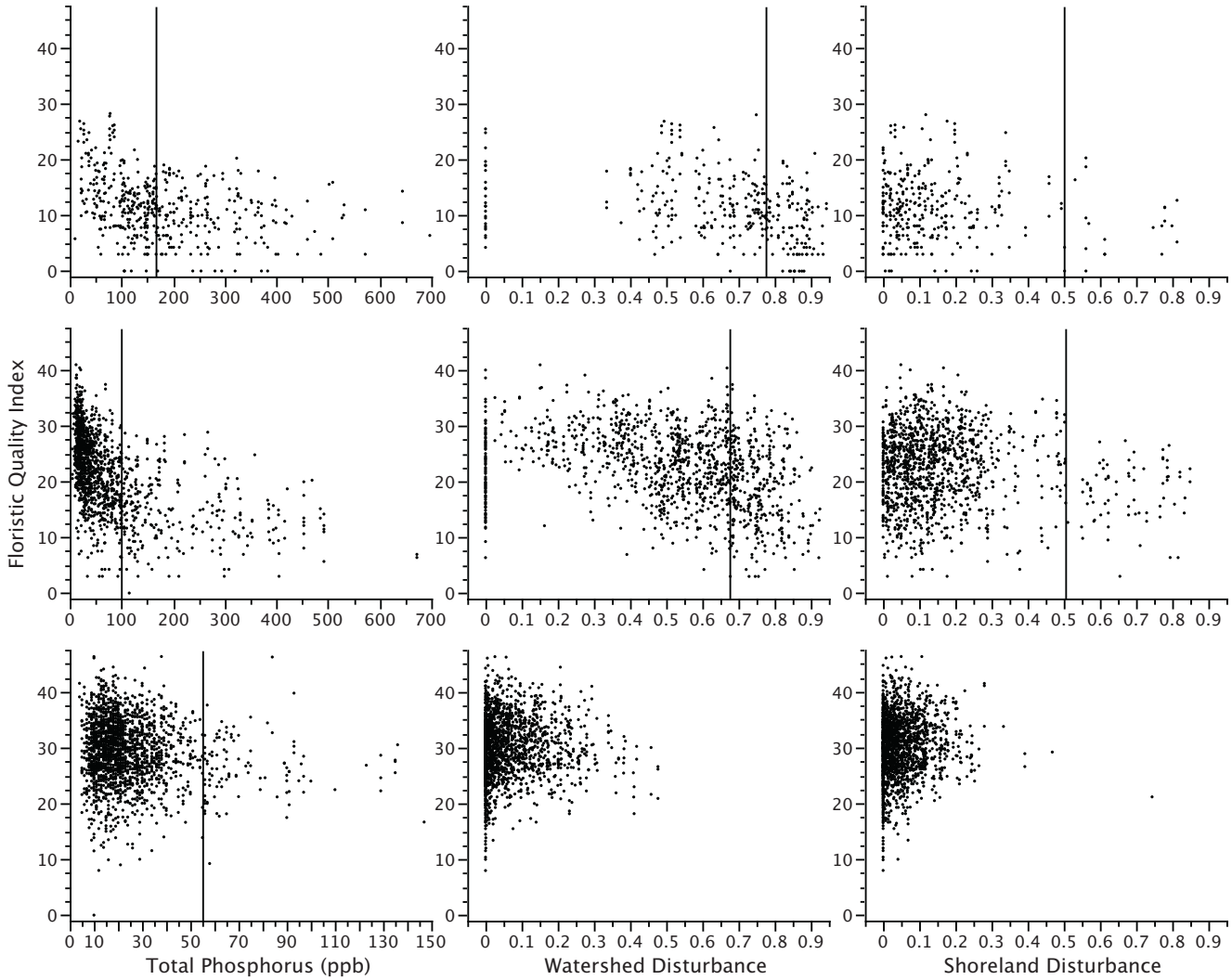

Fig. 8. Scatterplots of floristic quality and predictor variables with breakpoints (vertical line) by Omernik Level I ecoregion (top, Great Plains; middle, Eastern Temperate Forest; and bottom, Northern Forests).

likely to alternative between above and below threshold, which was expected, as these lakes are most prone to alternate between a clear and turbid state. Shallow lakes, especially in the Northern Glaciated Plains, Western Corn Belt Plains, and North Central Hardwoods ecoregions, were most likely to be classified as impaired.

