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Preliminary Analysis of Biological Assessment Thresholds for Determining Aquatic Life Use Attainment Status in the Upper Mississippi River Mainstem

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Introduction

The Midwest Biodiversity Institute (MBI) is engaged in a project through the Upper Mississippi River Basin Association (UMRBA) to aid the states of the Upper Mississippi River (UMR) as they consider the integration of biological tools and assessment approaches into their Clean Water Act (CWA) programs on the River. Specifically, the principal aim of this project is a UMR CWA Biological Assessment Implementation Guidance Document.

Within the project's scope, significant work has already been performed to identify the most promising assemblages, methods, and indices for use in determining attainment of the aquatic life use on the River under the CWA (Yoder et al. 2010). A major emphasis of the project is to identify "potential impairment thresholds for the UMR main channel in determining the attainment of aquatic life uses," as identified in the project's work plan (MBI 2010). To that end, we conducted a preliminary assessment of the derivation of biological thresholds for early review by the UMRBA Water Quality Task Force (WQTF) in January 2011. Our initial analyses relied heavily upon data gathered and research performed by the U.S. EPA EMAP-GRE program, and we initially employed the condition classes defined by Angradi et al. (2009a,b) to assess aquatic life use attainment within the already established CWA minimum assessment reaches in the UMR mainstem.

Feedback received from the WQTF in January 2011 indicated an interest in: 1) examining alternate approaches to establishing thresholds using EMAP-GRE indices (i.e., beyond the three condition classes developed by GRE), 2) comparing GRE-based thresholds to those developed using alternate approaches and indices, including reference condition-based approaches, 3) further exploring the sensitivity of the GRE suite of indices and other available indices to a set of stressor gradients, and 4) evaluating the possibility of incorporating a submersed vegetation index into a UMR bioassessment.

Therefore, in this revised final version of the preliminary thresholds determination, we build on our previous work by comparing EMAP-GRE indices and thresholds to other indices and thresholds potentially applicable to the UMR, examining each for their responsiveness to gradients of aquatic life stressors and their ability to accurately characterize the UMR main channel. We used multiple statistical methods to derive potential threshold values for the indices examined and evaluated the appropriateness of those thresholds in light of aquatic life use attainment realities in the UMR main channel.

The goals of this report are to:

1. Examine the sensitivity and suitability of various biological indices for assessing CWA aquatic life use attainment in the main channel of the UMR, including the identification of proximate stressors;
2. Aid the WQTF in visualizing the likely outcomes of a biological-based assessment of aquatic life use attainment for the UMR;

3. Compare the EMAP-GRE developed indices and thresholds to other available approaches to aid the WQTF in its consideration of EMAP-GRE tools as the leading candidates for recommended bioassessment approaches on the UMR; and,
4. Assess the potential for integrating an additional aquatic assemblage (submersed vegetation) into a UMR biological assessment.

Technical Approach

The preliminary analysis of biological condition thresholds for fish and macroinvertebrate assemblages found in the UMR main channel was conducted using data from the U.S. EPA Environmental Monitoring and Assessment Program for Great Rivers (EMAP-GRE; Angradi et al. 2009a, 2009b). The EMAP-GRE dataset was one of two system-wide databases for the main channel UMR that were identified in the project's initial scoping process and report (Yoder et al. 2010). EMAP-GRE was selected for our preliminary analyses because it represented the spatially most comprehensive database, it is of relatively recent origin (2004-6), and the collective rigor of the methods and data analyses conforms to the highest standards for a bioassessment program (i.e., it is a Level 4 program after Yoder and Barbour 2009).

Both fish and macroinvertebrate assemblages were included in a dual-indicator approach. Although a vegetation-based index has recently been developed for the UMR, we focused primarily on fish and macroinvertebrates because both were collected at similar spatial densities and over the same length of river (Yoder et al. 2010). We did, however, examine the potential utility of the submersed aquatic vegetation index, though our analyses were necessarily perfunctory given that SMI scores were available only for Reaches 0 through 5, and were matched to only 3 sites each in Reaches 1 and 4 where fish or macroinvertebrate indicators were also collected, compared to 14 sites in those reaches for the fish and macroinvertebrate indicators. Thus, the number of sites available for a multi-indicator assessment that includes submersed vegetation would have been spatially too limited.

Methods

Condition Assessments

Using EMAP-GRE data, we conducted biological condition assessments of the UMR by: 1) using EMAP-GRE derived thresholds and indices, 2) using alternate thresholds for EMAP-GRE indices, and 3) using Regional EMAP (REMAP) indices and thresholds (see Table 1). We also examined the effect of integrating a submersed aquatic vegetation index (SMI) into the condition assessment where that data was paired with the fish results.

Assessment Using EMAP-GRE Developed Indices (GRFIn and GRMIn) and Thresholds
<u>Use of EMAP-Derived Thresholds</u>: The EMAP-GRE program had derived biological condition thresholds for the GRFIn and GRMIn indices based on biological responses against an empirical stressor gradient constructed from land use, population density, habitat quality, and water chemistry indicators (Angradi et al. 2009a). Unlike a regional reference condition approach

where percentiles of least or minimally disturbed reference populations forms the basis for a set of condition thresholds, the empirical approach sets the baseline to the y-intercept in the relationship between the empirical stressor gradient and the respective biological assemblage endpoint (i.e., fish or macroinvertebrate assemblage index or attribute). Once the upper limit is defined (i.e., by the 95^{th} percentile regression line y-intercept) the data range was trisected, thus forming three disturbance classes - least, intermediate and most disturbed (re: Figure 7 in Angradi et al. 2009a). An empirical approach was initially used for the main channel UMR because the extent of anthropogenic modifications precludes a direct reference condition approach - essentially the navigable mainstem from the Twin Cities to St. Louis is a series of regulated, modified navigational impoundments and a highly modified open river segment.

<u>Initial Choice of “Most Disturbed” Threshold</u>: In our analysis, we initially considered sites to be failing to meet the minimum CWA goal for beneficial aquatic life uses if either the fish or macroinvertebrate indicators were less than their respective EMAP-derived, most disturbed threshold. Sites with both assemblage indices classed at or above intermediately disturbed were considered in full attainment of the minimum CWA aquatic life use goal.

<u>Site-Based Assessment</u>: Assessments were made at the site level to specifically track longitudinal trends in status (see Figure 13). Attainment status for the UMR from the confluence with the Ohio River upstream to St. Anthony Falls, MN was apportioned to miles of attainment/non-attainment by UMR assessment reach (using EMAP-GRE river mileage) and by state using the weighted probability baseline of the original EMAP-GRE design and as a linear interpolation of attainment status by contiguous sampling site.

<u>Alternative Calculations</u>: We also conducted alternative analyses using least disturbed as the CWA threshold, comparing a distribution of index scores from the lower St. Croix River as a least impacted analog for the UMR main channel, change point analysis, and an extrapolation from the reference condition of a regionally relevant assessment of Upper Mississippi and Ohio River tributary rivers (REMAP).

<u>Potential Causes of Non-Attainment</u>: Potential causes of non-attainment were identified by examining associations between biological index scores and ambient water chemistry, habitat, and stressor indicators (e.g., population density, upstream distance to wastewater treatment plants, etc. all based on data supplied by Dave Bolgrien, U.S. EPA). Statistical methods included rank-order correlations, classification and regression trees (CART), linear regression, and discriminant analyses. For the latter, condition classes formed the categorical variables, and only data from the impounded UMR were selected to allow comparisons between the two biological assemblages. Data from the unimpounded UMR (Open River) were not included because the GRMIn is not calibrated for that section of the UMR. Rank-order correlations revealed broad patterns in the data. Multiple regression goes a step further in assessing combinations of variables contributing explanatory power. CART models may reveal nonlinear and threshold responses undetected by linear regression, and discriminant analysis helps assess the results of the previously listed methods in terms of how well the stressors contribute to forming narrative classes.

Where stressors were identified as accounting for significant variation in the GRFIn or GRMIn scores, those stressors were noted as *proximate* causes of non-attainment in the assessment reaches having extreme values for those stressors (i.e., low for habitat variables, high for water quality variables) . For example, Figures 6 and 7 show the results of the CART analyses as regression trees. In Figure 6, habitat variables (channel complexity, substrate quality and high-quality vegetation) form a nested branch partitioning variation in the GRFIn scores. Similarly, linear combinations of habitat variables discriminate condition classes of GRFIn scores (see Table 4 in Results). A scatter plot of GRFIn scores against channel complexity (Figure 1) clearly illustrates the importance of habitat quality. In Figure 1, GRFIn scores falling below the most disturbed threshold coincidentally with low channel complexity scores are shaded black for likely being impaired due to poor habitat quality, or shaded gray for being less than the disturbance threshold in Figure 6. The gray shading indicates that habitat quality is potentially a cause of impairment (or very likely a contributor), but further inspection of the position of those points in relation to other stressors is necessary to assess whether another stressor is, perhaps, more proximate. Interpretation of water quality variables is slightly more straightforward, as also illustrated in Figure 1, where extreme values coinciding with impaired GRFIn or GRMIn scores are flagged.

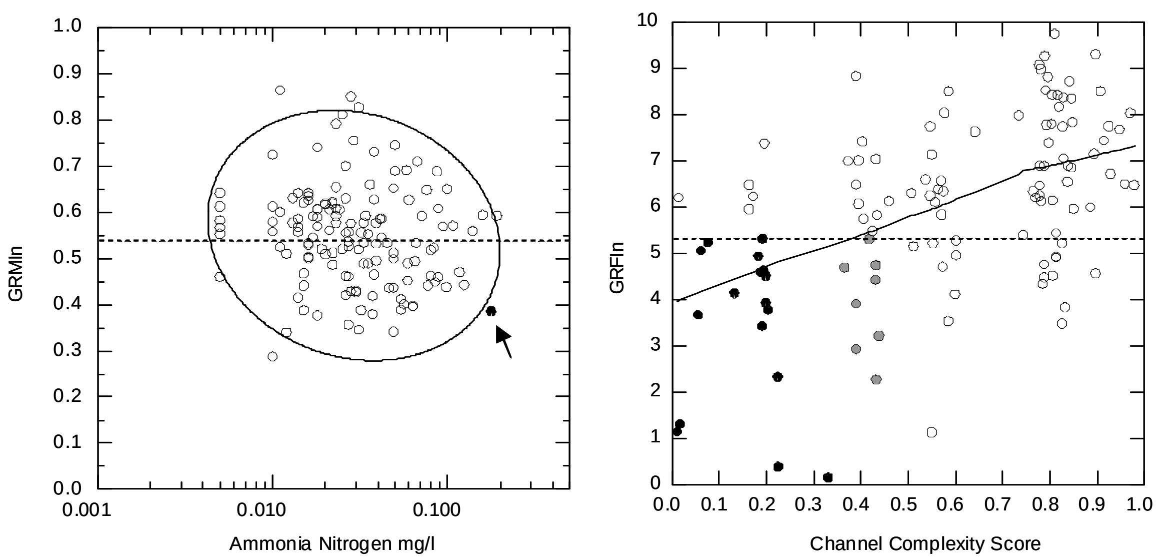

Figure 1. Example plots visually depicting the tacit algorithm used to assess causes of impairment. Horizontal lines extending from the y-axis in each plot show the intermediate disturbance threshold. The ellipse in the left panel bounds 2 standard deviations from the sample mean. The line following the local central tendency in the right panel is from LOWESS (q=0.5). The solid dot in the ammonia nitrogen plot deviates from the normal distribution and is lower than disturbance threshold. The shaded dots in the channel complexity plot are lower than the disturbance threshold and at the lower end of the gradient implied by channel complexity scores.

Table 1. Methods for determining aquatic life use thresholds.

Threshold	Indices Used	Rationale/Method	Description
Most Disturbed	GRE	Based on an empirical relationship, the boundary between most and intermediate disturbed should be achievable.	Biological index scores are plotted against a stressor gradient composed of land use, habitat, water quality and other environmental variables; the y-intercept of a quantile regression sets the upper bounds for index expectations, and the 5th percentile the floor. Trisection of the resulting range defines least, intermediate and most disturbed conditions.
Quadrisection	GRE	An *ad hoc* method that sets achievable expectations based on peers.	The 95th to 5th percentiles of index scores for a given river (in this case, scores from the upper impounded reach and the open river) from the ceiling and floor values, and the resulting range is quadrisected. The midpoint or first section boundary can set the threshold for attainment.
Quadrisection of Similar Rivers	REMAP	This method provides a partially independent method for determining a realistically achievable benchmark.	Index scores from similar rivers (size, drainage area, fauna, physical alteration) are quadrisected, and the midpoint sets the threshold.
Reference 25th Percentile	REMAP	Reference condition-based.	Reference sites were identified for the NMACI and FACI based on the 25th percentile of a stressor gradient (defined by environmental variables).
Change Point	REMAP & GRE	Least arbitrary of the methods for identifying an impairment threshold.	Index scores were plotted against the stressor index and a locally weighted regression line fit through the points. A deviance reduction method (classification and regression tree [CART]) was used to partition scores along the stressor index, and the point where the regression line crossed the partition was used as a threshold (see Figure 2).

Table 6 summarizes the number of habitat and water quality variables by assessment reach that were "flagged" as being proximate causes of impairment. Note that the mere presence of a parameter in a particular reach does not necessarily result in it being listed as a proximate cause of impairment. Take the example of ammonia-N in Figure 1, where several values exceed 0.1 mg/l, but only one is outside the ellipse bounding two standard deviations from the group centroid and less than the most disturbed threshold[1].

Assessment Using Alternate Thresholds for GRE Indices

Quadrisection: Quadrisection was used as an alternate method to define an assessment threshold, wherein the 95th and 5th percentiles of GRFIn and GRMIn scores (GRE Indices) from the entire UMR (the upper impounded reach and the open river) set the ceiling and floor values, and the midpoint of the resulting range set as the impairment threshold (Figure 2). Data from the entire UMR were then included to expand the potential stressor gradient. The midpoint of the quadrisection was chosen as the impairment threshold because it sometimes functions as a boundary between fair and good narrative quality (all internally-derived boundaries are necessarily arbitrary). A brief description and rationale for each of the methods used to draw thresholds is given in Table 1.

Change Point: Another method for defining an impairment threshold entailed arraying GRFIn and GRMIn scores along the stressor gradient (Angradi et al. 2009) and testing for a change point via the method of deviance reduction (i.e., the first split from a classification and regression tree [CART]). A locally weighted smoothing line (LOWESS, α=0.5) was fitted to the plot of GRFIn and GRMIn scores against the stressor index to find the respective index score corresponding to the change point (Figure 2). In both cases, the change point method resulted in a threshold similar to either the one given by quadrisection (GRFIn), or the existing disturbance threshold (GRMIn).

Assessment Using REMAP Indices (NMACI and FACI)

Indices Used: A separate set of biological indices developed for large Midwestern rivers via a Regional EMAP (REMAP) project (Emery et al. 2007) were applied to the UMR data to identify a disturbance threshold, and to provide a second set of condition assessments for comparison to the condition estimates given by the GRFIn and GRMIn indices. The indices, the Non-wadeable Macroinvertebrate Assemblage Condition Index (NMACI; Blocksom and Johnson 2009) and the Fish Assemblage Community Index (FACI; Emery et al. 2007), were developed from sites sampled during the REMAP Large Rivers project.

Thresholds Used: Potential thresholds for each index were derived in the following ways: 1) quadrisection of scores calculated from the UMR data, 2) the 25th percentile from REMAP reference sites, (the 25th percentile of reference sites for the FACI was estimated from Figure 12 in Emery et al. [2007], and for the NMACI, from Figure 4 in Blocksom and Johnson [2009]), 3)

[1] Ellipses drawn using Systat v9.0 software.

the change point against the stressor index, and 4) quadrisecting FACI and NMACI scores from similar large Midwestern Rivers[2].

Assessment Submersed Aquatic Vegetation Index (SMI)
Submersed aquatic vegetation is a third assemblage that recently became available for reaches 0 through 5 of the impounded UMR. The Submerged Macrophyte Index (SMI[3]) was developed based on sampling main and side channels (Moore et al. 2011). A threshold for the SMI was established by quadrisection, using the midpoint of the range between the 95th and 5th percentiles. Because the macroinvertebrate based GRFIn tended to have a compressed range of response, the SMI was experimentally used to over-ride condition assessments that were rated as not attaining based on GRMIn or NMACI scores *for sites where the SMI was available*. The over-ride approach was used because the spatial coverage of the SMI did not match that for the fish and macroinvertebrate assemblages, thus precluding a site based assessment of the SMI within the context of the present study. Therefore the SMI was used as supplemental indicator, rather than a substitute or third assemblage. This does not preclude its use in future UMR bioassessments provided the sampling data is available.

Sensitivities

Sensitivity of Biotic Indices to Measured Environmental Gradients
How well the various index scores (GRFIn, GRMIn, NMACI, SMI, and FACI [calculated at 0.5 and 1.0 km distances]) responded to measured environmental gradients was examined first by correlating the indices against water chemistry variables, habitat variables, a stress index score, a hydrology index score, population density, density of NPDES majors in the upstream catchment, density of NPDES majors in the 10 km channel riparian, and density of NPDES majors in the 100 km catchment upstream from a sampling point. Next, potential non-linear and local relationships were investigated using classification and regression trees (CART). Only data from the impounded UMR were used for the CART analyses, as channel complexity and substrate scores were not available for the unimpounded Open River reach. Index scores were plotted against the environmental variables forming the first node of the tree and a locally weighted regression line fitted to the respective plots to visually assess the nature of the relationship suggested by the first node.

Results

Condition Assessments

GRE Indices (Using Most Disturbed/Intermediate Disturbed Threshold)
Attainment Percentages: Based on the dual indicator (GRFIn and GRMIn) approach, for the mainstem as whole, 47 percent of the miles were in full attainment of the aquatic life use, and

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[2] The St. Croix R. below Taylor Falls, the Wisconsin R. below Lake Wisconsin, the Minnesota R. downstream from New Ulm, the Wabash R. downstream from the confluence with the Vermillion R., the Illinois R., and one impounded site on the Muskingum R. (FACI only).
[3] SMI data were supplied by Heidi Langrehr, Wisconsin DNR.

53 percent were in non-attainment. By assessment reach (Table 2) the percentage of miles in full attainment ranged from 0 to 78 percent. Assessment reaches 2 through 5 had the highest proportion of attainment, ranging from 64 to 78 percent of the miles in full attainment, which coincided with comparatively lower levels of anthropogenic stress relative to adjacent reaches (Figure 3). By state, Wisconsin and Iowa had more miles in full attainment than non-attainment, whereas the converse was true for Minnesota, Illinois and Missouri (Table 3).

Response to Stressor Gradient: Plots of fish and macroinvertebrate index scores (Figures 4 and 5) by river mile suggest that the GRFIn was generally more responsive to the stressor gradient. Although the stressor index explained similar variation in either GRFIn or GRMIn scores (18% and 15%, respectively), and both indexes followed a broadly similar longitudinal pattern, the slope of the GRFIn-stress relationship was steeper, coinciding with the broader range of response seen in Figure 4. GRFIn condition classes were classified with greater efficiency compared to GRMIn condition classes in the discriminant analysis (Table 4). Similarly, a greater proportional reduction in error was accounted for in GRFIn scores compared to GRMIn scores by CART (Figures 6 and 7). Lastly, about one-half of the variation in GRFIn scores was accounted for in the linear regression compared to less than a third for GRMIn scores (Table 5). These results support the observation that GRFIn is generally more responsive to the stressor gradient than was GRMIn.

