

## The impact of rare taxa on a fish index of biotic integrity

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### ABSTRACT

The index of biotic integrity (IBI) is a commonly used bioassessment tool that integrates abundance and richness measures to assess water quality. In developing IBIs that are both responsive to human disturbance and resistant to natural variability and sampling error, water managers must decide how to weigh information about rare and abundant taxa, which in turn requires an understanding of the sensitivity of indices to rare taxa. Herein, we investigated the influence of rare fish taxa (within the lower 5% of rank abundance curves) on IBI metric and total scores for stream sites in two of Minnesota's major river basins, the St. Croix ( $n = 293$  site visits) and Upper Mississippi ( $n = 210$  site visits). We artificially removed rare taxa from biological samples by (1) separately excluding each individual taxon that fell within the lower 5% of rank abundance curves; (2) simultaneously excluding all taxa that had an abundance of one (singletons) or two (doubletons); and (3) simultaneously excluding all taxa that fell within the lower 5% of rank abundance curves. We then compared IBI metric and total scores before and after removal of rare taxa using the normalized root mean square error (nRMSE) and regression analysis. The difference in IBI metric and total scores increased as more taxa were removed. Moreover, when multiple rare taxa were removed, the nRMSE was related to sample abundance and to total taxa richness, with greater nRMSE observed in samples with a larger number of taxa or sample abundance. Metrics based on relative abundance of fish taxa were less sensitive to the loss of rare taxa, whereas those based on taxa richness were more sensitive, because taxa richness metrics give more weight to rare taxa compared to the relative abundance metrics.

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### 1. Introduction

In any snapshot of a biological community there will be a suite of taxa at varying degrees of abundance. The relative contribution of taxa richness and abundance to the integrity of ecosystems is a central question in ecology (Loreau, 2000; Gessner et al., 2004), and has important implications for the design of management tools that track ecosystem quality. In particular, the significance of rare taxa (i.e., few in numbers or absent from most samples) to assessments of aquatic system health has been the object of some debate (Cao et al., 1998; Marchant, 1999, 2002; Cao and Williams, 1999; Cao et al., 2001; Van Sickle et al., 2007; Lavoie et al., 2009). On the one hand, common or abundant taxa may provide an adequate indication of ecosystem health, with rare taxa not

contributing significant additional information (Marchant, 1999, 2002; Van Sickle et al., 2007; Lavoie et al., 2009). Moreover, efforts to account for the true abundance of rare taxa in the field are prone to large sampling variance, which in turn may create statistical noise that bias observed patterns in biological communities; more time is also required to identify rare taxa (Gauch, 1982). Alternatively, rare taxa may exhibit higher sensitivity to environmental disturbances and have more specialized habitat requirements, and serve as early indicators of water quality degradation or of the loss of particular habitat types (Cao et al., 1998; Cao and Williams, 1999).

The index of biological integrity (IBI) is a multi-metric tool used to evaluate aquatic ecosystem health and water quality (Karr, 1981; Karr et al., 1986; Karr and Chu, 1997). The component metrics of the IBI typically measure either taxa richness (i.e., number of taxa) or relative abundance of specific taxa; and are selected to represent different aspects of community ecology, such as tolerance to pollution, trophic structure and the health of individual organisms (Karr and Chu, 1997). The values or scores of the component metrics vary in response to human disturbance, and together, provide an overall picture of ecosystem condition.

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The IBI has been widely adopted by water management agencies in the US to support water quality impairment decisions under the Clean Water Act (Environmental Protection Agency, 2002). In Minnesota, for example, the IBIs for fish assemblages (F-IBI) are used in conjunction with other relevant habitat, water chemistry and catchment data to determine whether stream sites should be listed as impaired as required by the section 303(d) of Clean Water Act.

The effect of rare taxa on bioassessments has been studied most commonly for macroinvertebrate assemblage indicators (reviewed by Lavoie et al., 2009), but the influences of rare taxa on a fish assemblage index are relatively unknown. The objective of this study was to investigate the sensitivity of fish metrics and F-IBI scores to rare taxa. To conduct this analysis, we used F-IBIs developed by the Minnesota Pollution Control Agency (MPCA) for stream systems in two of Minnesota's major river basins (Niemela and Feist, 2000, 2002). We quantified the effect of rare taxa by comparing F-IBI metric and total scores calculated from original field samples with those calculated after rare taxa had been removed. For each sample in our dataset, we assessed the influence of rare taxa using three different approaches: (1) separately excluding each individual taxon that fell within the lower 5% of rank abundance curves; (2) simultaneously excluding all taxa that had an abundance of one (singletons) or two (doubletons); and (3) simultaneously excluding all taxa that fell within the lower 5% of rank abundance curves.