4. Discussion

There are numerous factors that result in different estimates of lake macrophyte richness, such as difference in survey type (methods and protocol), aquatic macrophyte community dynamics, taxonomic resolution by surveyors, varying survey times within the summer survey period, and differences in extent of search and survey area. These differences are likely to be more important in lakes with moderate to high species richness and heterogeneous habitats. If a lake has few species that are rather evenly distributed throughout the littoral zone – all surveyors are likely to find a similar number of species, regardless of their method.

We found that some species occurred across a wide of range of lake plant richness, whereas, some species appeared to be good indicators of high richness. Sago pondweed (*Stuckenia pectinata*), curly leaf pondweed (*Potamogeton crispus*), and coontail (*Ceratophyllum demersum*) were wide ranging species that are known to be tolerant of turbidity, and grow in soft and hard water lakes (Nichols, 1999b). Whitestem pondweed (*Potamogeton praelongus*) and water marigold (*Bidens beckii*) are not tolerant of turbidity, have narrow water chemistry tolerances (Nichols, 1999b), and they generally occurred only in high aquatic plant diversity lakes. Thus, these species appear to be good indicators of high biological integrity in Minnesota lakes. Species with widespread geographic range and abundance within that range are typically considered more appropriate as indicator species than species that occur infrequently (Rabinowitz, 1981; Hutcheson et al., 1999). Other widespread species that, on a statewide basis, appear to be good indicators of diverse aquatic plant lakes include Illinois pondweed (*Potamogeton illinoensis*) and water celery (*Vallisneria americana*). Additional species may be useful indicators at the ecoregion level or for specific lake types.

In our study lakes with high total phosphorus, watershed disturbance, and shoreland disturbance often had lower aquatic macrophyte richness and floristic quality. Total phosphorus breakpoints used to predict potential aquatic macrophyte community impairment reflect values that produce high levels of algal production. From a statewide perspective, the vast majority of lakes in the forested areas of Minnesota appeared to have unimpaired macrophyte communities, and no disturbance–response breakpoint pattern was obvious. Although the use of a percentile-based threshold might be viewed as arbitrary, these low threshold

Table 8
The potential thresholds applied to lakes with existing transect and point-intercept surveys.

Ecoregion	No. of lakes	Aquatic macrophyte richness		Floristic quality		Aquatic macrophyte richness and floristic quality
		No. of lakes with surveys above and below thresholds	No. of lakes potentially impaired based on thresholds	No. of lakes with surveys above and below thresholds	No. of lakes potentially impaired based on thresholds	No. of lakes potentially impaired based on thresholds
1A and 1B						
Deep	46	6 (13%)	20 (43%)	8 (17%)	13 (28%)	13 (28%)
Shallow	186	34 (18%)	121 (65%)	32 (17%)	73 (39%)	73 (39%)
1C						
Deep	12	1 (8%)	2 (16%)	0	0	0
Shallow	24	3 (13%)	12 (50%)	1 (4%)	5 (21%)	5 (21%)
2B						
Deep	580	42 (7%)	214 (37%)	33 (6%)	173 (30%)	169 (29%)
Shallow	190	12 (6%)	136 (72%)	14 (7%)	116 (61%)	116 (61%)
3B West						
Deep	516	3 (<1%)	26 (5%)	1 (<1%)	12 (2%)	11 (2%)
Shallow	92	1 (1%)	4 (4%)	0	1 (1%)	1 (1%)
3B East						
Deep	267	0	9 (3%)	0	5 (2%)	5 (2%)
Shallow	135	1 (<1%)	11 (8%)	0	4 (3%)	4 (3%)
All ecoregions						
Deep	1421	52 (4%)	271 (19%)	42 (3%)	203 (14%)	198 (14%)
Shallow	627	51 (8%)	284 (45%)	47 (7%)	199 (32%)	199 (32%)
Total	2048	103 (5%)	555 (27%)	89 (4%)	402 (20%)	397 (19%)

values could represent outliers that may be reflecting a biological response to stress and disturbance. Aquatic macrophyte thresholds for this ecoregion may also reflect nutrient impairments. For example, Shagawa Lake, a lake that receives effluent from a wastewater treatment plant from the city of Ely, Minnesota, was below the first quartile for aquatic macrophyte richness and floristic quality for its ecoregion, and the lake was borderline on the nutrient criteria for impairment.