Explanatory Variables and Potential Causes of Impairment: Habitat quality figured prominently as an explanatory variable for GRFIn scores in all of the statistical models, and marginally so for GRMIn scores in the linear regression model. GRFIn scores were negatively associated with total nitrogen, and GRMIn scores were negatively associated with conductivity. More generally,

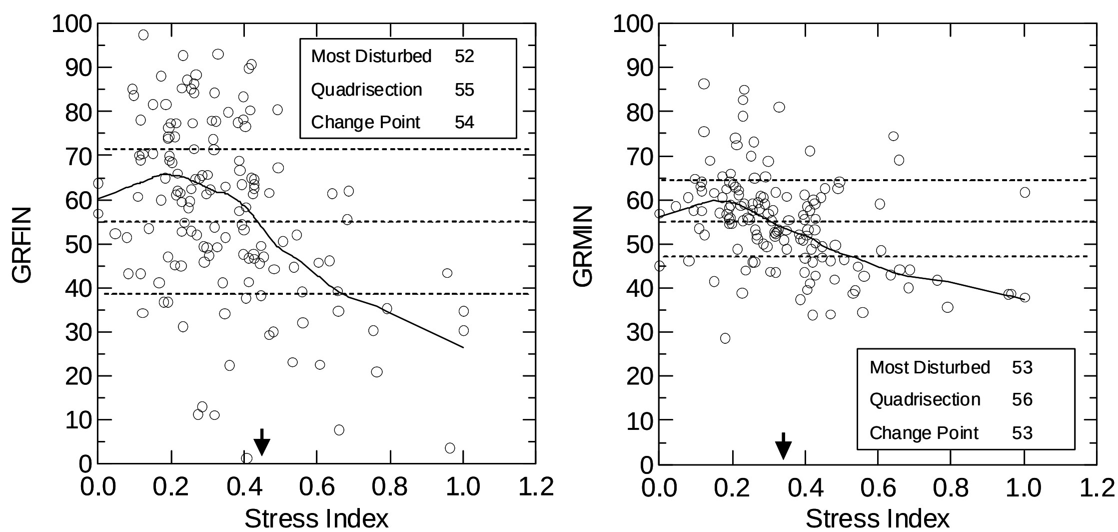


Figure 2. Quadrisection of GRFIn and GRMIn scores arrayed along an index of stress (Angradi 2009). The solid line fitted to the data in each plot is from LOWESS smoothing (q=0.5). Respective thresholds based on disturbance class, quadrisection and CART are inset in each plot. Arrows pointing to the x-axis show the respective change points suggested by CART.

both GRFIn and GRMIn scores were negatively associated with aggregations of water quality indicators indicative of wastewater loadings (e.g., ammonia-nitrogen, chloride) and nutrient enrichment (total nitrogen and phosphorus). For any of the explanatory variables statistically associated with the biological indicators, Table 6 summarizes the number of sites within a given reach where concentrations or measures of the explanatory variable were at levels that potentially contributed to impairment. For example, the presence of high quality vegetation was positively associated with GRFIn scores in multiple regression and CART analysis (see Table 5 and Figure 6), and within Reach 2, three sites had very poor metric scores for high quality vegetation, suggesting that for those sites, the vegetation component of the habitat may have been limiting to the fish community.

Alternate Thresholds and Condition Assessments

<u>Attainment Percentages</u>: The percent of sites in the UMR (and by extension, miles) categorized as impaired ranged from 53.0 percent using the most-intermediate disturbance threshold for GRFIn and GRMIn (Angradi et al. 2009a, 2009b), as described above, to 98.6 percent based on FACI and NMACI scores evaluated against the 25th percentiles of their respective REMAP reference populations (Table 7). Threshold scores, as a percentile rank of their respective index scores from the UMR, ranged from the 16th percentile for the quadrisection of FACI scores from similar rivers, to the 98th percentile for the NMACI based on the 25th percentile of the REMAP reference population. Fish index thresholds, again as percentile ranks, ranged from the 16th (quadrisection of similar rivers) to the 54th (FACI quadrisection of the UMR), and macroinvertebrate index thresholds ranged from the 41st (GRMIn most-intermediate disturbance) to the 98th (25th percentile of REMAP reference).

Overall, the most conservative assessment (in terms of impairment) resulted when using the 25th percentile of REMAP reference sites as the threshold for FACI and NMACI scores (Figure 8), and the most optimistic assessments resulted from the most-intermediate disturbance threshold for GRFIn and GRMIn scores and from quadrisection of the NMACI (UMR sites) and FACI (similar rivers).

At the reach level Reaches 2-5 had the lowest amount of impairment, and Reaches 0-1 and 9-13 had the highest amounts of impairment, with Reaches 6-8 intermediate between the two (Figure 10). Reach 3 appeared to be the least impaired, showing 25 percent impairment for six of the eight threshold combinations. Reaches 9 and 11 had the highest proportion of impairment, faring slightly worse than Reaches 12 and 13 in the Open River.

<u>Indicator Agreement</u>: Fish and macroinvertebrate indicators agreed on site-level condition status in roughly two-thirds of the cases when assessments were based on GRFIn and GRMIn scores, whereas condition assessments based on NMACI and FACI thresholds from similar REMAP rivers agreed in about one-third of the cases (Table 7). The highest frequency of agreement was between the NMACI threshold based on quadrisection of the UMR and the FACI threshold based on change point on the UMR stressor index.

Table 2. Aquatic life use attainment apportioned by miles for assessment reaches of the Upper Mississippi R. main channel based on EMAP-GRE and a weighted probability design. Segments with >50% non-attainment of the most disturbed/intermediate disturbed biological threshold are shaded. See Appendix Table 1 for the number of sites in each reach and the frequency of times each indicator was rated as impaired within a reach.

Assessment Reach	GRE River Miles[1]	Reach Length	MBI Assessment		Proximate Stressors[2]	2008 303(d) ALU Attainment[3]
			Full (percent)	Non (percent)		
Non-interstate UMR	870.5 – 812	58.5	0.00 (0)	58.5 (100)	Ammonia Habitat[4] TN[5] Conductivity	Turbidity
1 St. Croix River to Chippewa River	812 – 763	49	3.50 (7)	45.5 (93)	Habitat TN	Turbidity/ TSS
2 Chippewa River to Lock and Dam 6	763 – 714	49	38.1 (78)	10.9 (22)	Habitat Nutrients	Full
3 Lock and Dam 6 to Root River	714 – 694	20	15.0 (75)	5.0 (25)	None Detected	Full
4 Root River to Wisconsin River	694 – 631	63	40.5 (64)	22.5 (36)	Habitat	Full
5 Wisconsin River to Lock and Dam 11	631 – 583	48	34.3 (71)	13.7 (29)	Habitat	Full
6 Lock and Dam 11 to Lock and Dam 13	583 – 523	60	23.1 (38)	36.9 (62)	Habitat	Full
7 Lock and Dam 13 to Iowa River	523 – 434	89	34.2 (38)	54.8 (62)	Habitat	Al & Nutrients
8 Iowa River to Des Moines River	434 – 361	73	39.8 (55)	33.2 (45)	Habitat TN	Al
9 Des Moines River to Lock and Dam 21	361 – 325	36	12.0 (33)	24.0 (67)	Habitat TN	Full
10 Lock and Dam 21 to Cuivre River	325 – 237	88	35.2 (40)	52.8 (60)	Habitat TN	Full
11 Cuivre River to Missouri River	237 – 196	41	0.0 (0)	41.0 (100)	Habitat TN	Full
12 Missouri River to Kaskaskia River	196 – 118	78	26.0 (33)	52.0 (67)	Conductivity	Pb & Zn
13 Kaskaskia River to Ohio River	118 – 0	118	81.1 (69)	36.9 (31)	Conductivity Nutrients	Full
Total Length	870.5 - 0	870.5	411.1 (47)	459.4 (53)		

[1] EMAP-GRE river miles – these are different from the ACOE RM system. [2] Proximate stressors were defined by statistical associations between stressors and biological indicators. [3] Aquatic life use attainment reported by states based on chemical/physical indicators. [4] Habitat includes either channel complexity, substrate quality, or both. [5] Total nitrogen was strongly correlated with total dissolved solids along the entire mainstem; however, in the non-interstate reach, TN was also associated with common wastewater constituents such as chloride and ammonia-nitrogen. Conductivity was not strongly associated with TDS – see Discussion.

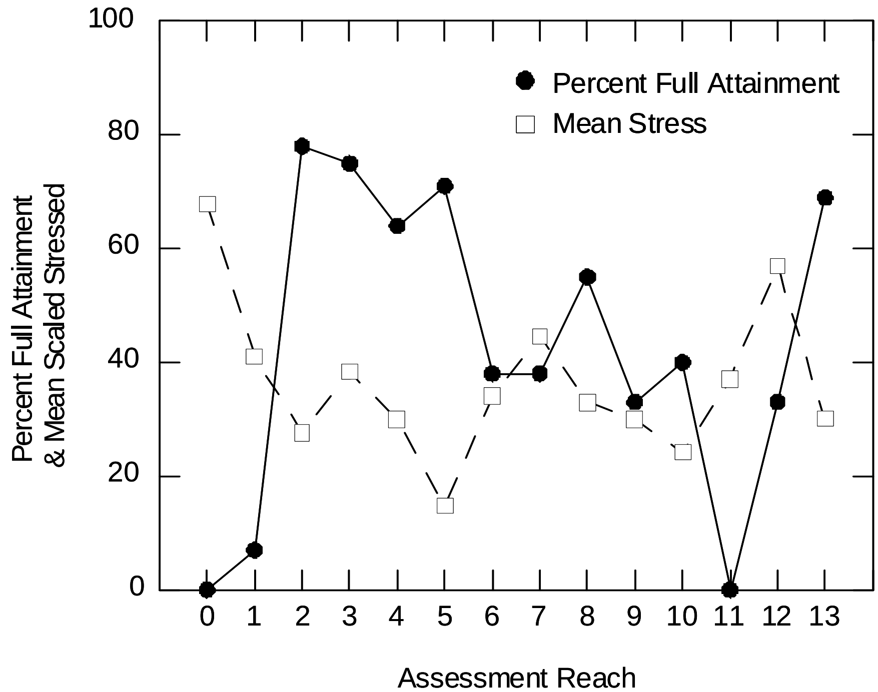


Figure 3. Percent full attainment by assessment reach in relation to the within reach mean stress index. The stress index was rescaled to 0-100 prior to averaging.

Table 3. Miles of attainment and non-attainment of aquatic life use for the Upper Mississippi River apportioned by state. Based on EMAP-GRE weighted probability design.

State	GRE River Miles[1]	Length	Full	Non
MN	870.5-695.2	175.3	69.8	105.50
WI	834.8-598.8	236.0	135.8	100.24
IA	695.2-372.0	323.2	166.8	156.44
IL	598.8-0.0	598.8	269.9	328.91
MO	372.0-0.0	372.0	169.1	202.91

[1] EMAP-GRE river miles – these are different from the ACOE RM system.

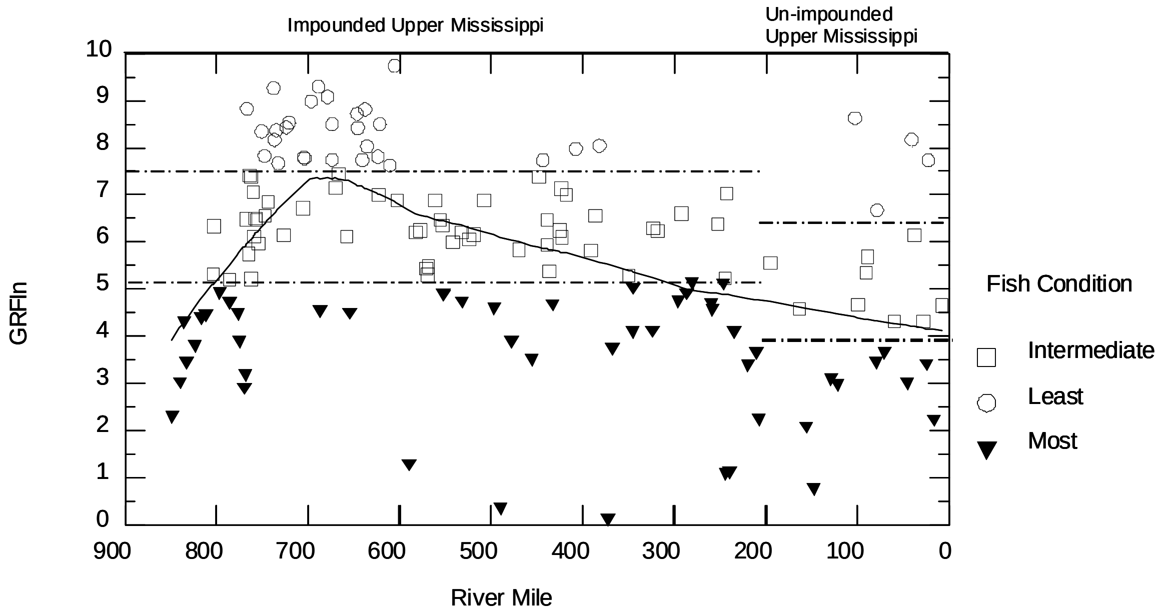


Figure 4. GRFIn scores plotted by EMAP-GRE river mile for the Upper Mississippi River mainstem. Condition class thresholds of Angradi et al. (2009a) are demarcated by horizontal stippled lines. The line tracking the central tendency of the data points is from LOESS (q=0.5).

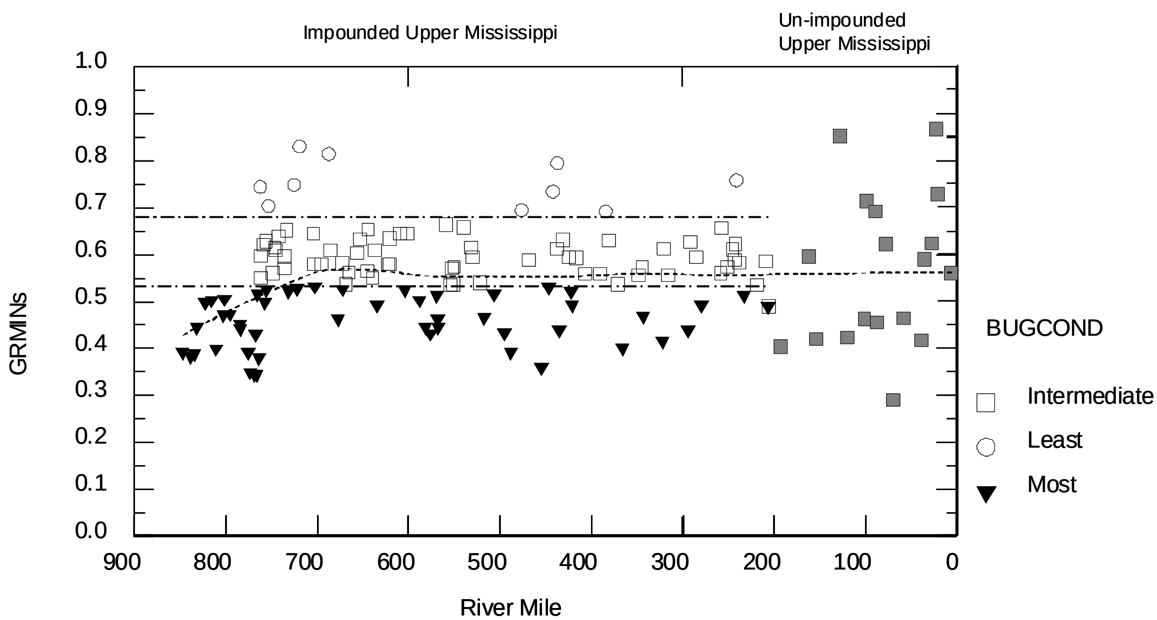


Figure 5. GRMIn scores plotted by EMAP-GRE river mile for the Upper Mississippi River mainstem. Condition class thresholds of Angradi et al. (2009b) are demarcated by horizontal stippled lines. The line tracking the central tendency of the data points is from LOESS (q=0.5). Note that condition classes for the open river reach have not been defined.

Table 4. Classification results of discriminant analyses based on fish and macroinvertebrate condition classes. The linear combination of stressor variables forming the discriminant function is shown above the respective classification matrices. Rows represent the actual classification (e.g., 27 sites were identified as least impacted based on fish); columns represent predicted classification.

	Predicted Least	Predicted Intermediate	Predicted Most	%correct
Fish: Channel Complexity + Substrate + Upstream Dam Distance + Total Nitrogen				
Actual Least	22	3	2	81
Actual Intermediate	12	30	10	58
Actual Most	4	4	28	78
Macroinvertebrates: Stress Index + Conductivity				
Actual Least	2	6	2	20
Actual Intermediate	16	36	8	60
Actual Most	14	7	23	52

Table 5. Linear regression models selected by stepwise regression (forward selection).

Effect	Coefficient	StdError	StdCoef	Tolerance	t	P(2Tail)
Fish R2 = 0.501, N=144						
Constant	22.11297	4.55552	0.00000	.	4.85410	0.00000
Chan. Complexity	2.03299	0.54307	0.27673	0.79436	3.74353	0.00029
Substrate	2.01372	0.58621	0.24929	0.82428	3.43516	0.00084
HQ Vegetation	0.14562	0.06011	0.16791	0.90377	2.42273	0.01705
Population Density	-1.27389	0.33811	-0.26766	0.86014	-3.76768	0.00027
Upstream Dam	-0.64026	0.31679	-0.13774	0.93461	-2.02110	0.04572
Total Nitrogen	-2.29553	0.72810	-0.22420	0.85841	-3.15277	0.00209
Macroinvertebrates[1] R2 = 0.297, N=122						
Constant	3.02658	0.91149	0.00000	.	3.32046	0.00122
Stress Index	-0.25284	0.05272	-0.42285	0.78622	-4.79570	0.00001
Substrate	0.07189	0.03340	0.17538	0.92061	2.15236	0.03353
Conductivity	-0.34487	0.16275	-0.17503	0.89589	-2.11902	0.03632
Total Phosphorus	-0.09375	0.03781	-0.20173	0.92334	-2.47937	0.01466

[1]Data from the open river reaches were excluded from these analyses.

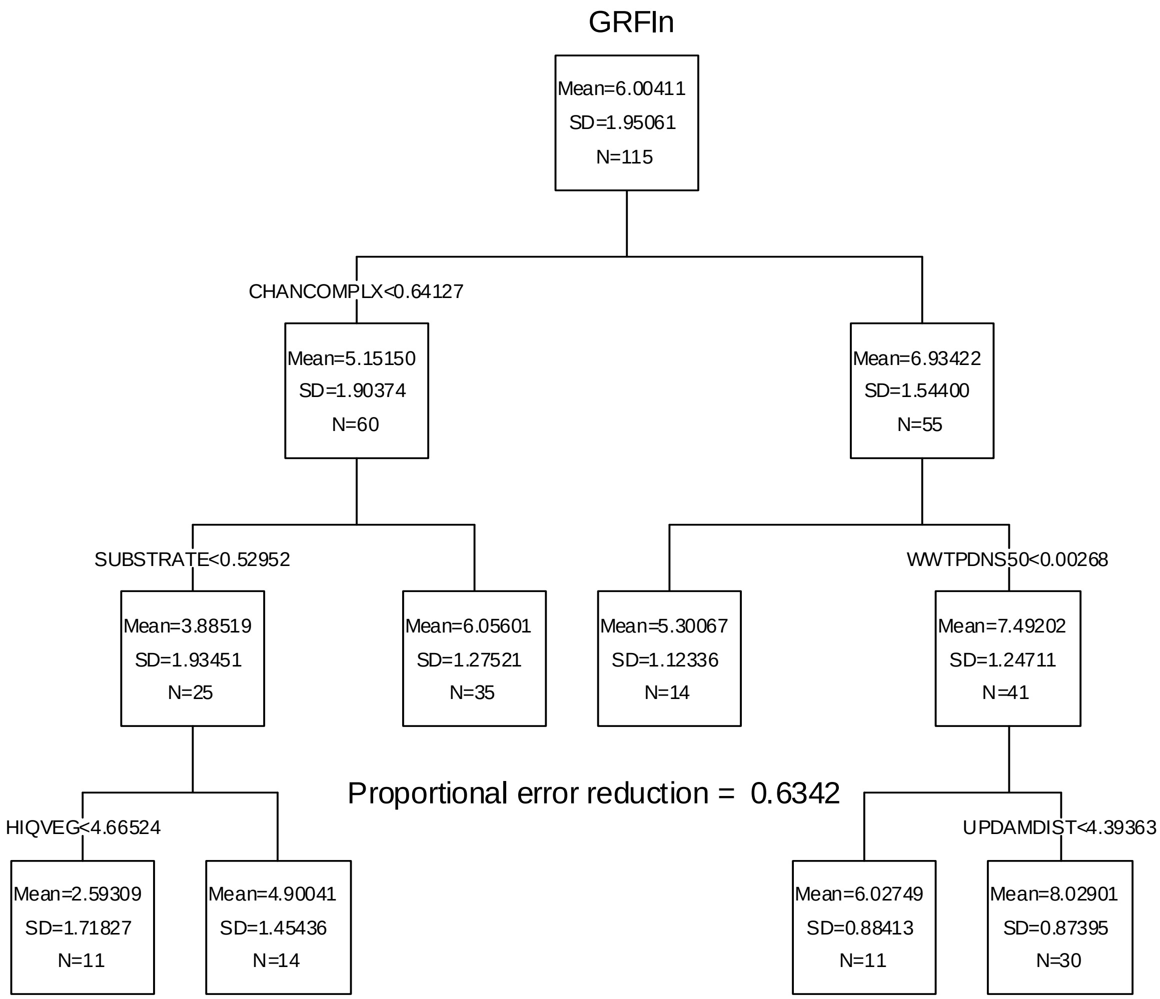


Figure 6. A regression tree for GRFIn scores in the impounded UMR. The mean GRFIn score listed at the top of each box is the mean after portioning by the variable forming the split. For example, channel complexity scores form the first split, and divide GRFIn scores into two groups with means of 5.15150 and 6.93422. Standard deviation (SD) of the mean and the number of scores (N) falling into the box are noted. The proportional reduction in error is the amount of variance reduced by allowing the data to be partitioned as shown.[4]

[4] Quick analogy by obvious example: If you took a random selection of college students and recorded their times in the 100m dash along with those for members of the track team, hours spent training would be a predictor variable that would separate the times into two relatively homogeneous groups based on variance – the randomly chosen college students would have much more variable times than the track athletes. So, although the mean times may be different, as one might expect, CART forms partitions based on partitioning variance.

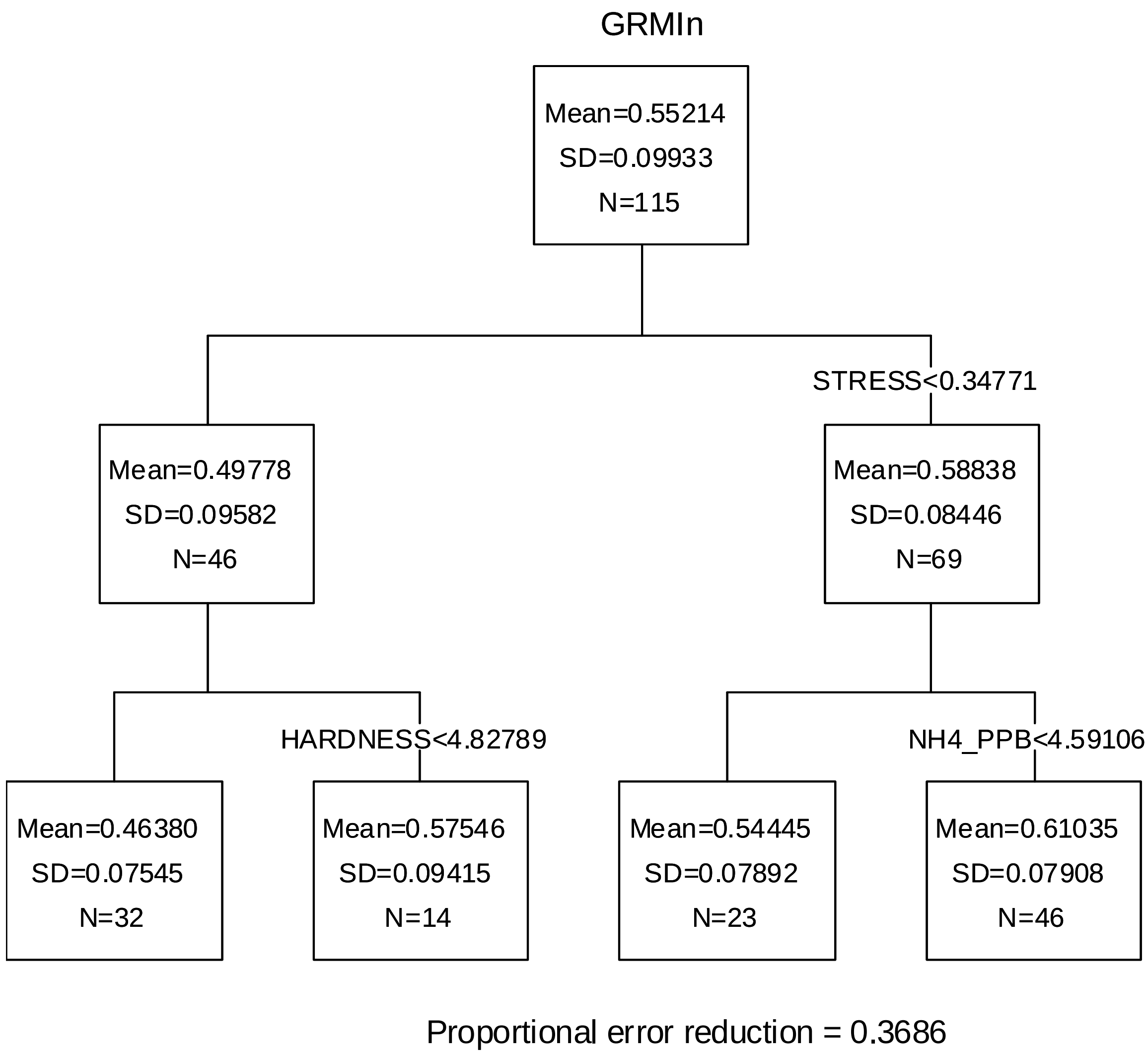


Figure 7. A regression tree for GRMIn scores from the impounded UMR. The mean GRMIn score listed at the top of each box is the mean after portioning by the variable forming the split. For example, the stress index formed the first split, and divided GRMIn scores into two groups with means of 0.49778 and 0.58838. Standard deviation (SD) of the mean and the number of scores (N) falling into the box are noted. The proportional reduction in error is the amount of variance reduced by allowing the data to be partitioned as shown. Note that chemical concentrations are given as log10 of µg/l.

Table 6: Summary of the number of habitat and water quality variables by assessment reach that were identified by statistical analyses as being potential causes of impairment. For distributions of the listed parameters by reach, see Appendix 2.

	Habitat Indicators			Water Quality Indicators					
Reach	Channel	Substrate	Vegetation	Sp.Cond.	TP	TN	NH4	TDS	Total
0	0	1	1	3	1	3	2	3	14
1	0	1	0	1	2	0	1	1	6
2	0	0	3	0	3	0	1	0	7
3	0	0	0	0	0	0	0	0	0
4	0	1	2	0	0	0	2	0	5
5	1	1	0	0	0	0	0	0	2
6	1	2	1	0	0	0	0	0	4
7	2	1	2	0	0	0	0	0	5
8	0	2	0	0	0	0	0	3	5
9	1	0	1	0	0	1	1	2	6
10	6	3	1	0	1	0	0	1	12
11	1	0	1	0	0	3	0	3	8
12	0	0	0	3	1	0	1	0	5
13	0	0	0	4	7	0	0	0	11

Submersed Aquatic Vegetation

When paired against condition assessments obtained from NMACI and GRMIn scores set against their respective quadrisection thresholds, the SMI generally agreed with the macroinvertebrate indicator on condition status, but where the two macroinvertebrate indicators disagreed, the SMI tended to indicate attainment. The NMACI and SMI differed in 12 of 32 cases, of which the SMI indicated attainment in 8. In these 8 cases where the GRMIn and SMI differed, 6 of the sites were considered in attainment by the SMI. Paired with GRFIn (again, based on quadrisection of the UMR for thresholds), the SMI agreed more frequently, but where it differed, the direction was always on the side of impairment (FACI, 6 cases; GRFIn 4 cases). At the reach level, the SMI override of the macroinvertebrate indicator did not result in an overall change in status for Reach 0 and 1, but suggested that impairment would be less extensive in reaches 2-5 (Table 8).

Table 7. Percent non-attainment given various index combinations and scoring thresholds (values in parentheses show percentile rank of the given index score from the UMR).

			GRE Indices GRMIn and GRFIn		REMAP Indices NMACI and FACI					
			Most-Intermediate Disturbed Threshold	UMR Quadri-section[1]	NMACI & FACI quadrisection Peer Rivers	NMACI – UMR quadrisectio; FACI – quadrisection Peer Rivers	NMACI & FACI UMR quadrisection	NMACI & FACI (@1 km) UMR quadrisection	NMACI & FACI 25th% REMAP Reference	NMACI – UMR quadrisection; FACI – change point on stressor index
REACH	**Number of Sites**	**Length (miles)**	**%Non-attainment Results**							
0	6	58.5	100.0	100.0	83.3	66.7	83.3	83.3	100.0	83.3
1	14	49	92.9	92.9	85.7	42.9	57.1	57.1	100.0	42.9
2	18	49	22.2	38.9	83.3	50.0	50.0	50.0	100.0	50.0
3	4	20	25.0	25.0	75.0	25.0	25.0	25.0	100.0	25.0
4	14	63	35.7	64.3	78.6	35.7	50.0	50.0	100.0	42.9
5	7	48	28.6	28.6	42.9	28.6	42.9	42.9	85.7	42.9
6	13	60	61.5	69.2	61.5	46.2	84.6	69.2	100.0	53.8
7	13	89	61.5	69.2	84.6	61.5	84.6	84.6	100.0	76.9
8	11	73	45.5	63.6	63.6	36.4	72.7	72.7	90.9	54.5
9	3	36	66.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0
10	15	88	60.0	73.3	86.7	66.7	86.7	86.7	100.0	80.0
11	4	41	100.0	100.0	100.0	75.0	100.0	100.0	100.0	100.0
12	6	78	83.3	100.0	100.0	83.3	100.0	100.0	100.0	100.0
13	16	118	56.3	81.3	93.8	81.3	100.0	100.0	100.0	100.0
All	144	870.5	**56.3**	**69.4**	**80.6**	**54.9**	**72.9**	**71.5**	**98.6**	**65.3**
Indicator Thresholds										
Macroinvertebrates			53(41)	56(53)	47(79)	40(50)	40(50)	40(50)	57(98)	40(50)
Fish			52(38)	55(45)	38(16)	38(16)	51(54)	55(53)	48(46)	45(36)
% Agreement on Condition			64.8	69.7	34.7	55.6	56.9	72.2	46.5	77.8

[1] Thresholds derived from quadrisection of GRFIn and GRMIn scores for the entire UMR (IR and OR).

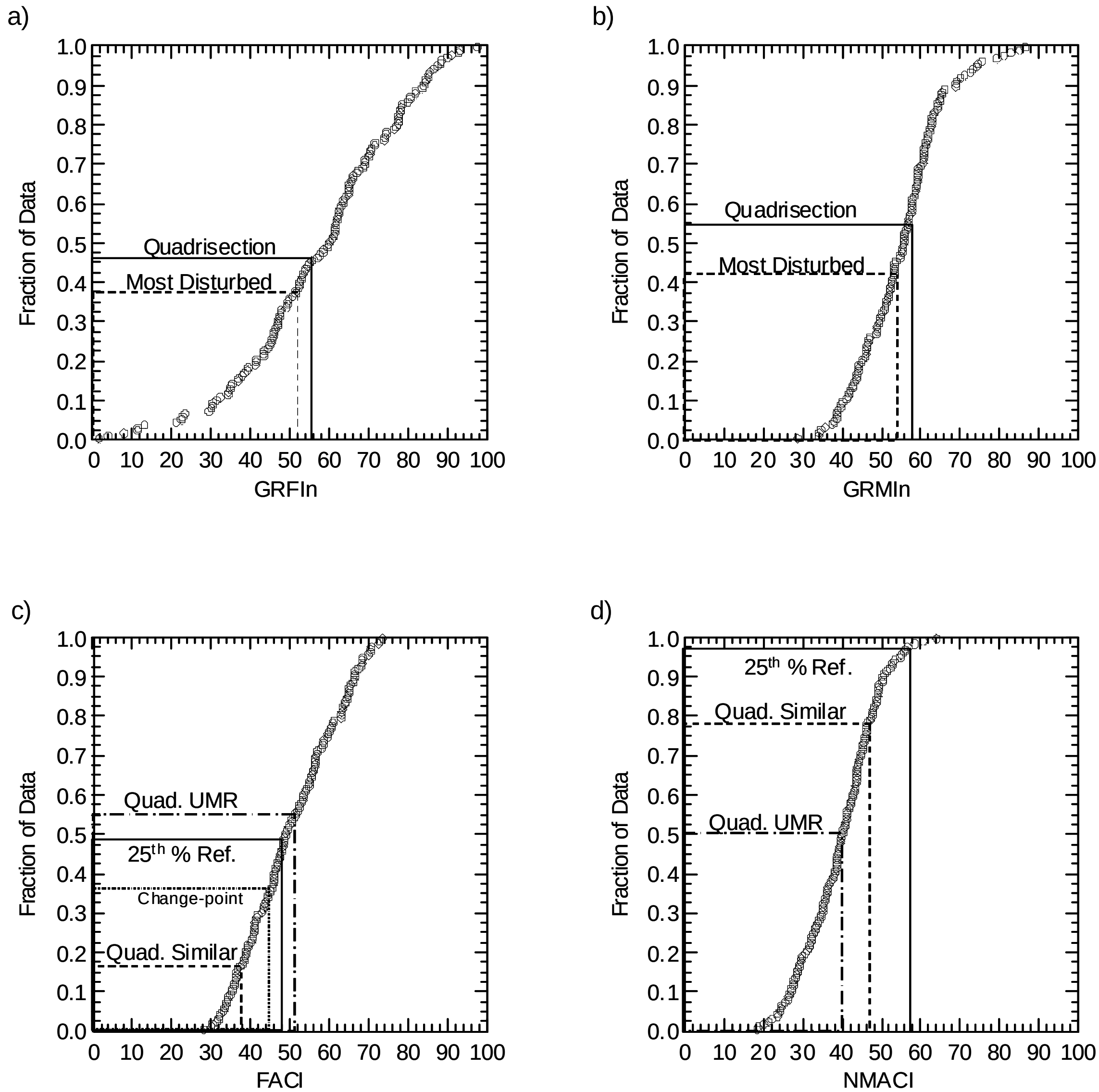


Figure 9. Graphical depiction of various thresholds discussed in the text for the a) GRFIn, b) GRMIn, c) FACI, and d) NMACI.

Table 8. Comparison of assessment statistics using Submerged Vegetation Index (SMI) scores to override the macroinvertebrate result in cases where both the SMI and fish index agree on attainment, but the macroinvertebrate index suggests impairment. Assessments based on the dual fish and macroinvertebrate indicators are done in the usual manner where non-attainment is indicated if either indicator fails their respective threshold. Assessment statistics are based only on sites that had SMI scores. Note that vegetation for the SMI was sampled 2006—2008 and also in selected reaches in 2009 and 2010. Fish and Macroinvertebrates were sampled 2006-2009 for EMAP-GRE.

	Number of Sites		**Assessment Based on GRFIn and GRMIn (Quadrisection Threshold)**		**Assessment Based on GRFIn and SMI (Quadri-section of UMR)**	**Assessment Based on FACI and NMACI (Quadrisection Threshold)**	
Reach	Fish and Bugs	Plants	Percent Non-attainment	Percent Non-attainment with SMI Override of GRMIn	Percent Non-attainment*	Percent Non-attainment	Percent Non-attainment with SMI Override of NMACI
0	6	6	100.0	100.0	100	83.3	83.3
1	14	3	100.0	100.0	100	66.7	66.7
2	18	11	36.4	0.0	0	54.5	0.0
3	4	4	25.0	0.0	25	25.0	25.0
4	14	3	66.7	33.3	33.3	66.7	33.3
5	7	5	20.0	20.0	40	40.0	20.0

*Statistics based on paired samples of fish and plants

Sensitivities

The fish indices showed greater sensitivity to measured environmental variables compared to the macroinvertebrate indices, as suggested by the correlation matrix in Table 9. The GRFIn and FACI responded similarly to stressors, with the FACI being most sensitive to measures of suspended sediment (TSS, Secchi, and turbidity) and the channel complexity score. Similarly, the SMI was sensitive to measures of transparency and channel complexity, and proximity to wastewater treatment facilities (negatively so). GRFIn scores were more strongly associated with channel complexity than they were with any of the other environmental variables. GRMIn scores tracked the stressor index, and the NMACI was lacking association with most of the environmental variables, and only weakly so for turbidity, TSS, and near-shore variation in depth.

Although the NMACI did not show a strong linear response to any of the environmental variables, partitioning of NMACI scores in the nonparametric CART model resulted in ~ 25 percent reduction in variance (Table 10). A plot of the NMACI over littoral depth variation (Figure 10a) shows that scores tended to be slightly higher when there was less variation in

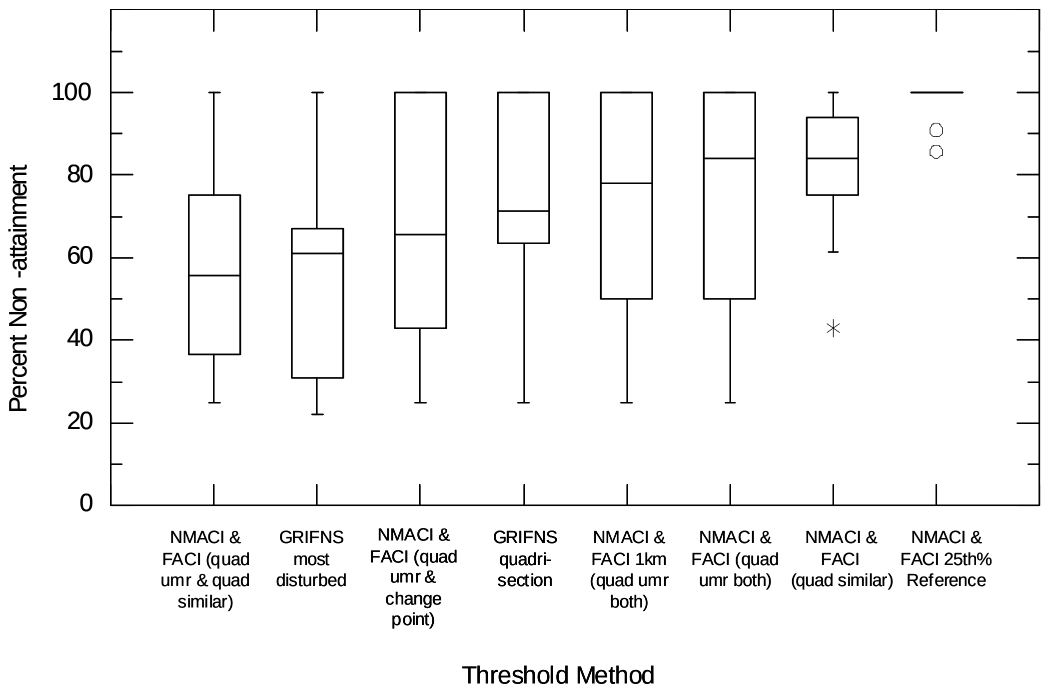


Figure 8. Graphical representation of Table 7 showing the distributions of percentages classed in non-attainment by threshold method.

depth. The hydrology index partitioned significant amounts of variance in the FACI scores. The relationship between the FACI and hydrology index appeared to have a distinct threshold (Figure 11b), with no linear tendency between the two at the lower half of the hydrology index. The hydrology index tended to score the highest in Reaches 2-5, along with the top carnivore, round-bodied sucker, darter, and sensitive species components of the FACI. The GRFIn tracked channel complexity in a more continuous, linear fashion (Figure 11c). Detritivores and exotic species were the GRFIn components becoming more abundant with an increasingly uniform channel, and darters being more frequently encountered where the channel was more complex. GRMIn again followed the stressor index (Figure 11d), with tolerant taxa increasing with increasing levels of stress. Note that indicators of wastewater loadings also partitioned variance in the GRMIn scores, as did proximity to treatment plants for GRFIn scores. Overall, more variation in both of the fish index scores was accounted for in the CART models compared to the macroinvertebrate indices and attributes.