In oligotrophic and mesotrophic lakes nutrient loading increases tolerant aquatic macrophyte species abundance. Garrison and Wakeman (2000), using paleolimnological techniques, determined that aquatic macrophyte increases in northern Wisconsin lakes coincided with early shoreland development and associated increases in nutrient loading. However, in more fertile eutrophic lakes, increases in nutrient loading can have profound influence on algal production, which reduces aquatic macrophyte distribution and abundance (Wetzel, 2001). Ramstack et al. (2004) reconstructed past water chemistry for 55 Minnesota lakes and found substantial declines in water quality, with higher total phosphorus levels at present, compared to the 1700s, especially in the agricultural areas of the west-central and southern part of the state.

In this review of aquatic macrophyte communities, we found that many shallow lakes had degraded aquatic macrophyte communities, with a greater proportion degraded compared to deeper water lakes. The degradation of Minnesota shallow lakes has been broad-based, cumulative and persistent (MNPCA, 2004). The portion of shallow lakes as potential impaired in this study was comparable to the estimate from the MNDNR Shallow Lakes Program. About 2/3rds of the shallow lakes were identified as poor from a habitat and water quality perspective (MNDNR, 2010). The majority of the lakes in central or southwest Minnesota are non-supporting of aquatic recreational uses. The reasons for non-support of swimmable use vary. Many northern and north central Minnesota shallow lakes do not support swimmable use due to some past or present source of excess phosphorus loading in their watershed, such as a wastewater treatment plant discharge. The vast majority of shallow lakes in the southwest or northwest have highly agricultural watersheds. Runoff from these agricultural lands is typically very high in phosphorus. This high nutrient loading from the watershed and shallowness of the lakes (which promotes poor retention of phosphorus by lake sediments and internal recycling of phosphorus) typically leads to high in-lake phosphorus concentrations and subsequently nuisance algal blooms and low transparency.

Minnesota's shallow lakes with high phosphorus levels likely have switched or alternate from a relatively clear, macrophyte-dominated condition to a cloudy, algal-dominated condition. Shallow lakes are known to exhibit two alternating stable states (Scheffer et al., 1993). The first state is characterized by clear water, abundant aquatic vegetation and shallow bays covered with emergent vegetation, desirable for fish, invertebrates, and with excellent waterfowl production. The second state, equally stable, is less species-rich and less diverse with very turbid water, little or no submerged vegetation, heavy algal blooms, poor fish communities, and reduced waterfowl production. These shallow lakes can exist for years as either clear or turbid waters. It takes a major perturbation to move from one state to another. In addition, the combination of high watershed nutrient loading and the limited assimilative capacity of shallow lakes often limit the degree to which water quality of these lakes might be improved.

In this study, phosphorus and alkalinity appeared to influence aquatic macrophyte composition, and, by ecoregion, lakes with higher levels of total phosphorus and disturbance in both the watershed and shoreland had lower aquatic macrophyte richness. Moyle (1945) had noted earlier the importance of alkalinity and aquatic plant relationships for Minnesota lakes. For Wisconsin

lakes, Alexander et al. (2008) reported total phosphorus and alkalinity to be important predictive variables influencing lake plant communities, and lakes with high shoreland disturbance had lower macrophyte abundance. Mikulyuk et al. (2011) found that environmental, land-use, and spatial patterns explained 31% of the variation in lake plant assemblages, with environmental factors, such as alkalinity and watershed soils, most important. Croft and Chow-Fraser (2009) found higher macrophyte species richness for all life forms in "pristine" wetlands than in "degraded" wetlands. Cheruvelil and Soranno (2008) found that lake macrophyte cover was correlated with land use. In addition, Sass et al. (2010) found that aquatic macrophyte richness was negatively related to watershed development, with agricultural development explaining more of the relationship than urban land use. While Sass et al. (2010) found that species richness of all aquatic macrophyte life forms declined with increasing levels of watershed development at both the whole-lake and near-shore scales, they found no significant correlation to land use when relations were assessed in separate ecoregions, which, given the sample size of their study, may have been due to low statistical power. We found that lake plant richness was influenced by land use for most ecoregions, but with the Northern Lakes and Forest ecoregion no clear relationship was noted. We speculate that aquatic plant communities in this ecoregion may be in reasonably good condition with insufficient number of lakes in a degraded condition. If this speculation is true, then most of the variability in richness and floristic quality for these lakes is due to alkalinity variability (with many soft water lakes), nutrient availability, and lake morphological differences.