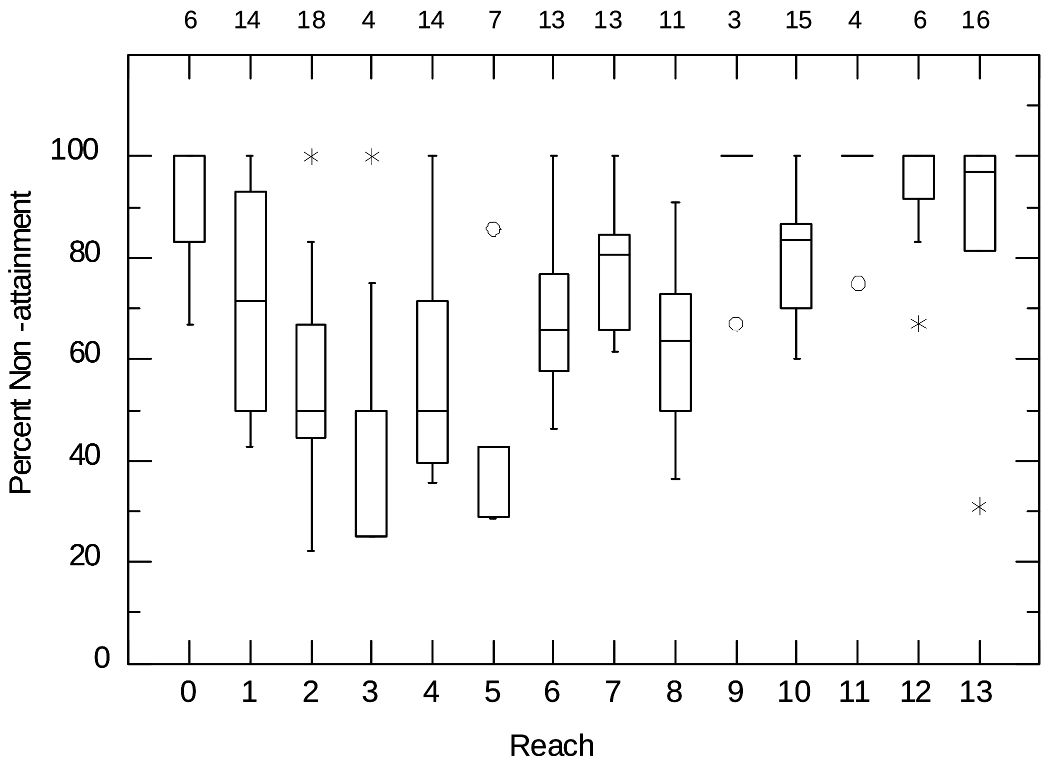


Figure 10. Distributions of percent non-attainment generated by the various threshold combinations plotted by reach. The number of sampling sites within each reach is indicated at the top of the graph.

Table 9. Spearman correlations between condition indices and environmental variables. Data are from the UMR upper impounded reach and the open river. Shaded cells have an absolute value of Spearman rho ≥ 0.3. Acronyms are found in Appendix Table 3.

Environmental Variable	NMACI	FACI	FACI1000	GRFIn	GRMIn	SMI	Ad Hoc
STRESS	-0.06034	-0.10479	-0.12094	-0.38191	-0.42716	-0.37874	-0.11612
POPDENS	-0.10183	-0.22194	-0.24023	-0.39952	-0.33994	-0.4205	-0.19687
WWTPDNS100	-0.06104	-0.32857	-0.29845	-0.33542	-0.31859	-0.45077	-0.28548
ANC	-0.04002	-0.23666	-0.26142	-0.2781	-0.30519	-0.27746	-0.20283
WWTPDNS50	-0.14158	-0.26867	-0.26131	-0.3196	-0.28669	-0.48249	-0.25035
HARDNESS	-0.05311	-0.31753	-0.33046	-0.33821	-0.28051	-0.19562	-0.22802
TOC_PPM	-0.01382	0.36183	0.32882	-0.00074	-0.23798	-0.18161	0.31998
SOBC	-0.11125	-0.42167	-0.41747	-0.3137	-0.22337	-0.18633	-0.4122
SI_PPM	0.03181	0.40235	0.39201	0.05239	-0.20738	-0.17214	0.27033
COND	-0.12267	-0.37129	-0.36379	-0.30699	-0.20105	-0.06434	-0.39441
ANSUM	0.0648	-0.39287	-0.38203	-0.38669	-0.19939	-0.23721	-0.21921
NTL_PPB	0.05386	-0.32412	-0.31407	-0.35794	-0.198	-0.31824	-0.12208
TDS	0.08229	-0.34589	-0.33665	-0.36624	-0.19651	-0.24876	-0.16388
NO3_PPB	0.08856	-0.32308	-0.31427	-0.35841	-0.18939	-0.2618	-0.13637
SO4_PPM	-0.12217	-0.30848	-0.31653	-0.26964	-0.18605	-0.11109	-0.42057
NH4_PPB	0.11023	-0.08647	-0.05042	-0.11342	-0.15617	-0.2767	-0.06242
WWTP10C400	-0.1507	-0.05004	-0.05544	-0.13187	-0.12464	-0.22813	-0.14244
LMXDEPTH	0.15653	0.19217	0.17645	-0.00834	-0.11486	-0.18449	-0.01505
LMXSLOPE	0.11675	0.09488	0.09393	-0.01797	-0.113	-0.18933	-0.102
PHEOA	-0.09701	-0.45199	-0.42981	-0.37544	-0.11225	-0.45605	-0.39961
TEMPC	-0.0049	-0.4389	-0.46115	-0.32617	-0.0635	-0.17214	-0.27735
TURBIDITY	-0.20119	-0.52527	-0.51902	-0.34399	-0.0574	-0.58717	-0.4965
TSS_MGL	-0.20485	-0.57749	-0.57399	-0.35126	-0.04696	-0.57984	-0.50525
CHLA	0.03699	-0.0291	-0.0318	-0.10274	-0.02098	-0.12191	0.01713
HYDROLOGY_SC	0.19648	0.35207	0.31889	0.2696	-0.00559	0.44253	0.28766
POC_MGL	-0.07181	-0.26729	-0.24773	-0.23495	0.00204	-0.42428	-0.23336
PH	0.04305	0.1848	0.18072	0.10259	0.03248	0.10452	0.17708
PON_MGL	-0.05924	-0.20241	-0.18412	-0.17737	0.03734	-0.36551	-0.13906
TOTAL_METALS	-0.11124	-0.15206	-0.13949	-0.13979	0.04786	-0.34103	-0.31962
PTL_PPB	-0.1054	-0.11031	-0.10007	0.0552	0.05746	0.35063	-0.21211
CL_PPM	-0.05357	-0.19608	-0.14954	-0.04238	0.06517	0.02255	-0.33287
ORTHOP_PPB	-0.03192	0.03964	0.03912	0.17072	0.11328	0.48877	-0.02368
SECCHI	0.20286	0.46216	0.46867	0.31511	0.12075	0.57127	0.44937
LMCVDEPTH	-0.24451	-0.19412	-0.1608	0.12268	0.15653	0.00807	-0.2043
DO	0.11434	0.11641	0.1052	0.13913	0.17282	0.1773	0.26187
LMCVSLOPE	-0.05182	0.11708	0.10881	0.08254	0.19217	0.074	-0.00852
HIQVEG	0.13267	0.17034	0.19627	0.29057	0.20467	-0.21265	0.15906
XWIDRATIO	-0.10123	-0.09709	-0.10203	0.1474	0.21348	0.20972	-0.16589
CHANCOMPLX	0.10086	0.50404	0.53533	0.48847	0.2463	0.57002	0.41084
SUBSTRATE	-0.16872	0.04568	0.02042	0.3897	0.24713	0.33118	0.09345
Number of Variables with Spearman Rho ≥ 0.3 and median Rho	0 0.10145	18 0.251975	17 0.25452	18 0.27387	4 0.179435	17 0.25528	11 0.21566

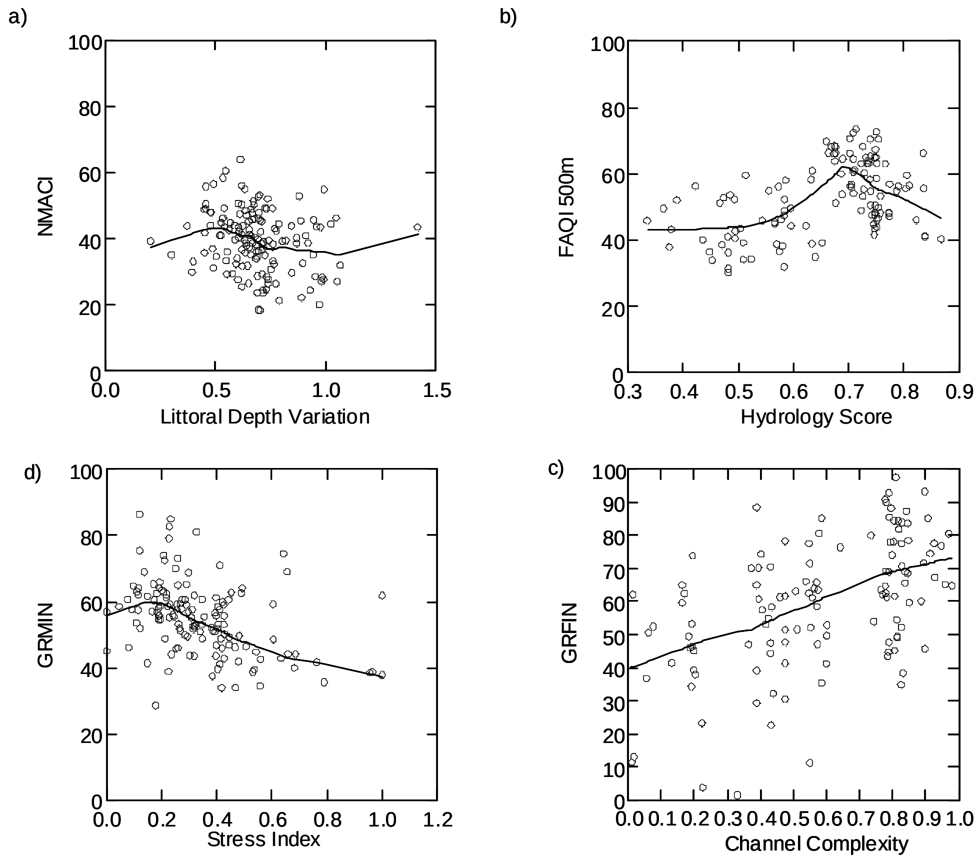


Figure 11. Plots of index scores against environmental variables forming first node in classification trees: a) NMACI vs. coefficient of variation in littoral zone depth; b) FACI vs. Hydrology Score; c) GRFIn vs. Channel Complexity; d) GRMIn vs. Stress Index. The plot of 1 km length FACI scores against Hydrology Scores was omitted as it was redundant. Lines fitted to the data points are from LOWESS smoothing (q=0.5).

Table 10. Environmental variables forming nodes in classification and regression trees. The proportional reduction in variance accounted by a node is listed under the Improvement column.

Split	Variable	Improvement
NMACI		
1	LMCVDEPTH	0.08645
2	TSS_MGL	0.09914
3	TOC_PPM	0.07320
FAQI1km		
1	HYDROLOGY_SC	0.31016
2	TEMPC	0.12698
3	SI_PPM	0.06734
4	LMXDEPTH	0.06481
FAQI500		
1	HYDROLOGY_SC	0.35071
2	LMXDEPTH	0.06594
3	HYDROLOGY_SC	0.11673
4	LMXSLOPE	0.05252
GRFIn		
1	CHANCOMPLX	0.21595
2	WWTPDNS50	0.10789
3	SUBSTRATE	0.12919
4	HIQVEG	0.06719
GRMIn		
1	STRESS	0.22582
2	COND	0.08965
3	CL_PPM	0.0947
4	NH4_PPB	0.05166

Discussion

Condition Assessment Based on the GRFIn and GRMIn

GRFIn and GRMIn Comparison

The rationale for a dual indicator approach is that it enhances the redundancy of the resulting bioassessment because individual assemblages have different temporal responses and sensitivities to and within various stressors existing along a disturbance gradient. Hence, the accuracy of the overall bioassessment is improved with two assemblages. However, the dual assemblage approach is equally important for determining proximate causes of non-attainment. As such we evaluated the sensitivities of the two primary assemblage indicators, fish and macroinvertebrates, to environmental stressors in the UMR main channel. Optimally, indicators based on different assemblages will each track the overall stressor gradient in a generally similar manner, but will show differing sensitivities to individual stressors along the disturbance gradient.

The longitudinal plots of the GRFIn and GRMIn scores by river mile for the UMR (Figures 5 and 6) suggest that both were tracking the overall stressor gradient, and, indeed, both the GRFIn and the GRMIn were correlated with the general stressor index for the upper impounded UMR (Spearman rho = -0.44 and -0.50, respectively). As for sensitivities to individual stressors, the GRFIn appeared to be more sensitive to habitat and total nitrogen while the GRMIn appeared more sensitive to ammonia and conductivity (Table 5). Although the variation in GRFIn scores was generally better explained by combinations of the individual stressors that, in itself, does not necessarily suggest that the GRFIn is better calibrated than the GRMIn. Rather it may demonstrate the value of the dual indicator approach because the GRFIn scores were clearly tracking habitat quality, especially channel complexity (Figure 12), which would have been missed or perhaps underrated by using the GRMIn alone. While habitat quality is clearly important for macroinvertebrate assemblages, fish assemblages, as an indicator, are more likely to integrate reach-level habitat quality simply owing to the greater breadth of niches inhabited by fish as well as habitat scale issues in the responses of fish and macroinvertebrates.

Sequential Interpretation of Index Scores along Linear Reaches

The sampling locations for the UMR EMAP-GRE survey were based on a weighted probability sample. Probabilistic sampling presumably offers the advantage of providing population estimates of known precision for less effort than a complete census. The goal of most aquatic resource monitoring programs, however, goes well beyond the need of a simple population estimate of condition and general status. Point sources and other localized stressors clearly are not uniformly distributed across the land and riverscape, and are best managed with information drawn from sufficiently designed targeted sampling. Although the EMAP-GRE samples were not drawn to elucidate longitudinal patterns consistent with pollution gradients in relation to point sources or other known stressors, the samples were weighted to provide representative spatial coverage by mostly avoiding large gaps between sampling points, thereby affording a spatial frame of reference for where impairment is most severe (i.e., thus

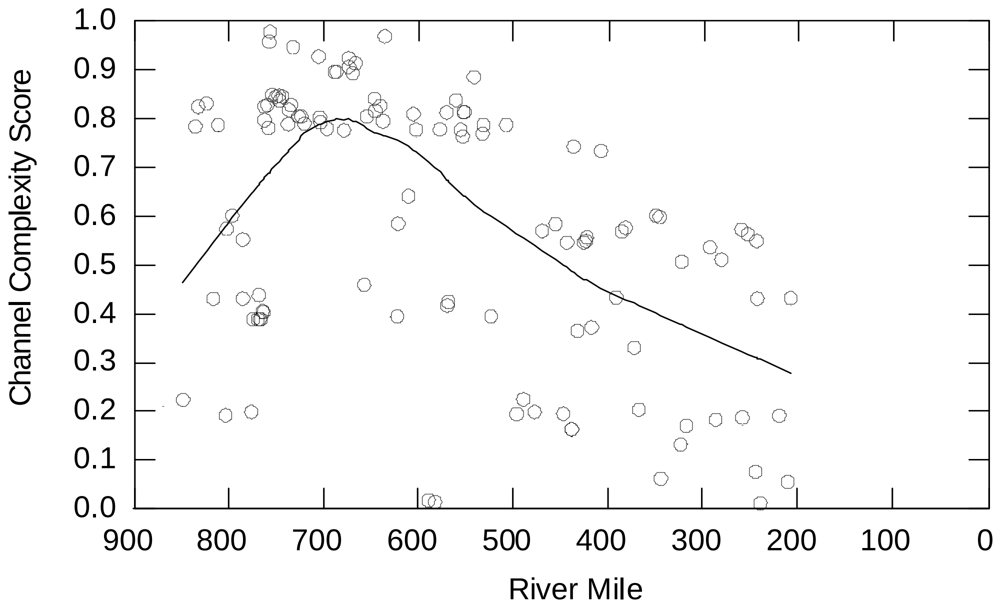


Figure 12. Channel complexity scores for the impounded UMR. The line drawn through the data points is derived from a locally weighted least squares regression (LOWESS, q=0.5). EMAP-GRE rivers miles are used.

detecting and characterizing pollution gradients), and which segments have the best conditions. In short, the spatial sampling density along the UMR main channel was sufficient to emulate that which would have otherwise been produced by a longitudinal "pollution survey" type of sampling design.

One advantage of a sequential linear interpretation of sampling results is the ability to compare the magnitude of departure of the assemblage index scores, either positively or negatively, from an attainment threshold, thus forming a standardized unit relating the "severity" of departures (i.e., the area of a graphed line connecting sampling points either below a threshold) or the degree to which condition is above a threshold. Termed the Area of Degradation Value and the Area of Attainment Value (ADV and AAV; after Yoder et al. 2005), these standardized units can then be compared between reaches, in relation to stressors, or over time to evaluate the quantity and trajectory of changes in response management actions. Figure 13 shows the magnitude of ADV and AAV departures for GRMIn and GRFIn scores summed by assessment reach and in relation to the EMAP-GRE Least and Intermediate disturbance thresholds. Readily apparent is the relatively low amount of impairment suggested by the macroinvertebrate indicator when the Intermediate-Most disturbance boundary is used as the attainment threshold (on a single assemblage basis). Conversely, setting the Least-Intermediate boundary as threshold has both indicators suggests more widespread impairment. Also note that Reach 7 is the most degraded on a per unit basis, despite the visual impression suggested by Figures 4 and 5 of the non-interstate reach (i.e., Reach 0) and Assessment Reach 1 as being the most degraded in terms of miles.

Lastly, a linear interpretation of impairment can yield a reasonable population estimate of impairment, given a spatially robust enough sampling effort. Tables 11 and 12 compare miles of attainment based on a linear interpolation of GRFIn and GRMIn site scores to the proportional estimates given by the weighted probability sample. The estimates track each other remarkably well, obviously owing to the weighting used in the probability draw. However, a well-designed targeted effort would, by default, have a similar spatial representation along a reach and would have filled some gaps in reaches with comparatively few sites (i.e., ≤3 sites).

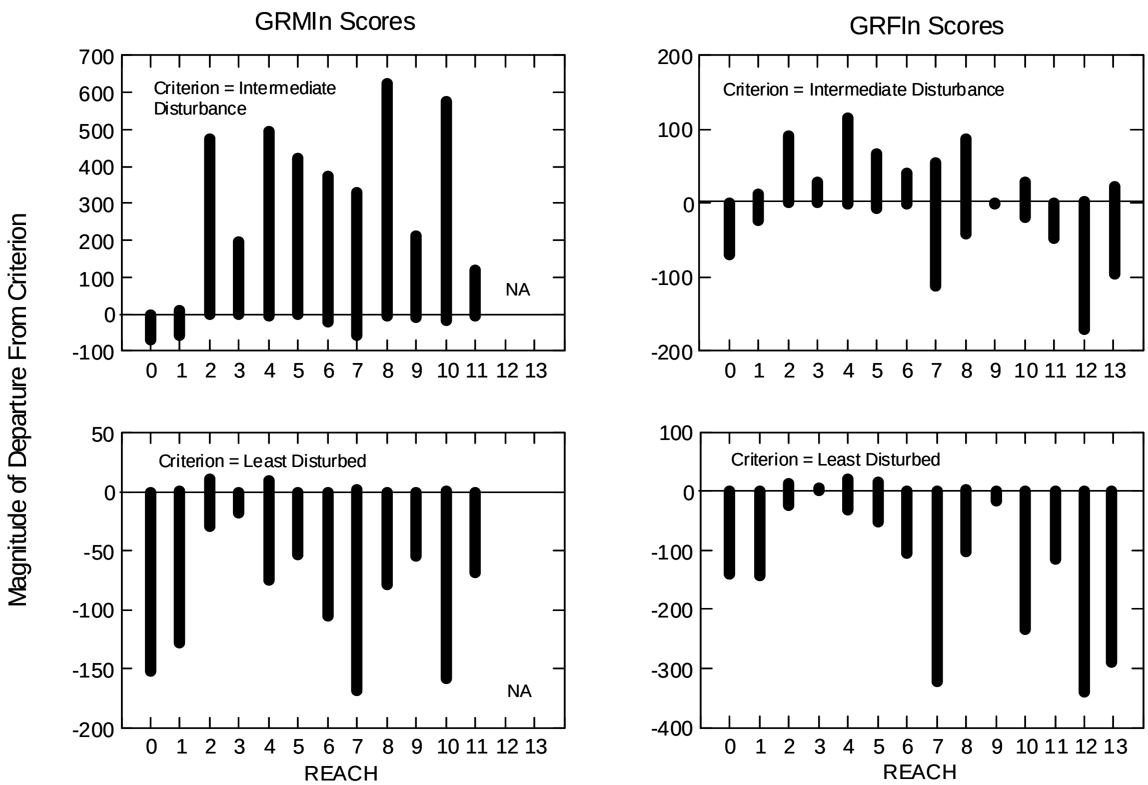


Figure 13. Magnitude of departure from disturbance thresholds for GRMIn (left panels) and GRFIn (right panels) scores grouped by assessment reaches. The magnitude of departure is calculated based a summing the area above (positive) or below (negative) a given threshold formed by a longitudinal plot of data points by river mile. The area above the threshold value (Area of Attainment Value) can be compared to the corresponding area below the value (Area of Degradation Value) for visual assessment of the magnitude of impairment for a given reach, and can be used to compare difference between reaches, and differences over time (e.g., to track restoration effectiveness).

Table 11. Miles of impairment based on linear interpolation of GRFIn site scores, and the corresponding proportional estimates from the probability sample for two disturbance thresholds. Miles are based on measured miles used by USEPA GRE.

Reach	Length	Miles Impaired Based on Disturbance Thresholds			
		Intermediate-Most		Least-Intermediate	
		Proportional Estimate	Linear Interpolation	Proportional Estimate	Linear Interpolation
0 Non-interstate UMR	58.5	58.5	58.5	58.5	58.5
1 St. Croix River to Chippewa River	49	24.5	37.4	45.5	48.7
2 Chippewa River to Lock and Dam 6	49	0.0	0.0	27.2	25.1
3 Lock and Dam 6 to Root River	20	0.0	0.0	5.0	9.8
4 Root River to Wisconsin River	63	9.0	3.9	22.5	29.5
5 Wisconsin River to Lock and Dam 11	48	6.9	12.0	20.6	18.3
6 Lock and Dam 11 to Lock and Dam 13	60	13.8	7.2	60.0	60.0
7 Lock and Dam 13 to Iowa River	89	27.4	41.5	82.2	91.5
8 Iowa River to Des Moines River	73	19.9	18.2	59.7	61.5
9 Des Moines River to Lock and Dam 21	36	24.0	18.9	36.0	36.0
10 Lock and Dam 21 to Cuivre River	88	52.8	57.2	88.0	101.0
11 Cuivre River to Missouri River	41	41.0	41.0	41.0	41.0
12 Missouri River to Kaskaskia River	78	52.0	43.3	78.0	81.0
13 Kaskaskia River to Ohio River	118	36.9	22.3	88.5	93.7
Total	870.5	302.3	361.4	677.1	755.6

Table 12. Miles of impairment based on linear interpolation of GRMIn site scores, and the corresponding proportional estimates from the probability sample for two disturbance thresholds. Miles are based on measured miles used by USEPA GRE.

Reach	Length	Miles Impaired Based on Disturbance Thresholds			
		Intermediate-Most		Least-Intermediate	
		Proportional Estimate	Linear Interpolation	Proportional Estimate	Linear Interpolation
0 Non-interstate UMR	58.5	58.5	58.5	58.5	58.5
1 St. Croix River to Chippewa River	49	45.5	48.9	45.5	49.4
2 Chippewa River to Lock and Dam 6	49	10.9	1.2	40.8	37.2
3 Lock and Dam 6 to Root River	20	5.0	0.1	20.0	20.0
4 Root River to Wisconsin River	63	22.5	13.2	58.5	55.5
5 Wisconsin River to Lock and Dam 11	48	13.7	5.4	48.0	48.0
6 Lock and Dam 11 to Lock and Dam 13	60	36.9	22.5	60.0	60.0
7 Lock and Dam 13 to Iowa River	89	54.8	61.9	68.5	87.9
8 Iowa River to Des Moines River	73	33.2	10.4	66.4	70.8
9 Des Moines River to Lock and Dam 21	36	24.0	14.5	36.0	36.0
10 Lock and Dam 21 to Cuivre River	88	52.8	43.1	82.1	98.2
11 Cuivre River to Missouri River	41	41.0	28.7	41.0	41.0
Total	674.5	398.8	308.4	619.2	662.5

Condition Assessments using Alternate Indices and Thresholds

Index Comparisons

In five of the eight threshold scenarios (Table 7), the macroinvertebrate indicator defined more sites impaired compared to the fish indicator. Clearly this is a trivial truth given that threshold scores for the GRMIn and NMACI as a percentile of their range in the UMR were at the median or higher except for the most-intermediate disturbance threshold for the GRMIn. It is also likely an artifact of the comparatively narrow range of response in the current macroinvertebrate indicators. This begs the question of whether the narrow range of response is itself an artifact of index development and calibration, or that the main channel border littoral habitat as measured by EMAP-GRE has either a narrow range of condition for macroinvertebrates, highly uniform habitat with respect to macroinvertebrates, or a combination of the two. The range of response in both fish indices along the length of the UMR, and the fact that they exhibited sharper responses to individual stressors, taken as a whole, suggests that there is a range of condition in the UMR, and that the macroinvertebrate indices are less responsive by comparison. The low frequency of association between measured environmental variables and the macroinvertebrate indices also suggests a lack of sensitivity (Table 9), especially for the NMACI. The SMI, in contrast, was associated with at least 17 of the measured environmental variables, and therefore represents a viable alternative to the present macroinvertebrate indicators[5].

<u>Limitations of Current Macroinvertebrate Indices</u>: Whether a structural change to one or both of the macroinvertebrate indices would result in greater sensitivity (sensu Jackson et al. 2010) is a major question raised by our analyses. Further analyses show that simple correlations of all possible macroinvertebrate metrics analyzed against measured chemical and habitat variables[6] collected from EMAP-GRE sites throughout the UMR suggests that environmental information relevant to the UMR from the macroinvertebrate assemblage is not necessarily being captured by GRMIn. For example, the frequencies of rank order correlations with a coefficient $\geq$ 0.25 between any of the ~164 candidate GRMIn metrics, and any of the land use, habitat or chemical variables used in the present analyses were higher for many of the individual metrics than for the GRMIn, and higher for individual metrics not included in the GRMIn (Table 13) than for the present GRMIn metrics. These latter metrics are represented by several broad categories, notably amphipods/crustaceans, Asian clams/zebra mussels, mayflies, water mites, non-insects, hydrosychid caddisflies, swimmers, climbers, and pollution tolerant organisms.

<u>*Ad Hoc* Macroinvertebrate Index</u>: An *Ad Hoc* index was created from a subset of the aforementioned metrics along with several of the GRMIn component metrics (Table 14). It shows a similar longitudinal pattern along the length of the UMR compared to the *GRFIn and FACI* (Figure 14). The non-GRMIn metrics in Table 14 were selected to be representative of the broad categories mentioned above, and were selected by inspecting individual correlations with the environmental variables. The four original GRMIn component metrics were retained

[5] The frequency of correlations between the biotic measures and environmental variables, when restricted to Reaches 0-5, were similar to those presented in Table 9.
[6] An ad hoc analysis done post hoc for this Discussion section.

because the Hemipteran metric showed a high frequency of correlation with environmental variables, and the other three metrics trended positively with the stressor index. Scoring for this Ad Hoc macroinvertebrate index was done using discrete scoring based on inspection of quantile plots for data from the UMR (e.g., Figure 15). The point of this exercise was not merely to create an alternate index; it was done simply to help diagnose why the GRMIn (and by extension, the NMACI) was comparatively less responsive to the stressor gradient, and it suggests a possible direction for improving the GRMIn.

In going through this exercise, what was first apparent is that the amphipod, non-insect, and tolerant metrics were counterintuitive to known responses. For example, the tolerant metric (i.e., Tolerant [TOLVAL 7.0-10] % Individuals) was positively associated with channel complexity. Angradi et al. (2010) noted that tolerance values were not available for many UMR taxa, and those that do exist may not necessarily be valid for great rivers. Also, these systems are naturally predominated by organisms generally considered tolerant or facultative (e.g., oligochaetes and chironomids). In this case, an alternate explanation of what the tolerant metric actually represents instead of its usual sense is that it may simply be representing functional biomass. Similarly, amphipods are generally considered an indicator of nutrient enrichment, and they were positively correlated with nitrogen and phosphorus in the UMR. However, they were negatively correlated with turbidity, and well correlated with channel complexity (Spearman rho>0.6). An alternate explanation in this case might be that amphipods represent where nutrients are transferred through the food web. There is little point in further speculation, but clearly a better understanding of the ecological functions of benthic macroinvertebrates in great rivers is needed, and is likely necessary to construct a more robust index. On a related note, the association between NMACI scores and littoral depth variation, wherein the NMACI tended to score higher at sites with less depth variation, might suggest an artifact of sampling efficiency. Not to overplay this association, but, quite simply, shallower, more uniform shorelines are likely easier to sample using the EMAP-GRE method.

Missouri River GRE Scoring for Open Mississippi Reach

GRFIn and GRMIn scores for the lower two reaches (12 & 13) in the Open River were computed using the scoring algorithms from the Lower Missouri River (Angradi et al. 2009a and b), as the two environmental settings are similar. The possible advantage of using the Lower Missouri indices is that the algorithms were derived from a larger number of sites than that for the Mississippi River OR, so it is presumably more robust. Add to that the Missouri River adds substantial flow to the UMR this alternative also has more ecotype relevance. For the GRFIn, the Missouri River scoring did not change the local central tendency (two-sample t-test, $p=0.25$), although the scores based on the Missouri River were less variable than those based on the Mississippi River OR (compare Figure 4 and Figure 16, and see Figure 18). For the GRMIn, the Missouri River scoring also resulted in less variation in scores, but significantly lowered the local central tendency (two-sample t-test, $p<0.002$; compare Figure 5 and Figure 16, and see Figure 18).

One caveat in using the Missouri River scoring for the GRMIn is that some of the GRMIn component metrics for the Missouri River tend to correlate with distance from the river mouth

(Angradi et al. 2009c). No adjustment to account for this was made for the GRMIn scores computed for the Mississippi River OR, and although a longitudinal pattern is evident for the GRMIn scores in the OR, that pattern is also evident with the unadulterated GRFIn (Figure 4), the FACI (Figure 14c,) the GRFIn with Missouri River scores (Figure 16), and the Ad Hoc index (Figure 17). Residuals from a regression of the Ad Hoc Index on river mile of the UMR suggest that after adjusting for the longitudinal pattern, scores in Reaches 12 and 13 still tend to under-perform (Figure 17d).

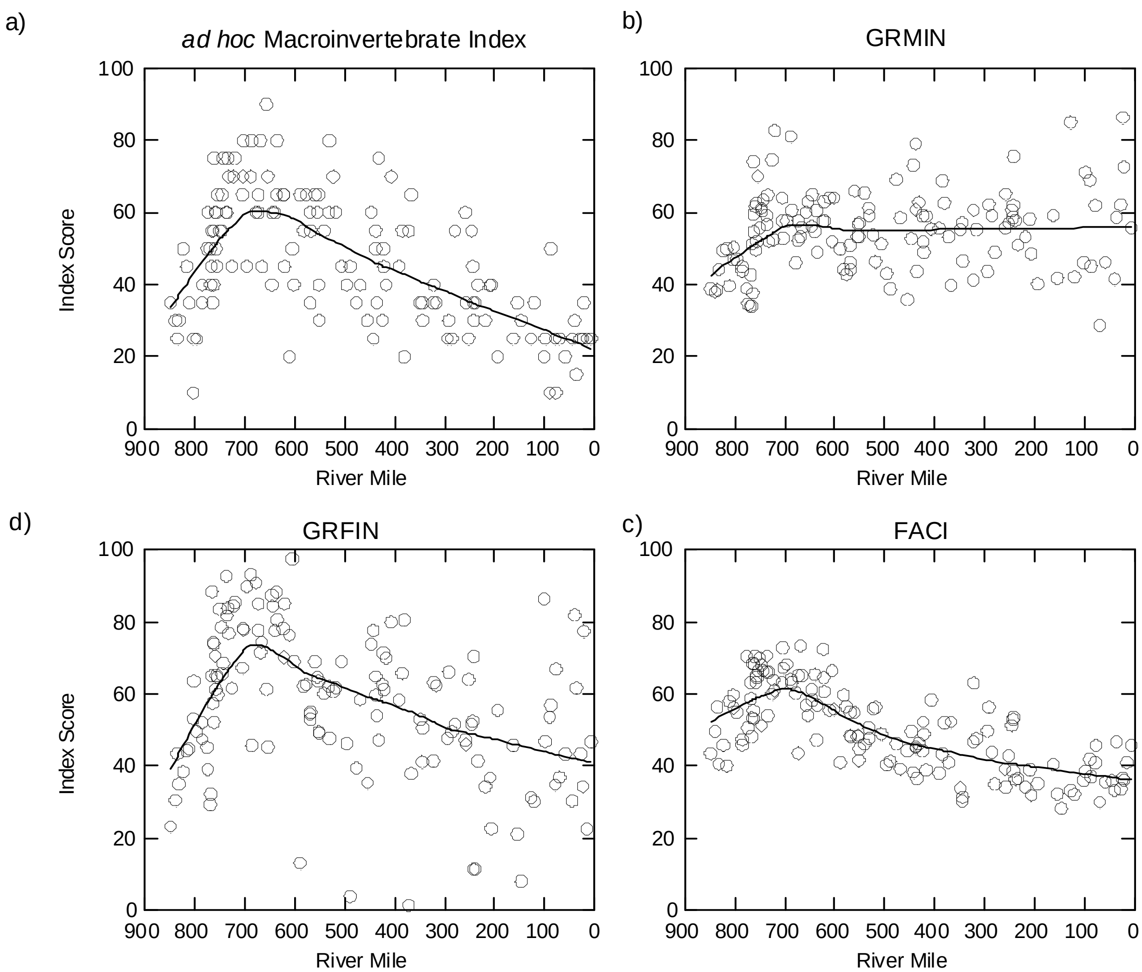


Figure 14. Longitudinal performance of a) the *Ad Hoc* macroinvertebrate index, b) the GRMIn, c) the FACI, and d) the GRFIn for the UMR. The line in each plot following the local central tendency is from LOWESS (q=0.5).

Table 13. Frequency of rank order correlations ≥ 0.25 between macroinvertebrate metrics and environmental variables (number of variables in category).

Metric	Land Use (78)	Habitat (67)	Water Chemistry (36)
UMR Impounded GRMIn Metrics			
Hemiptera Distinct Taxa Richness	38	16	12
Scraper % Distinct Taxa	34	12	4
Clinger % Individuals	20	7	7
Cricotopus/ Cricotopus/Orthocladius % Individuals	16	5	5
Climber % Individuals	9	9	0
Odonata % Distinct Taxa	7	2	1
Collector-Gatherer % Individuals	6	6	0
Scraper % Individuals	5	15	0
Collector-Gatherer % Distinct Taxa	4	0	0
TOLVAL 0-1.9 % Distinct Taxa	0	0	1
GRMIN	10	4	4
Other Macroinvertebrate Metrics			
Amphipod % Distinct Taxa	42	18	2
Amphipod % Individuals	44	19	14
Amphipod Distinct Taxa Richness	42	13	11
Climber Distinct Taxa Richness	14	6	10
Collector-filterer Trichoptera % Individuals	34	17	6
Corbicula/Dreissena % Distinct Taxa	50	24	0
Corbicula/Dreissena % Individuals	51	17	0
Corbicula/Dreissena Distinct Taxa Richness	52	18	0
Crustacean % Distinct Taxa	47	17	2
Crustacean % Individuals	47	21	12
Ephemeroptera % Distinct Taxa	39	17	5
EPOT % Distinct Taxa	40	17	3
Hemiptera % Individuals	46	12	8
Hydrachnidia % Individuals	15	6	10
Hydrachnidia Distinct Taxa Richness	6	4	12
Hydropsychidae % Individuals	39	17	6
Mollusc % Distinct Taxa	46	14	6
Mollusc % Individuals	48	11	3
Mollusc Distinct Taxa Richness	45	12	10
Non-Insect Distinct Taxa Richness	42	9	11
Scraper Distinct Taxa Richness	7	7	9
Scraper/Piercer Distinct Taxa Richness	10	5	9
Swimmer % Distinct Taxa	49	7	0
Swimmer % Individuals	46	8	1
Swimmer Distinct Taxa Richness	49	11	7
Tolerant (TOLVAL 7.0-10) % Distinct Taxa	32	18	0
Tolerant (TOLVAL 7.0-10) % Individuals	21	5	9
TOLVAL 8-10 Distinct Taxa Richness	18	12	9
TOLVAL 8-10 Distinct Taxa Richness	18	12	9

Table 14. Metrics and scoring threshold used in the *Ad Hoc* macroinvertebrate index.

Metric	1	5
Hemiptera Distinct Taxa Richness	<1	>2
Clinger % Individuals	>40	<7.852
Cricotopus/ Cricotopus/Orthocladius % Individuals	>1.4	=0
Collector-Gatherer % Distinct Taxa	>50	<40
Amphipod % Individuals	<1.6	>14.46
Climber Distinct Taxa Richness	<4	>6
Hydrachnidia % Individuals	=0	>4.545
Mollusca Distinct Taxa Richness	<3	>5
Non-Insect Distinct Taxa Richness	<11	>20
Tolerant (TOLVAL 7.0-10) % Individuals	<37.68	>73.84

Lastly, the discrete scoring used to draw the Ad Hoc index lends itself to threshold identification. Applying the Ad Hoc index in a dual indicator approach, and using trisection to draw the threshold would yield approximately 53 percent of the impounded UMR in non-attainment – a result similar to the most-intermediate disturbance thresholds for the GRE-derived indices (Table 15). Furthermore, using other potential impairment thresholds (e.g., the 16th percentile of REMAP, UMR quadrisection) for the Ad Hoc index yields between 26 and 62 percent non-attainment for the impounded UMR (Table 15). Although the total amount of impairment suggested using either the Ad Hoc index or the GRMIn is similar, the Ad Hoc index suggests greater variation in condition down the run-of-river, a result that generally comports the GRFIn, FACI, and SMI.