Lake macrophyte communities are also degraded through human removal and control. Payton and Fulton (2004) documented that many Minnesota lakeshore property owners reported removing aquatic vegetation. For Minnesota lakes, Radomski and Goeman (2001) found a 20–28% decrease in emergent and floating-leaf vegetative cover along developed shorelines compared to undeveloped shorelines, and Radomski (2006) estimated the total vegetative cover loss of these aquatic plant communities at 15%. Elsewhere, estimates of aquatic vegetative cover have been higher (e.g., Meyer et al., 1997). Elias and Meyer (2003) found that the mean number of macrophyte species were lower along developed shorelines than along undeveloped shorelines. Hatzenbeler et al. (2004) determined that aquatic macrophyte communities declined with increasing lakeshore development. They found that the number of plant species per lake, number of highly intolerant plant species per lake, species richness and frequency of occurrence of floating-leaf vegetation lower on more-developed lakes. In a study of northern lakes, Hicks and Frost (2011) noted a negative correlation between aquatic macrophyte richness and lakeshore development density.

Several plant IBIs have been developed for lakes (Nichols et al., 2000; Clayton and Edwards, 2006; Rothrock et al., 2008; Beck et al., 2010) and impounded portions of rivers (Moore et al., 2012). The statistical model approach that we used to identify outliers with regard to aquatic macrophyte richness and floristic quality provides another way to assess biological impairment in Minnesota lakes. This approach is a cost effective way to complete biological assessments since it uses existing aquatic plant survey protocols that are used for multiple purposes to estimate simple response variables – aquatic macrophyte richness and FQI. In addition, aquatic macrophyte richness and floristic quality used in this approach was correlated to Beck et al.'s (2010) macrophyte-based and Drake's fish-based (Drake and Pereira, 2002; Drake and Valley, 2005) indices of biotic integrity. In this study, from a statewide perspective, the vast majority of lakes in the forested areas of Minnesota appeared to have unimpaired aquatic macrophyte communities. Lakes with high total phosphorus, watershed disturbance, and shoreland disturbance had lower aquatic macrophyte richness and floristic quality. Shallow lakes often had degraded aquatic macrophyte communities. These lakes are clustered in agricultural areas of the state, and many of these lakes have reduced water clarity due to nutrient loading.

There are three general approaches that can be used to determine biological impairment. The first is a comparative approach that uses traditional IBI development techniques or statistical models to identify extremes in species richness, composition, or abundance. This approach, which was used here, has benefits and shortcomings. One benefit is that biological extremes can be relatively easy to identify, especially, as in this case, when a large number of waterbodies are analyzed. The obvious shortcoming is that such an approach may as easily find biological extremes due to natural conditions as due to human disturbance. Given this shortcoming, it is often necessary to employ a decision support system or a decision tree to bring additional information to bear on whether to proceed with designating a waterbody as impaired. We provided a decision tree for use with the aquatic macrophyte integrity indices developed (Radomski and Perleberg, 2012). The decision tree includes a series of questions for the natural resource manager on such matters of data quality and likelihood of human disturbance as the probable factor in exceeding the threshold. The second approach is an individual waters approach, where the undisturbed condition is known and the lake or river is monitored through time. When the biological integrity falls below a pre-determined condition, then the waterbody is designated as impaired. This approach, which is ideal, is rarely used because the availability of biological data sufficient to set an undisturbed baseline is rare. Finally, the third approach uses the concepts of the first two approaches. An example of this approach includes use of measures of taxonomic completeness (e.g., Wright et al., 2000; Hawkins, 2006). The ratio between the observed and expected taxonomic composition is the IBI, where the expected number of taxa is estimated by models developed with data collected from a set of reference or high quality sites. The benefit of this approach is that the metric need not be validated against any stressor gradient (similarly, the statistical model approach used here included the stressors within the model so no post hoc validation was necessary). The shortcoming of this approach is that good predictive models are necessary to estimate the expected number of taxa, which can be challenging due to variability in natural conditions and differences in likelihood of a species colonizing a particular waterbody. The approach does have merit and additional research in its application may be useful.