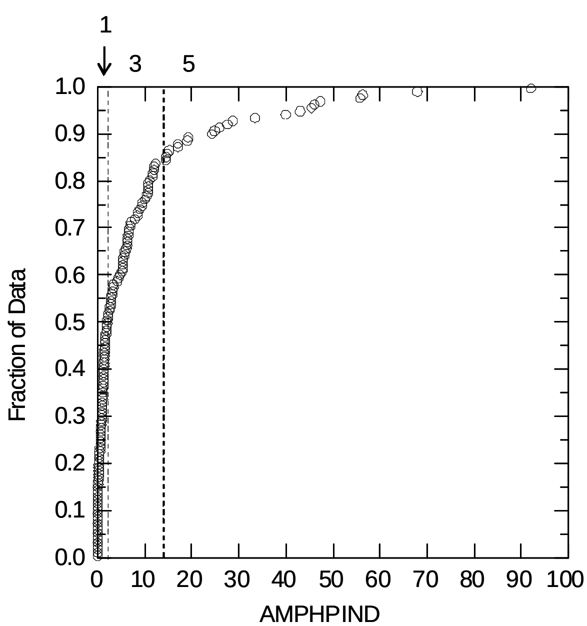
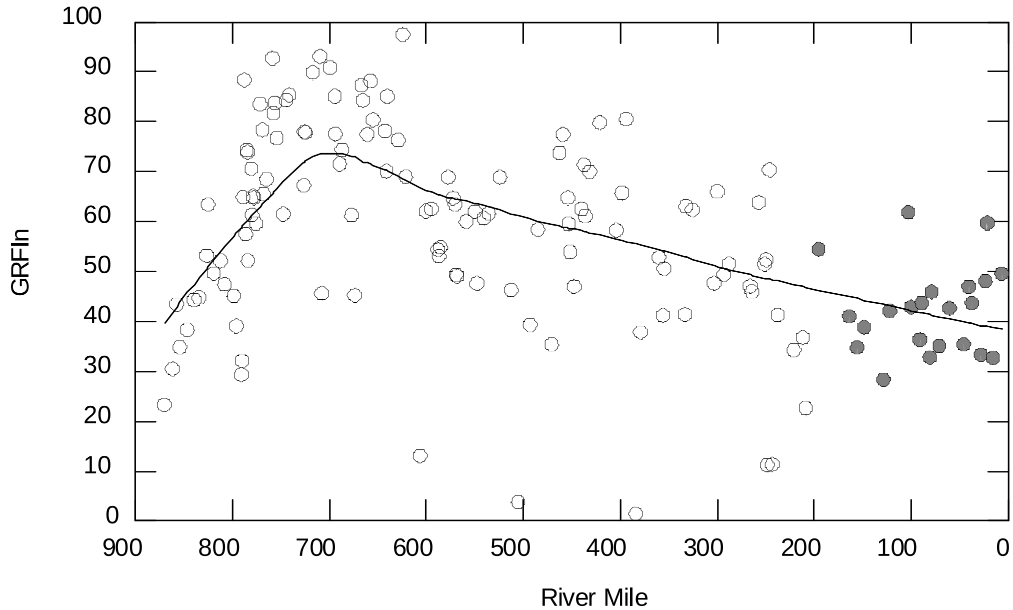


Figure 15. Example of how the Ad Hoc macroinvertebrate index component metrics were scored.



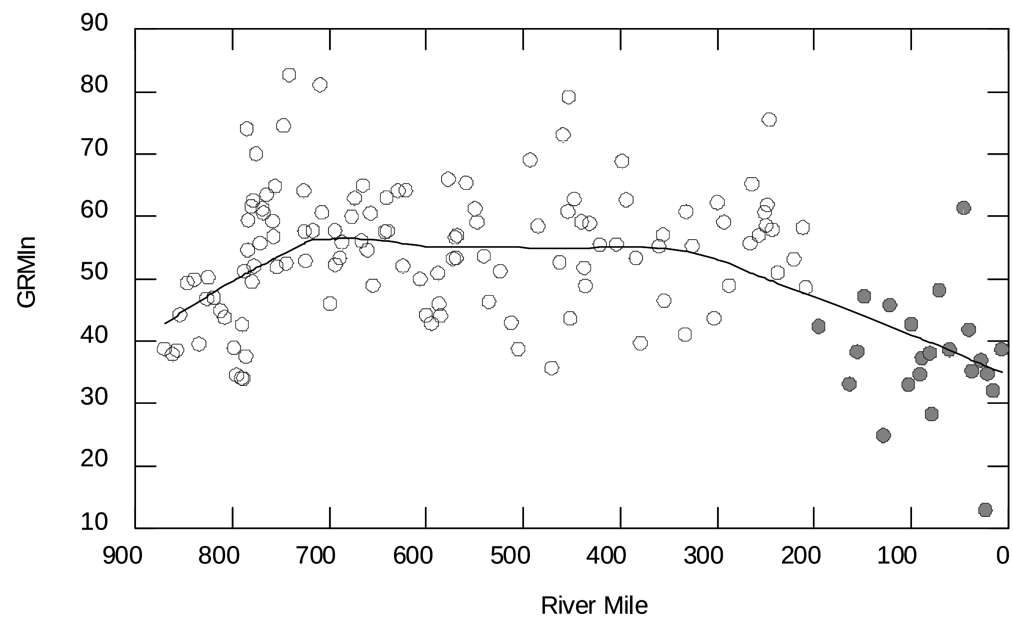


Figure 16. Plots of GRFIn and GRMIn scores by river mile for the Upper Mississippi River. Shaded points were scored using the scoring algorithm from the Lower Missouri River. The line following the local central tendency in each plot is from LOWESS (q=0.5).

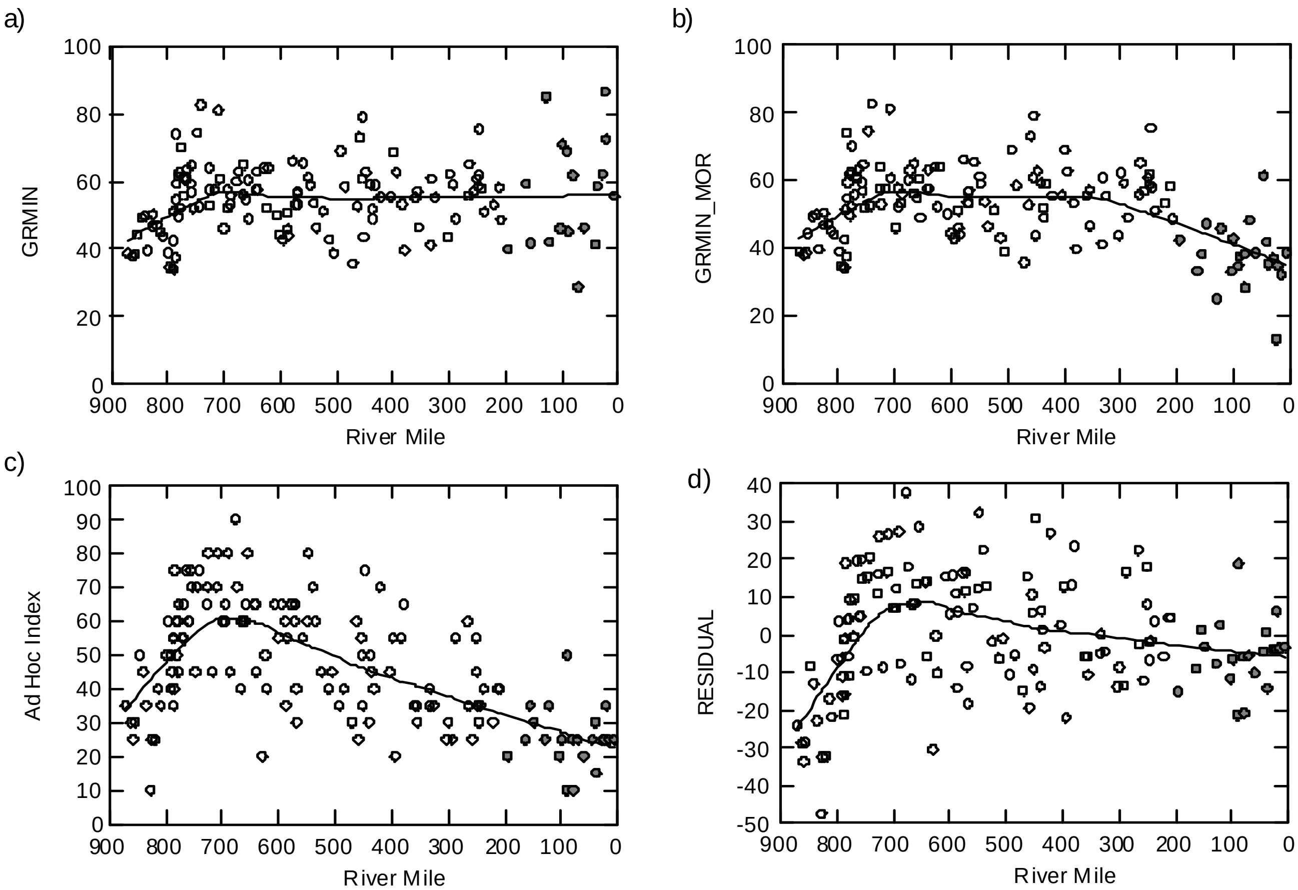


Figure 17. a) GRMIn scores based on UMR scoring, and b) GRMIn scores adjusted for Missouri River scoring (applied to Reaches 12 & 13 only). C) the Ad Hoc Index, and d) residuals from the regression of the Ad Hoc Index on river mile. The shaded points denote sites in Reaches 12 and 13.

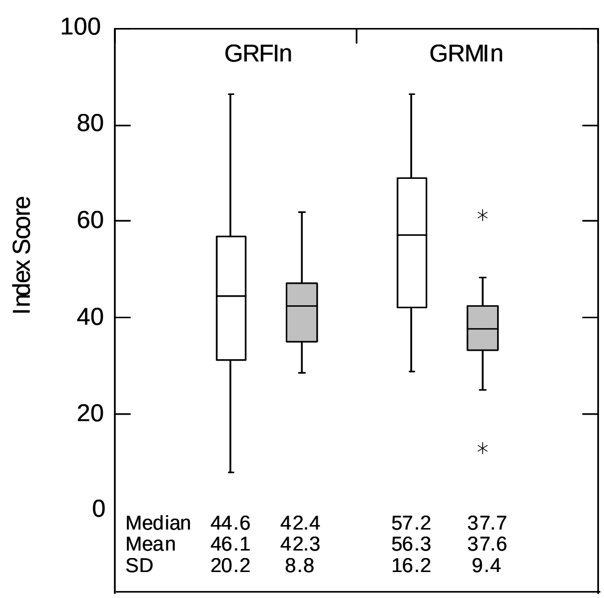


Figure 18. Distributions of GRE index scores for Reaches 12 and 13 of the Open River UMR. Hollow box plots show score distributions computed using the Mississippi River scoring algorithms, shaded box plots show the score distributions using the Missouri River scoring algorithm (Angradi 2009a).

Appropriate Threshold

This all leads up to the overarching question of identifying an appropriate CWA condition threshold for the UMR. While this report cannot answer all pertinent questions regarding the choice of appropriate threshold(s) for the UMR, it can provide some valuable insights to answering the question, as described below. It also needs to be understood that this report emphasizes the statistical relationships in the various data that we examined. This does not necessarily address the policy issues surrounding the establishment of impairment thresholds, but it is an essential quantification of the available data and measures of biological condition. A companion document (Rankin and Yoder 2011) examined alternate means to determine a threshold via the development of a Biological Condition Gradient (BCG) for the UMR and together with the analyses accomplished herein it provides ecological based insights as to what each of the statistically derived thresholds mean in terms of relative quality of the UMR biological assemblages. The goal of that report is to know how the current indices correspond to the breadth of the BCG and what the different thresholds represent in terms of contemporary and historical quality.

Attainability/Percent Attainment

The biological assessment using GRE indices set at the boundary between Intermediate and Most Disturbed resulted in a little less than one-half (47%) of the mainstem being classified in attainment for aquatic life. Had the Least-Intermediate boundary been chosen as the threshold, only ***three*** sites would have been in full attainment. Similarly, the most stringent assessment based on the REMAP indices and a threshold set at the respective 25th percentiles of their reference sites clearly resulted in a similar outcome from the standpoint of attainment status. Keep in mind, however, that the 25th percentile for the REMAP reference sites included rivers with a distinctly different character than the UMR (e.g., the upper St. Croix above St. Croix Falls, etc.). Nevertheless, this begs the question of where to set the threshold if the degree of anthropogenic modification in the UMR and similarly sized rivers is so extensive as to preclude the development of a threshold based on contemporary reference conditions. Again, the companion BCG report was developed to aid in that understanding. However, the congruence of thresholds identified by the change point method, most-intermediate disturbance boundary, and 2nd section boundary of quadrisection lends support to an internally-derived biocriteria tier near the 40th percentiles of the respective indexes.

Comparison to Other Large River Systems

The absence of contemporary reference conditions in the UMR does not necessarily mean that the observed range of condition is heavily skewed toward a degraded state. In this light, one can compare the existing range of conditions in the UMR to a presumably less stressed system, or to a collection of its regional peers. The REMAP study provides us with that opportunity.

Lower St. Croix River: Distributions of GRFIn scores from the lower St. Croix River (data collected by the Minnesota Pollution Control Agency), a river with presumably less disturbance than the UMR, were compared with those from the UMR (Figure 18). The results suggest that the Intermediate-Most boundary is a reasonable threshold in that it is *attainable* for aquatic life use support in the UMR main channel, *if* the lower St. Croix River is considered as an analog for a desired condition in the UMR. Although the central tendencies of the distributions from the UMR and the St. Croix do not differ significantly (Kruskal-Wallis test, P>0.1), the distribution of scores from the St. Croix have less variation than those from the UMR (Kolmogorov-Smirnov test, P<0.01), and few of the St. Croix GRFIn scores were less than the Intermediate-Most threshold. This means that if the GRFIn is reflecting condition in the St. Croix accurately, portions of the UMR are performing at a level consistent with a presumably less disturbed system.

<u>Collection of Similar (Peer) Rivers</u>: Another approach is to consider a group of regional rivers as a benchmark against which to gauge the attainability of UMR threshold choices. Herein we used the FACI and NMACI to conduct this analysis since each were suited to the collection methods used in the REMAP project. Because of sampling methodology differences, principally with the fish sampling site distance, it was not possible to calculate GRFIn for the REMAP data. However, it was possible to calculate a FACI score for the UMR because the GRE sites were split into two 0.5 km reaches whereas REMAP consisted of single 0.5 km sites. The assessment based on a quadrisection of similar rivers for the FACI paired with the quadrisection of the UMR for the NMACI appeared equally realistic for the UMR, especially considering that the FACI threshold used here was derived from its regional peers, and therefore represents a regionally attainable expectation. Comparing the distribution of FACI scores for the UMR to its regional peers (Figure 20), provides context for where the UMR is positioned along the biological condition continuum, and lends further support to choosing these thresholds. In this context, at least half the GRFIn UMR scores compare favorably with those from the lower St. Croix (Figure 19), and in terms of the FACI, the UMR compares favorably to a broader collection of its regional peers (Figure 20).

The impairment threshold drawn by the quadrisection of FACI scores from similar rivers equates to a FACI score of 38. For the UMR, a FACI score of 38 is at the 16^{th} percentile (Table 15). Given that this threshold is derived from a wider collection of rivers, and from an index that appears to be robust in response to the UMR stressor gradient, it is perhaps the most defensible threshold given the absence of contemporary reference conditions in the UMR itself. In that light, applying the 16^{th} percentile of GRFIn and GRMIn scores from the UMR as an impairment benchmark is plausible, given that the FACI and GRFIn are well correlated in the UMR (Figure 21), and using the 16^{th} percentile of GRMIn may guard against the tendency toward type I errors presented by the relatively compressed range of scores. Using the Ad Hoc macroinvertebrate index as a benchmark to pair with the FACI, and applying the 16^{th} percentile to the Ad Hoc index yielded similar estimates of impairment on a reach-basis to that when the 16^{th} percentile is applied to the GRE indices (Table 15).

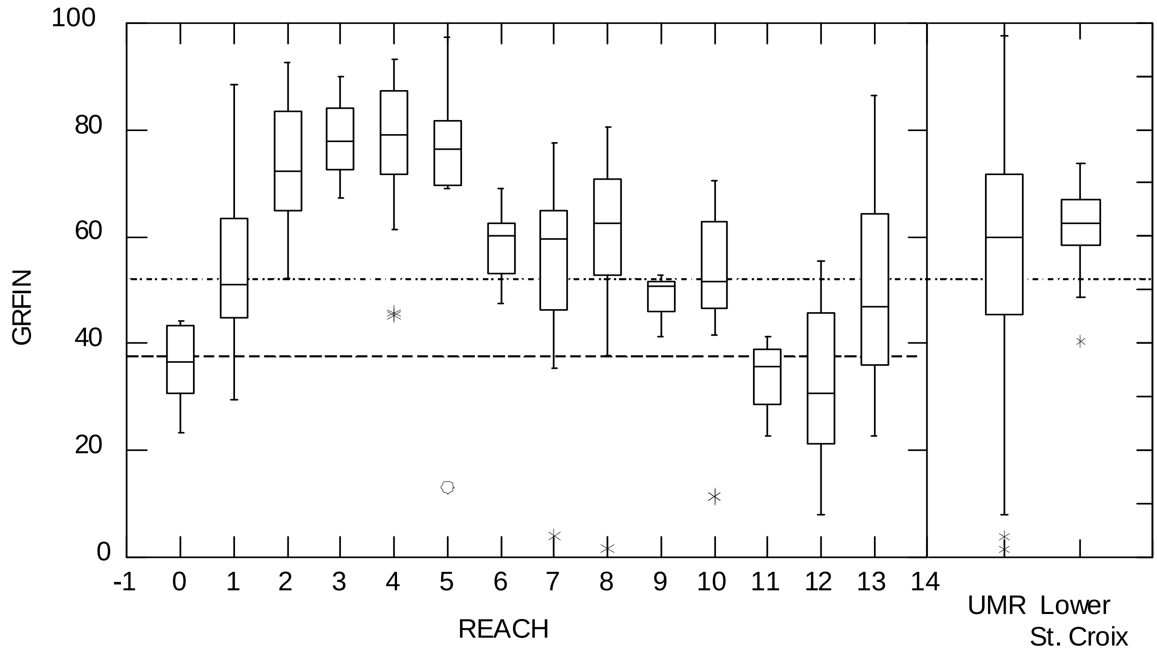
Because the precision of the GRE indices have not been determined with repeat sampling (to the best of our knowledge at this time), setting the condition threshold at the 16^{th} percentile is conservative (i.e., with respect to rejecting the null hypothesis of no impairment) within the context of the dual indicator approach where one failing index will drive non-attainment, given that failure of either indicator at that level would reasonably signify stress. As such, adopting the 16th percentile approach could present a first step in threshold-setting which would avoid erroneously categorizing reaches as impaired, given the uncertainties with the GRMIn. While this begs the question of whether this results in setting the minimum CWA threshold at an un-protective level, especially considering that the other methods resulted in higher thresholds, it opens the opportunity to consider tiered thresholds for reaches of the UMR that perform well above this minimum threshold. This would entail setting a higher threshold or thresholds for reaches that score well above the 16th percentile-derived threshold as a means to preserve an already existing higher quality.

<u>Inclusion of Lower Missouri River Scoring</u>: Because GRMIn scores computed using the Lower Missouri River scoring were significantly lower than those computed using the Mississippi River OR algorithm, the choice of threshold (first tier quadrisection, midpoint quadrisection, trisection, or most disturbed threshold – Table 15b) has little consequence, as all but two sites would be rated as impaired by the GRMIn under the most liberal/least stringent criterion (i.e., the first tier of quadrisection). Relaxing the criterion to the 16th percentile would result in slightly less impairment, as the GRMIn would then rate 7 sites as passing. The tendency for the Missouri River-scored GRMIn to track the Ad Hoc index through Reaches 12 and 13 is due to the fact that both indexes share several metrics (or closely related metrics).

Index Sensitivity

Lastly, because the original component metrics of the GRFIn and GRMIn were chosen to respond to a stressor index developed from various abiotic variables, the sensitivity of the GRE indices to other gradients, or combinations thereof, that were not adequately included in the index may be questionable. However, for the impounded UMR GRFIn at least, the component metrics are a familiar set of metrics and attributes seen in many other fish IBIs. Given the history and use of fish IBIs, it is axiomatic that the GRFIn responded well to multiple stressors, and did not simply act as a gauge of the specific set of stressors and the resulting stressor index from which it was calibrated. However, that cannot be stated with the same degree of confidence for the GRMIn, given the multiples of candidate metrics that are generally available for macroinvertebrate IBIs. Indeed, the GRMIn is more strongly correlated to the stressor index than it is to other stressors, and where it shows association with other stressors, the association is fairly weak. This, however, does not necessarily obviate its usefulness as a gauge of condition in a dual indicator approach, especially when the companion fish index was demonstrably more sensitive to the wide range of conditions in the UMR. The GRMIn at least provides a “read” about overall stress, and if the threshold is set at an appropriately conservative level, it can complement the GRFIn. That said, exploring further refinements of the GRMIn is clearly warranted, and the Ad Hoc index introduced here is a start in that process.

The SMI is another alternative for a companion index for use in a dual/multi-indicator assessment. Note that it was sensitive to roughly the same number of stressors as was the GRFIn, but was particularly sensitive to TSS and turbidity. Turbidity is considered an existing, primary impairment on the UMR for which a TMDL exists for Pools 2, 3 and 4 thru upper Lake Pepin. SMI is a key response indicator for that latter water body.



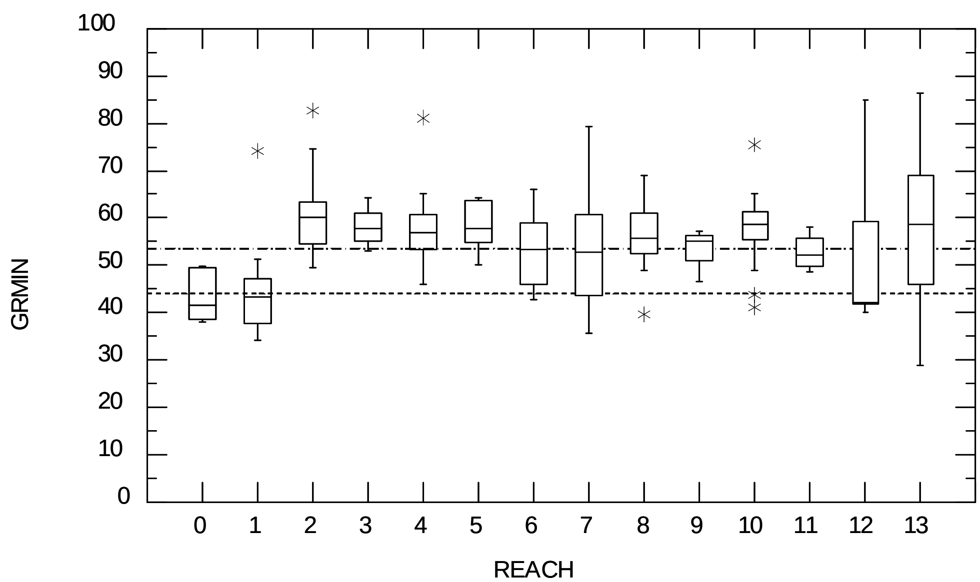


Figure 19. Upper panel, distributions of GRFIn scores by reach for the UMR, for the entire UMR compared to those from the lower St. Croix River (i.e., downstream from Taylor Falls). The stippled line joining the y axis at 52 is the most-intermediate disturbance boundary from Angradi (et al. 2009a), and the dashed line joining at 38 is the 16th percentile. Lower panel, distributions of GRMIn scores by reach in the UMR. The stippled line joining the y axis at 53 is the most-intermediate disturbance boundary from Angradi (et al. 2009b), and the dashed line joining at 44 is the 16th percentile.

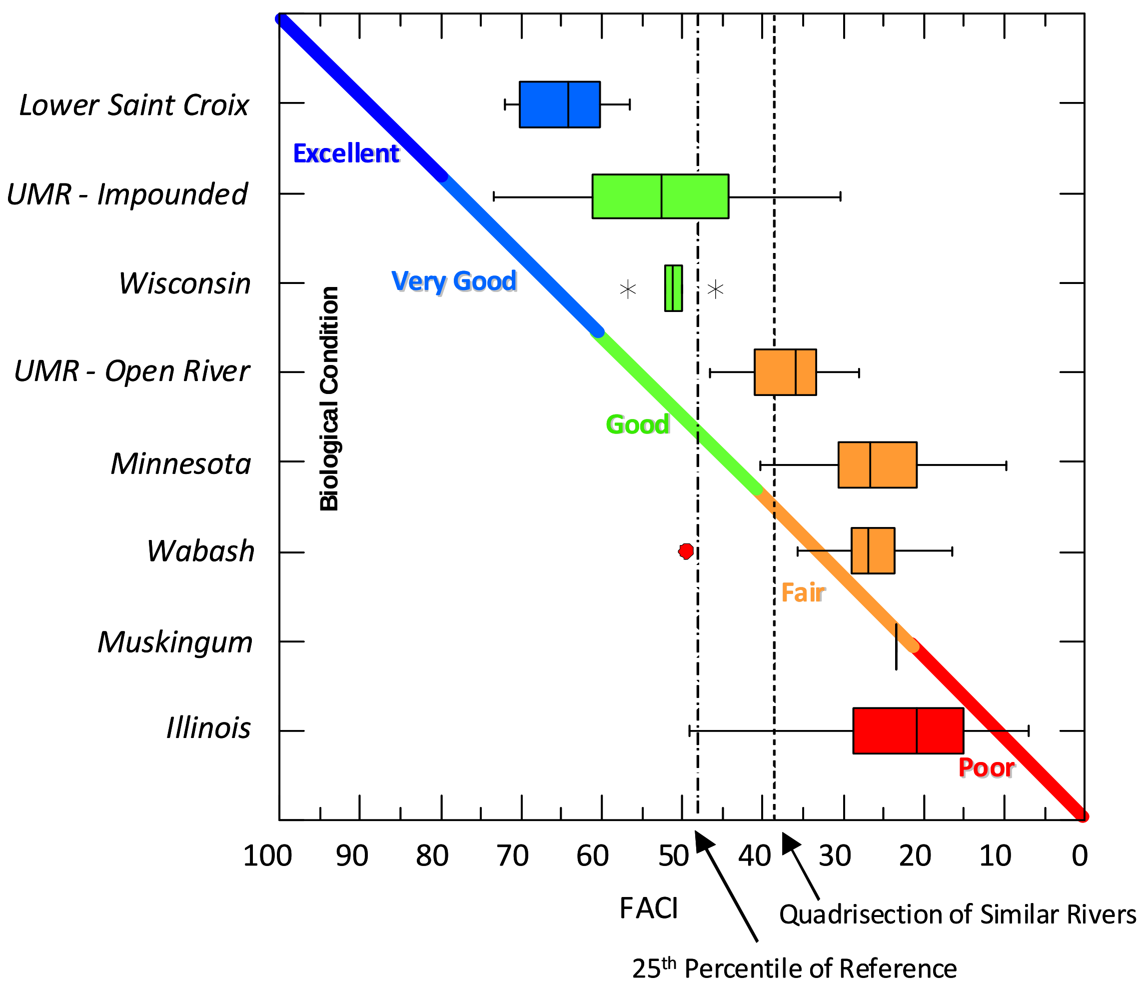


Figure 20. Box plots of FACI scores from large, similar rivers used to draw the quadrisection. The rivers are: the Lower St. Croix downstream from Taylor Falls, the Muskingum River near Marietta, the Wisconsin River downstream from the Lake Wisconsin, the Wabash River downstream from the confluence with the Vermillion River, the Minnesota River downstream from New Ulm, and the Illinois River. Distributions of FACI scores for the Impounded UMR and UMR Open River are shown for comparison. Thresholds suggested by quadrisection (i.e., the 16th percentile option) and 25th percentile of REMAP reference sites are shown for comparison.

Table 15a. Recapitulation of impairment statistics given by the GRE indices based on the most-intermediate disturbance boundaries of Angradi et al. (2009a and b) compared to statistics given by quadrisection of the UMR, the 16th percentile for GRE indices, the Ad Hoc macroinvertebrate index used in lieu of the GRMIn, and the Ad Hoc index paired with the FACI.

		GRE-Indexes Most Disturbed		GRE-Indexes Quadrisection of UMR[2] (2nd section boundary)		GRE-Indexes Quadrisection of UMR[2] (1st section boundary)		GRE-Indexes Quadrisection of UMR[3] (1st section boundary)		GRE-Indexes at 16th %		GRFIn Most Disturbed & Ad Hoc Trisection		GRFIn & Ad Hoc Quadrisection (2nd section boundary)	
		GRFIn[1]	GRMIn	GRFIn	GRMIn	GRFIn	GRMIn	GRFIn	GRMIn	GRFIn	GRMIn	GRFIn	Ad Hoc[4]	GRFIn	Ad Hoc[4]
Threshold Score		52	53	55	56	39	47	46	44	38	44	52	53	55	60
Percentile of Range in UMR		*38*	*41*	*45*	*53*	*17*	*34*	*27*	*17*	*16*	*16*	*38*	*37*	*45*	*48*
REACH	Length														
0	57	100.0		100.0		83.3		100.0		66.7		100.0		100.0	
1	47	92.9		92.9		78.6		57.1		50.0		78.6		85.7	
2	47	22.2		38.9		0.0		0.0		0.0		5.6		22.2	
3	19	25.0		25.0		0.0		0.0		0.0		0.0		25.0	
4	61	35.7		64.3		7.1		14.3		0.0		21.4		28.6	
5	47	28.6		28.6		14.3		14.3		14.3		42.9		57.1	
6	58	61.5		69.2		30.8		7.7		7.7		30.8		38.5	
7	86	61.5		69.2		38.5		38.5		30.8		53.8		61.5	
8	71	45.5		63.6		18.2		18.2		18.2		54.5		72.7	
9	35	66.7		100.0		33.3		33.3		0.0		100.0		100.0	
10	85	60.0		73.3		26.7		33.3		26.7		93.3		100.0	
11	40	100.0		100.0		75.0		100.0		75.0		100.0		100.0	
12	76	83.3		100.0		66.7		83.3		66.7		83.3		83.3	
13	115	56.3		81.3		31.3		43.8		31.3		56.3		62.5	
All	844	56.3		69.4		31.9		32.6		24.3		52.8		61.8	

Table 15a. Continued.

		GRFIn & Ad Hoc Quadrisection (1st section boundary)		GRFIn & Ad Hoc at 16th Percentile		FACI Quadrisection of Similar Rivers & Ad Hoc at 16th %		FACI & Ad Hoc Quadrisection of UMR		GRFIn Most Disturbed & SMI Quadrisection (Reach 0-6; GRFIn & GRMIn Most Disturbed (Reach 7-11); GRFIn Most Disturbed (Reach 12-13)		
		GRFIn	Ad Hoc[4]	GRFIn	Ad Hoc[4]	FACI	Ad Hoc[5]	FACI	Ad Hoc[5]	GRFIn	SMI	GRMIn
Threshold Score		39	50	38	48	38	48	51	60	52	44	53
Percentile of Range in UMR		*17*	*27*	*16*	*16*	*16*	*16*	*54*	*48*	*38*	*41*	*41*
REACH	Length											
0	57	83.3		66.7		50.0		100		100.0		
1	47	57.1		35.7		21.4		71.4		57.1		
2	47	0.0		0.0		0.0		16.7		11.1		
3	19	0.0		0.0		0.0		25		25.0		
4	61	0.0		0.0		0.0		28.6		28.6		
5	47	28.6		28.6		14.3		57.1		42.9		
6	58	15.4		7.7		7.7		69.2		61.5		
7	86	38.5		23.1		23.1		84.6		61.5		
8	71	36.4		27.3		18.2		72.7		45.5		
9	35	100.0		33.3		100		100		66.7		
10	85	66.7		46.7		53.3		93.3		60.0		
11	40	75.0		75.0		75.0		100		100.0		
12	76	66.7		66.7		100		100		83.3		
13	115	31.1		31.3		100		100		56.3		
All	844	35.4		26.4		34.0		68.8		51.4		

1 The disturbance threshold for the impounded UMR was extended through the open river reach.
2 Thresholds derived from quadrisection of 95^{th}-5^{th} percentile range of GRFIn and GRMIn scores for the entire UMR (IR and OR)
3 Quadrisections using the y-intercept values of GRFIn and GRMIn scores on the stressor index (Angradi 2009a and b) to set ceiling and floor values.
4 Ad Hoc macroinvertebrate index and GRFIn thresholds applied to impounded UMR; GRFIn only applied to OR.
5 FACI and Ad Hoc thresholds extended through the OR.

Table 15b. Impairment statistics given by the GRE indices with GRFIn and GRMIn scores for the Open River (Reaches 12 and 13) computed based on the scoring algorithm for the Lower Missouri River (Angradi et al. 2009a and b). GRFIn and GRMIn scores for Reaches 0 – 11 are based on the impounded UMR. Thresholds are drawn based on the most-intermediate disturbance boundaries of Angradi (2009a and b) for the impounded UMR, trisection of the data (lower tier), quadrisection of the data (lower tier and middle tier), and the 16th percentile of the data.

		GRE Indices at 16th Percentile of Data Range		**GRE Indices Quadrisection (1st section boundary)**[1]		**GRE Indices Trisection (1st section boundary)**		**GRE Indices Most Disturbed Impounded UMR**		**GRE Indices Quadrisection (2nd section boundary)**	
		GRFIn	GRMIn	GRFIn	GRMIn	GRFIn	GRMIn	GRFIn	GRMIn	GRFIn	GRMIn
Threshold Score		38	39	43	43	48	46	52	53	58	52
Percentile of Range in UMR		*16*	*16*	*23*	*23*	*36*	*30*	*42*	*49*	*49*	*45*
REACH	Length										
0	58.5	66.7		83.3		100.0		100.0		100.0	
1	49	35.7		50		64.3		92.9		92.9	
2	49	0.0		0		0.0		22.2		16.7	
3	20	0.0		0		0.0		25.0		0.0	
4	63	0.0		0		21.4		35.7		28.6	
5	48	14.3		14.3		14.3		28.6		14.3	
6	60	0.0		7.7		38.5		61.5		61.5	
7	89	15.4		30.8		38.5		61.5		53.8	
8	73	18.2		18.2		27.3		45.5		45.5	
9	36	0.0		33.3		33.3		66.7		100.0	
10	88	13.3		20		40.0		60.0		66.7	
11	41	75.0		100		100.0		100.0		100.0	
12	78	50.0		100		100.0		100.0		100.0	
13	118	87.5		100		100.0		100.0		100.0	
All	870.5	25.0		34.7		46.5		61.8		59.7	

[1] Thresholds derived from quadrisection of 95th-5th percentile range of GRFIn and GRMIn scores for the entire UMR (IR and OR).

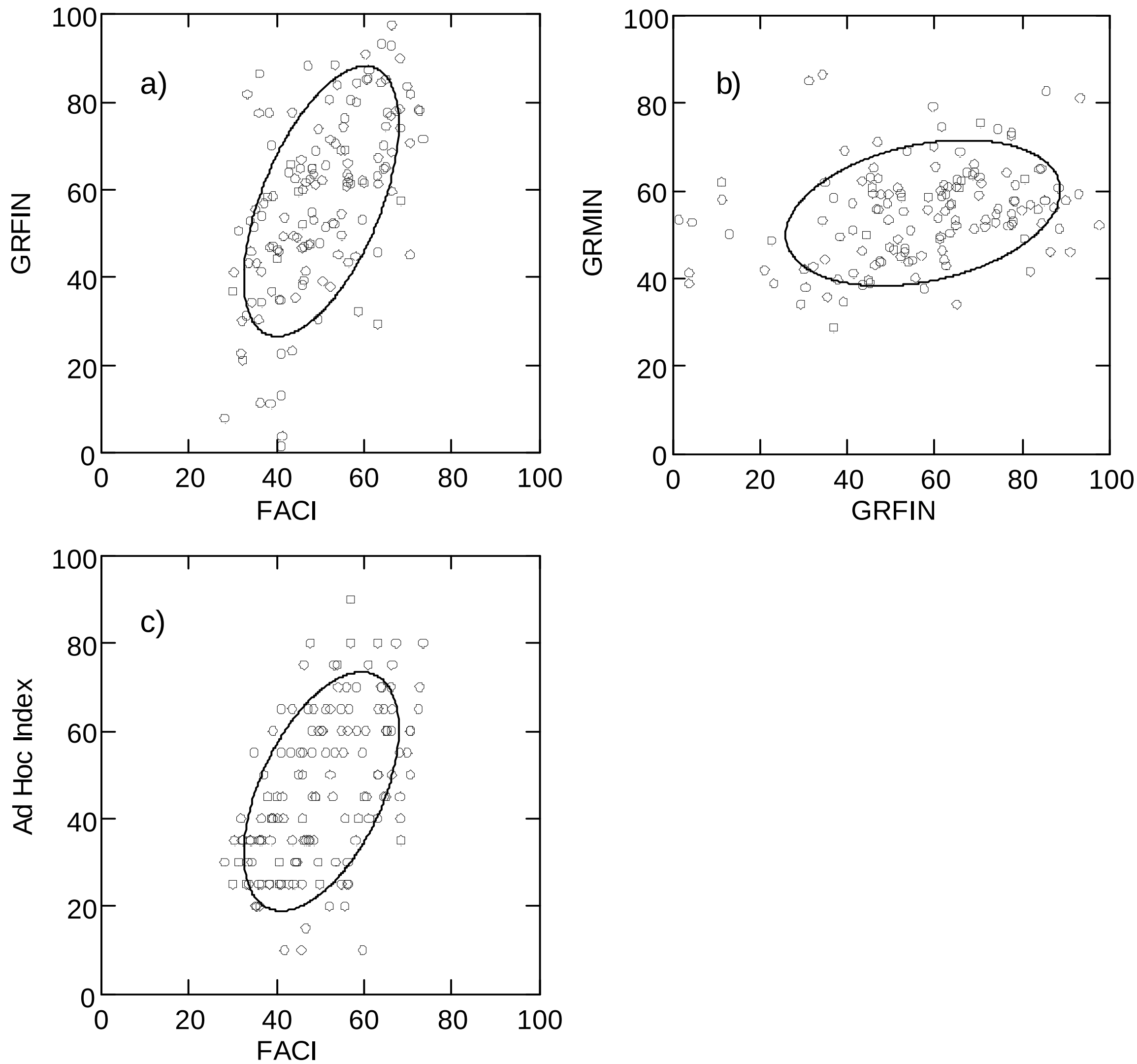


Figure 21. Scatter plots of a) GRFIn on FACI, b) GRMIn on GRFIn, and c) the ad hoc index on FACI score from the UMR.

Conclusions

Available Biological Assessment Tools

This report has examined the potential applicability of existing and modified biological indices in making UMR CWA assessments. The EMAP-GRE indices developed specifically for the UMR (GRFIn and GRMIn) have been examined in detail and the resultant thresholds compared to those from REMAP-developed large river indices (FACI and NMACI). The potential utility of a vegetation index (SMI) has also been examined. The following conclusions can be drawn from the examination of these indices:

- Both GRFIn and the GRMIn hold promise for CWA aquatic life use assessment on the UMR as both are calibrated specifically for the UMR. This holds especially true for the Impounded Reaches for both indicators, and for the GRFIn only in the Open River reach. The development of the Ad Hoc macroinvertebrate index holds promise for improving the GRMIn.
- GRFIn is responsive to the different types and gradients of stressors in the UMR main channel, and appears to track a wide range of condition.
- The GRMIn suggests a more narrow range of condition in the UMR compared to the GRFIn, and tended to be less responsive to measured environmental variables compared to the GRFIn. However, the GRMIn compliments the GRFIn by responding to different stressors (i.e., GRFIn was more responsive to habitat stressors, GRMIn to selected water quality stressors). The GRMIn needs to be tested via a longitudinal survey against a local, known stressor (e.g., a reach subject to combined sewer overflows or other such severe point source impacts) to evaluate whether the apparent narrow response is a function of the overall condition of the UMR (as read by the GRMIn) or a true limitation of the GRMIn. Individual components of the macroinvertebrate community were more responsive to environmental stressors than the composite GRMIn index, suggesting that structural improvements to GRMIn may improve its utility for UMR bioassessment. The Ad Hoc index developed herein is a good first step in fulfilling that need.
- The REMAP derived FACI was responsive to the range of stressors in the UMR much the same as GRFIn, and it correlated well with the GRFIn in the UMR. However, since it was derived from smaller, albeit large rivers, its application in the UMR needs to take that difference into account. This is particularly true for the OR reaches that may be outside of its derivation and calibration domain.
- The REMAP NMACI was the least responsive of all the indices examined against the stressor gradient for the UMR main channel, and is therefore not recommended for use at this time.
- SMI was sensitive to a number of different stressors and therefore represents another candidate assessment tool that can be used either with or in lieu of the GRMIn. However, because a direct evaluation of the SMI in a dual indicator approach was

limited by spatial differences between where vegetation and fish or macroinvertebrates were sampled, further study is encouraged to demonstrate the utility of the SMI to the remainder of the impounded UMR.