Other investigations may also prove productive. The interaction between nutrient loading and lake phosphorus concentrations on the integrity of lake macrophyte communities is large and clearly understood. Aquatic macrophyte thresholds from this study produced results consistent with designated nutrient impairments – over 65% of the designated nutrient impaired lakes had an aquatic macrophyte integrity index at or below the thresholds identified here. Since many aquatic macrophyte are perennials that are dependent on light transmission through the water column, they reflect the cumulative effects of water quality degradation on water clarity at the lake-wide scale and at meaningful time-scales. The importance of aquatic plant–phosphorus relationships and the associated fish–aquatic plant relationships has lead to discussions on the benefits of an integrated approach to identify biologically impaired lakes. Soranno et al. (2008, 2010) outlined several ways to integrate numerous biological thresholds to identify individual lake phosphorus criteria or designate lake impairment. Further analyses of these approaches may have merit. Development of an adaptive system to implement an integrated approach to biological impairment may reduce regulatory complexity while providing a robust assessment of impairment. Such an approach would allow the use of multiple biological integrity indices, perhaps including

one or more aquatic macrophyte integrity indices that have been developed for Minnesota waterbodies.

Biological integrity is a complex concept and may be viewed from both structural and functional perspectives (DeLeo and Levin, 1997). Integrity is not a discrete value and is often described in general terms or in comparison to pristine communities. DeLeo and Levin (1997) further suggest that ecosystem integrity reflects the capability of a system to support services of value to humans. This human value factor makes defining and describing integrity even more difficult. We suggest that in most Minnesota lakes, rather simple aquatic macrophyte indices can be used as a surrogate for plant community integrity. When macrophyte indices are above threshold values, we suggest that biological integrity has likely been retained. Managers may cite factors such as matted plant growth, dominance by a non-native species, or restriction of most species to isolated patches, as indicators that the aquatic macrophyte community has lost biological integrity, despite retaining sufficient plant richness. In these cases, we may not objectively evaluate whether or not the lake plant community has declined in functional integrity. Managers may elect to conduct more detailed plant assessments to evaluate if there have been recent changes in the spatial distribution and/or abundance of plants.

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Appendix A. R code for models

The R code for the best linear mixed-effects (lme) model to predict floristic quality index (FQIspp) using the observed data set (vegdata) and the best generalized linear mixed model (glmm) with a Poisson distribution with a logarithmic link (family = poisson) to predict aquatic macrophyte richness (numSPP) was as follows:

```
Preferred Model ← lme(FQIspp ~ TP + ALK + ACRESSQ +
MAXDEPTHSQ + Wdist + Shoredist + TP*ACRESSQ + TP*Level3M +
Wdist*Level3M + ALK*Wdist + SURV_TYPE + Level3M, random = ~1|lake, data = vegdata)
Preferred Model ← glmmML(numSPP ~ TP + ALK + ACRESSQ +
MAXDEPTHSQ + Wdist + Shoredist + TP*ACRESSQ +
TP*MAXDEPTHSQ + TP*Shoredist + TP*Level3M +
ACRESSQ*MAXDEPTHSQ + Wdist*Level3M + SURV_TYPE + Level3M,
cluster = lake, family = poisson, data = vegdata)
```

where ecoregion (Level3M) and survey type (SURV_TYPE) were analyzed as fixed effects; total phosphorus (TP), alkalinity (ALK), lake size square root transformed (ACRESSQ), maximum depth square root transformed (MAXDEPTHSQ), watershed disturbance (Wdist), and shoreland disturbance (Shoredist) were added as linear effects, and lake was used as the random effects variable.

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