- The Missouri River scoring resulted in less variation in GRFIn and GRMIn scores in the Open River reach of the Mississippi, suggesting that component metrics of the Missouri River indices could supplement or replace existing OR Mississippi River GRFIn or GRMIn metrics.

Bioassessment Threshold Options

Various threshold determination approaches (empirical/trisection, quadrisection, 25% of REMAP reference, best available "peer" examples [16th % option], best of UMR, change point) were applied using the GRFIn, GRMIn, FACI, and NMACI indices to the impounded UMR and unimpounded Open River UMR (Tables 16a and 16b). These examinations provide insight into options for setting one or more aquatic life use goals and evaluating their respective attainability. In this context, the following conclusions can be drawn:

- All internally-derived threshold analyses produced >50% non-attainment for the UMR main channel as a whole. This result is not surprising given that threshold scores for all indices as a percentile of their range in the UMR were near the median or higher, except for the most-intermediate disturbance thresholds for the GRMIn and GRFIn. This is an obvious change in the characterization of the UMR given that only 4 of 13 interstate assessment reaches are currently reported to have aquatic life use impairment using the current non-biological approach.
- Threshold calculations based on best available conditions from other rivers (i.e., NMACI & FACI 25th % of "reference") result in greater proportions of the UMR in non-attainment (>80%) and do not show good agreement on condition status between fish and macroinvertebrates. As such, these thresholds are not reasonably attainable nor are they effective approaches for a UMR CWA assessment.
- The percentages of non-attainment from all other threshold approaches ranged from 24.3% to 72.9% non-attainment. These approaches may offer more plausible options for threshold determination. Comparison to assessment outcomes from other peer rivers indicates that this range of thresholds (i.e., the 16th % option) would result in a condition assessment for the UMR comparable to (i.e., the lower St. Croix) or better than that of the other regional rivers.
- Among the more plausible options, the EMAP-GRE most disturbed/intermediate disturbed threshold presents a viable option for an "upper tier" threshold, as it produces an attainable upper tier goal in the context of comparing the UMR to its nearby "peer" rivers. Similarly, another suitably protective "upper tier" threshold (e.g., the 25th or 50th percentile) can be identified from the Lower St. Croix. These thresholds may be appropriate for establishing tiered biocriteria as a forerunner of tiered aquatic life uses (TALU) for the UMR.
- Given the uncertainties with the current GRMIn index, a workable lower-end threshold is suggested by comparing the UMR to the wider collection of its regional peers. Application

Table 16a. Options for numeric thresholds delineating condition boundaries for the impounded reached of the UMR. Shaded options are viewed as unrealistic due to issues with biological index non-responsiveness to UMR stressor gradient.

Option[1]	**Index/Threshold Scenarios**[2]	**Indices**	**Threshold**[3] **(Percentile Rank in UMR)**	**Rationale**
Impounded UMR (Reaches 0-11)				
1	"Peer Rivers" GRFIn & Ad Hoc at 16^{th} % of UMR Range	GRFIn	38 (16)	Most defensible threshold given the absence of contemporary reference conditions, derived based on performance relative to peers; Ad Hoc index in place of GRMIn
		Ad Hoc	48 (16)	
2	"Peer Rivers" GRFIn & GRMIn at 16^{th}% of UMR Range	GRFIn	38 (16)	Most defensible threshold given the absence of contemporary reference conditions, derived based on performance relative to peers; down-weights chance of Type I errors due to narrow response range of GRMIn
		GRMIn	44 (16)	
3a	GRFIn & GRMIn 1^{st} Section Boundary of Quadrisection UMR (95^{th} – 5^{th} percentile quadrisection)	GRFIn	39 (17)	Defensible in that high percentages of sites in the UMR surpass these internally-derived thresholds; 1^{st} section boundaryof quadrisection reduces tendency toward Type I errors
		GRMIn	47 (26)	
3b	GRFIn & GRMIn 1^{st} Section Boundary of Quadrisection UMR (empirical y-intercept quadrisection)	GRFIn	46 (27)	Upper boundary of quadrisection empirically derived by stressor index; 1^{st} section boundary of quadrisection reduces tendency toward Type I errors
		GRMIn	44 (17)	
4	GRFIn & Ad Hoc Quadrisection (1st section boundary)	GRFIn	39 (17)	Defensible in that high percentages of sites in the UMR surpass these internally-derived thresholds; 1^{st} section boundary of quadrisection reduces tendency toward Type I errors
		Ad Hoc	50 (27)	

Option[1]	Index/Threshold Scenarios[2]	Indices	Threshold[3] (Percentile Rank in UMR)	Rationale
Impounded UMR (Reaches 0-11)				
5	GRFIn & GRMIn Intermediate-Most Disturbed Threshold on GRE Stressor Gradient	GRFIn	53 (38)	Defensible based on relationship with stressor index; GRMIn threshold relatively high as percent of range in UMR
		GRMIn	52 (41)	
6	GRFIn Most Disturbed & Ad Hoc Trisection	GRFIn	53 (38)	Defensible based on relationship with stressor index; GRMIn threshold relatively high as percent of range in UMR
		Ad Hoc	53 (37)	
7	GRFIn & Ad Hoc Quadrisection (2nd section boundary)	GRFIn	55 (45)	Defensible in that high percentages of sites in the UMR surpass these internally-derived thresholds
		Ad Hoc	60 (48)	
8	GRFIn & GRMIn Mid-point of quadrisection UMR	GRFIn	56 (53)	Defensible in that high percentages of sites in the UMR surpass these internally-derived thresholds
		GRMIn	55 (45)	
9	GRFIn + SMI Reaches 0-5 Mid-point of quadrisection UMR; GRFIn & GRMIn Reaches 6 - 13	GRFIn	56 (53)	SMI is sensitive to multiple stressors in the UMR; only applicable to Reaches 0 – 5; higher thresholds suggested to coincide with best performing reaches
		SMI	44 (41)	
10	GRFIn @ median of Lower St. Croix; GRMIn at corresponding UMR percentile	GRFIn	62 (56)	Analog for higher TALU tier.
		GRMIn	57 (56)	
11	GRE Indices trisection of 95-5%ile of "Best UMR" Reaches 2&3	GRFIn	70 (69)	Sets higher threshold for impounded UMR based on demonstrated performance in least impacted reaches.
		GRMIn	59 (70)	
12	GRE Indices quadrisection of 95-5%ile of "Best UMR" Reaches 2&3	GRFIn	67 (65)	Sets slightly lower threshold at fair-poor boundary. Provides for upper tier threshold.
		GRMIn	58 (59)	
13	NMACI – quadrisection UMR; FACI change point on stressor index	NMACI	40 (50)	Non-response of NMACI to stressors in UMR obviates use as condition indicator[4]
		FACI	45 (36)	

Option[1]	Index/Threshold Scenarios[2]	Indices	Threshold[3] (Percentile Rank in UMR)	Rationale
Impounded UMR (Reaches 0-11)				
14	NMACI – quadrisection UMR; FACI quadrisection similar rivers	NMACI	40 (50)	Non-response of NMACI to stressors in UMR obviates use as condition indicator[4]
		FACI	38 (16)	
15	NMACI & FACI quadrisection UMR	NMACI	40 (50)	Non-response of NMACI to stressors in UMR obviates use as condition indicator[4]
		FACI	51 (54)	
16	NMACI & FACI quadrisection similar rivers	NMACI	47 (79)	Non-response of NMACI to stressors in UMR obviates use as condition indicator[4]
		FACI	38 (16)	
17	NMACI & FACI 25th% of REMAP Reference	NMACI	57 (98)	Non-response of NMACI to stressors in UMR obviates use as condition indicator[5]; 98th percentile is obviously unattainable.
		FACI	48 (46)	

1 These are listed in a general order of defensibility from most to least.
2 Combinations of thresholds are described in Table 1.
3 For the given index score the percentile rank from all scores in the UMR is listed.
4 The FACI works for the UMR, the NMACI doesn't; ergo, eliminating these scenarios as assessment thresholds does not obviate the useful inferences drawn from using each in condition estimates.

of this threshold (at the 16th percentile) to the GRMIn is recommended as a default until the uncertainties with the GRMIn can be sorted out.

- The analyses of threshold choice in this report have largely been driven by the comparison of outcomes (i.e., non-attainment percentages) across threshold options to determine attainability and comparability to other systems. It seems useful to integrate these outcomes with the BCG analysis (Rankin and Yoder 2011) for the selection of CWA threshold for the UMR.

Table 16b. Options for numeric thresholds delineating condition boundaries for the unimpounded Open River reaches of the UMR.

Option[1]	Index/Threshold Scenarios[2]	Indices	Threshold[3] (Percentile Rank in UMR)	Rationale
1	Missouri River GRFIn and GRMIn Reaches 12 – 13 16th percentile of UMR	GRFIn (UMR +MOR)	38 (16)	Conditions in lower Missouri River are analogous to that in OR reach of Mississippi. Defensible given the absence of contemporary reference conditions, derived based on performance relative to peers; down-weights chance of Type I errors.
		GRMIn (UMR +MOR)	39 (16)	
2	"Peer Rivers" GRFIn at 16th% of UMR Range	GRFIn (Imp.)	38 (16)	Most defensible threshold given the absence of contemporary reference conditions; derived based on performance relative to peers; extends impounded GRFIn to OR based on BCG analysis.
3	Missouri River GRFIn and GRMIn Reaches 12 – 13 1st Section Boundary of Quadrisection UMR	GRFIn (UMR +MOR)	39 (17)	Conditions in lower Missouri River are analogous to that in OR reach of Mississippi. Same rationales as above apply to each of the respective following thresholds – LB quadrisection has a high percent of sites above threshold.
		GRMIn (UMR +MOR)	47 (34)	
4	Missouri River GRFIn and GRMIn Reaches 12 – 13 1st Section Boundary of Trisection UMR	GRFIn (UMR +MOR)	44 (26)	Trisection – high percent of sites above threshold.
		GRMIn (MOR)	50 (40)	

Option[1]	Index/Threshold Scenarios[2]	Indices	Threshold[3] (Percentile Rank in UMR)	Rationale
5	GRFIn Intermediate-Most Disturbed Threshold on GRE Stressor Gradient	GRFIn (Imp.)	53 (38)	Defensible based on relationship with impounded UMR stressor index; GRFIn threshold relatively high as percent of range in UMR.
6	Missouri River GRFIn and GRMIn Reaches 12 – 13 Impounded UMR Most Disturbed	GRFIn (UMR +MOR)	52 (38)	Most disturbed threshold is empirically derived.
		GRMIn (UMR +MOR)	53 (49)	
7	Missouri River GRFIn and GRMIn Reaches 12 – 13 Middle Bounds of Quadrisection UMR	GRFIn (MOR)	55 (48)	Sets high threshold. Type I errors likely given high percentile threshold of GRMIn.
		GRMIn (UMR +MOR)	56 (59)	
8	GRFIn Intermediate-Most Disturbed Threshold on GRE Stressor Gradient	GRFIn (OR)	36 (14)	Defensible only based on relationship with OR UMR stressor index; attainability focuses on OR only.

1 These are listed in a general order of defensibility from most to least.
2 Combinations of thresholds are described in Table 1.
3 For the given index score the percentile rank from all scores in the UMR is listed.

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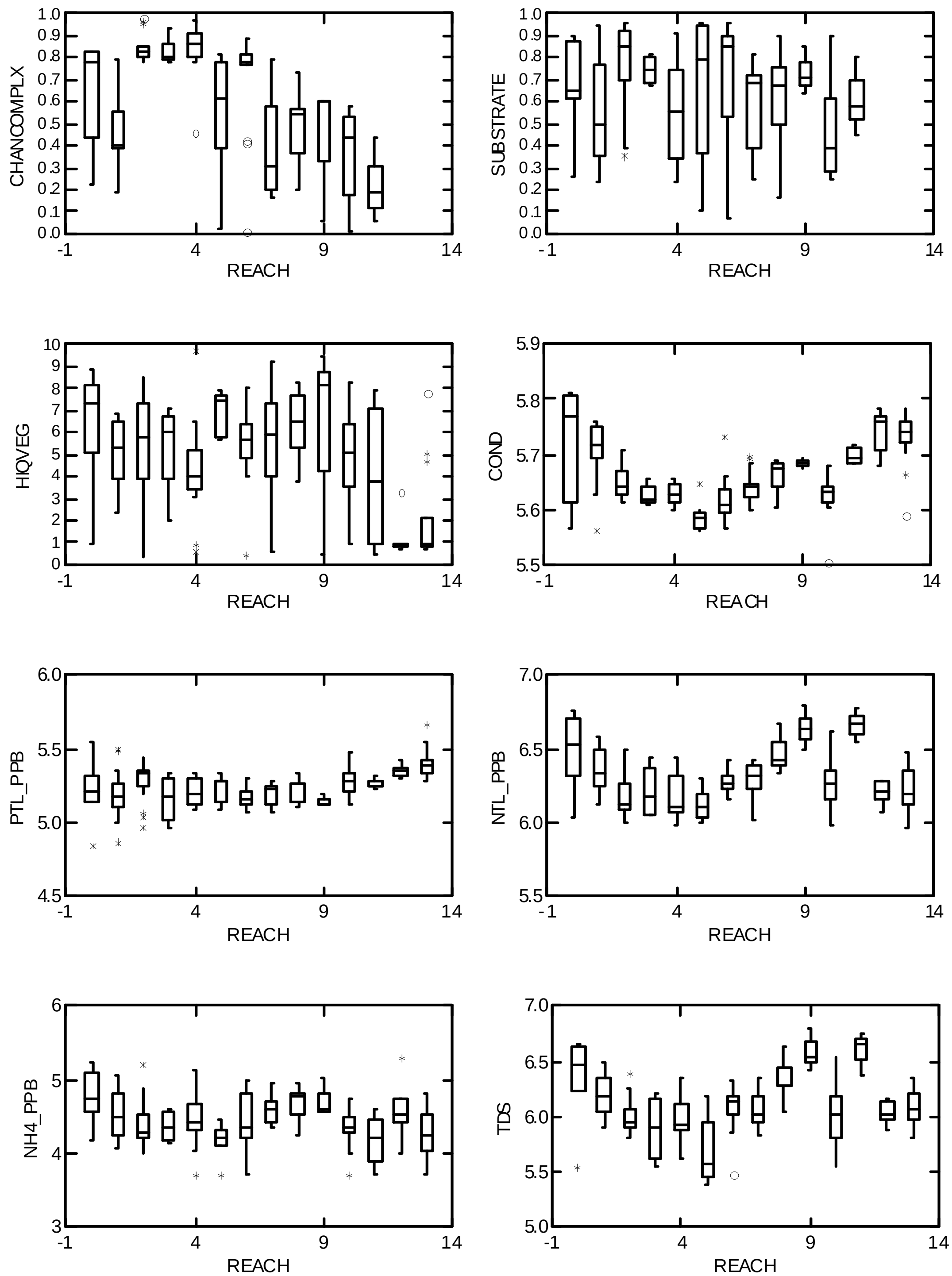
Appendix Table 1. Companion to Table 2.

Assessment Reach	River Miles	Number of Sites in Reach	Biological Indicator Frequency of Non-attainment	
			Fish	Bugs
Non-interstate UMR	870.5 – 812	6	6	6
1 St. Croix River to Chippewa River	812 – 763	14	6	7
2 Chippewa River to Lock and Dam 6	763 – 714	18	0	4
3 Lock and Dam 6 to Root River	714 – 694	4	0	1
4 Root River to Wisconsin River	694 – 631	14	2	3
5 Wisconsin River to Lock and Dam 11	631 – 583	7	1	1
6 Lock and Dam 11 to Lock and Dam 13	583 – 523	13	3	5
7 Lock and Dam 13 to Iowa River	523 – 434	13	4	7
8 Iowa River to Des Moines River	434 – 361	11	3	3
9 Des Moines River to Lock and Dam 21	361 – 325	3	1	2
10 Lock and Dam 21 to Cuivre River	325 – 237	15	9	3
11 Cuivre River to Missouri River	237 – 196	4	4	2
12 Missouri River to Kaskaskia River	196 – 118	6	5	na
13 Kaskaskia River to Ohio River	118 – 0	16	9	na

Appendix 2. Distributions of scores and concentrations of parameters appearing in Table 6.



Appendix Table 3. Acronym dictionary to supplement Table 9 in the Preliminary Analysis of Biological Assessment Thresholds for Determining Aquatic Life Use Attainment Status in the Upper Mississippi River Mainstem.

Environmental Variable	Definition
STRESS	Stressor_Gradient – An index that summarizes information on a variety of anthropogenic stressors. See Angradi et al. 2009.
POPDENS	National Atlas 2000 census data - population per sq mile
WWTPDNS100	Density (#/sq km) major PCS dischargers in 100 km catchment
ANC	Acid neutralizing capacity mg/L CaC03
WWTPDNS50	Density (#/km2) major PCS dischargers in catchment
HARDNESS	Hardness by Calculation (Ca_ppm * Mg_ppm)
TOC_PPM	Total organic carbon in parts per million in water
SOBC	Sum of base cations in water
SI_PPM	Silica in parts per million in sediment
COND	Average conductivity (uS/cm) by site (up to 9 subsamples)
ANSUM	Sum of anions
NTL_PPB	Total nitrogen in parts per billion
TDS	Total dissolved solids (mg/L) in water
NO3_PPB	Nitrate in parts per billion
SO4_PPM	Sulfate in parts per million in water
NH4_PPB	Ammonia in parts per billion
WWTP10C400	Density (#/sq km) major PCS dischargers in 10 km channel riparian
LMXDEPTH	Mean littoral depth shore to 30 m ('mean(fish_hab_depth), both fish transects)
LMXSLOPE	Mean % littoral slope, shore to 30 m ('mean(absolute value(fish_hab_depth(n)-fish_hab_depth(n+1))*100/3) where fish_hab_depth>0, both fish transects)
PHEOA	Pheophytin µg/l
TEMPC	Average water temperature (C) by site
TURBIDITY	Average turbidity (ntu) by site
TSS_MGL	Total suspended solids in mg/L
CHLA	Chlorophyll a µg/l
HYDROLOGY_SC	Hydrology Index – An index that summarizes information from measures of hydrologic condition given by various combinations of magnitude, variation and predictability in monthly and annual flows. See Taylor et al. (manuscript in review).
POC_MGL	Particulate organic carbon in mg/L
PH	Water column pH
PON_MGL	Particulate organic nitrogen in mg/L
TOTAL_META	Total Metals (ppb) in water
PTL_PPB	Total phosphorus in parts per billion
CL_PPM	Chloride in parts per million

Appendix Table 3. (continued)

Environmental Variable	Definition
ORTHOP_PPB	Ortho P in parts per billion
SECCHI	Average secchi depth (cm) by site
LMCVDEPTH	CV of littoral depth, shore to 30 m ('stdev(fish_hab_depth)/mean littoral depth for shore out to 30 m, both fish transects, a measure of littoral substrate unevenness)
DO	Average DO (mg/L) by site (up to 9 subsamples)
LMCVSLOPE	CV of % littoral slope, shore to 30 m, secondary transect ('stdev(absolute value(fish_hab_depth(n)-fish_hab_depth(n+1))*100/3)/mean % littoral slope where fish_hab_depth>0, secondary transects, a measure of littoral substrate unevenness)
HIQVEG	Riparian Quality Index – an index that combines measures of proximity-weighted human disturbances such as roads, structures, agriculture and industries, and the extent of multi-height woody vegetation cover along the banks. See Taylor et al. (manuscript in review).
XWIDRATIO	Mean bankfull/wetted width ratio
CHANCOMPLX	Channel Complexity Index - An index that summarizes information from measures of habitat condition including floodplain connectivity, presence of islands and backwaters, channel patterns, and channel constraints. See Taylor et al. (manuscript in review).
SUBSTRATE	Substrate Index - An index that summarizes information from measures of substrate size and bank stability. See Taylor et al. (manuscript in review).