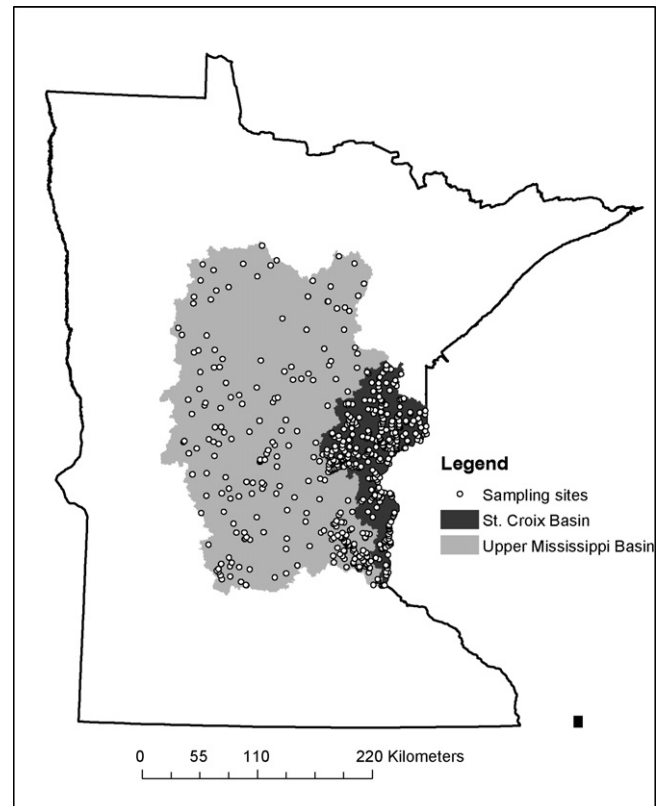
## 2. Data and methods

### 2.1. Data

We analyzed fish data and IBI scores from 378 stream sites in two major river basins of Minnesota: the St. Croix and Upper Mississippi (Fig. 1). Each sampling site was visited between one and seven times, for a total of 293 site visits for the St. Croix basin and 210 site visits for the Upper Mississippi basin (hereafter, we use sample and site visit interchangeably). There were 100 taxa in the dataset with a mean  $\pm$  SE:  $19.38 \pm 7.30$  per sample. The maximum and minimum numbers of taxa in one sample were 36 and 3, respectively. The IBIs were developed exclusively for warmwater streams; thus, no coldwater streams are included in our analysis. To stratify natural variability across warmwater stream sites, the MPCA classified the IBIs by major river basin, drainage area and ecoregion. The IBIs for sites in each of these classes include slightly different metrics and metric scoring criteria (Tables 1 and 2), and the total scores for IBIs in all classes were scaled to the range 0–100. Stream sites included in this study spanned a broad range of F-IBI scores and sizes of drainage area, as well as two ecoregions (Niemela and Feist, 2000, 2002; Table 2). Fish samples used in IBI calculations were collected by the MPCA during summer low-flow conditions from 1996 to 2006. Fish were captured using electrofishing and were subsequently identified to the lowest possible taxonomic level (typically species).

### 2.2. Statistical analyses

The concept of rare taxa is relative and is defined in practice according to taxonomic group, assessment method and specified research goal (Cao et al., 1998; Magurran, 2004). In this study, rare taxa were defined as those taxa within the lower 5% of the rank abundance curve for a sample (Murray et al., 1999). We assessed the influence of rare fish taxa on the Minnesota F-IBI metric and total scores using three different resampling procedures. In the first procedure, an individual taxon identified as rare was removed and the resampled IBI score was calculated. This procedure was repeated for each rare taxon in the sample and for all samples in



**Fig. 1.** Stream sites in the Upper Mississippi and St. Croix River basins that were used to estimate the effect of rare taxa on the fish index of biotic integrity in Minnesota.

the dataset ( $N = 3101$ ). The second procedure investigated the influence of all taxa that had an abundance of one (singletons) or two (doubletons). In this analysis, all the singletons and doubletons were removed from the fish sample and the resampled IBI score was calculated. The procedure was repeated for all the samples that contained singletons and/or doubletons ( $N = 467$ ). In the last procedure, we investigated the role that all rare taxa had on the IBI by removing all rare taxa from each sample in the dataset ( $N = 503$ ) and recalculating IBI scores.

We compared the resampled metric and total scores to the corresponding original scores using two methods. The first method calculated the normalized root mean square error (nRMSE) between the suite of metric scores in the original sample and the suite of metric scores in the resampled sample. This statistic is a relative measure of the difference between the original and resampled IBI scores. The nRMSE was defined as follows:

$$\text{nRMSE} = \frac{100 \times \sqrt{(\sum_{i=1}^k (IBI_{O,i} - IBI_{R,i})^2)/k}}{\max(IBI_i) - \min(IBI_i)}$$

where  $IBI_{O,i}$  is the IBI  $i$ th metric score from the original sample and  $IBI_{R,i}$  is the IBI  $i$ th metric score from the resampled sample, and  $k$  is the number of metrics employed. The  $\max(IBI_i)$  and  $\min(IBI_i)$  are the maximum and minimum metric scores across all the metrics in the original and resampled samples. The nRMSEs were then categorized by taxa richness and sample total abundance to examine the effects of sample size.

Although nRMSEs have the advantage of representing change by a single number, it does not capture possible trends in resampled IBI scores over the range of the original IBI scores. For this reason, we also determined the best fit linear regression ( $R^2$ , slope, and intercept) between the original IBI and the corresponding resampled scores for each of the IBI metrics, thereby providing

**Table 1**

IBI metrics used in the Minnesota fish index of biotic integrity. Metrics are ordered based on their types: taxa richness, normalized total abundance and relative abundance. Taxa richness indicates a suite of metrics used to assess the number of specified taxa in a sample, the only normalized total abundance metric standardizes the total abundance of a sample by distance, and relative abundance indicates a suite of metrics used to assess the proportion of the specified taxa in abundance.

Type	Name	Meaning
Taxa richness	BenInsect	The number of benthic insectivore taxa
	CountofTaxa	The total number of taxa
	Darter	The number of darter taxa
	DarterSculpNot	The number of taxa designated as darter, sculpin, and madtoms taxa
	Insect-Tolerant	The number of insectivore taxa minus the number of tolerant taxa
	Headwater-Tolerant	The number of headwater taxa minus the number of tolerant taxa
	Minnows-Tolerant	The number of minnow taxa minus the number of tolerant taxa
	Omnivore	The number of omnivore taxa
	Piscivore	The number of piscivore taxa
	Sensitive	The number of sensitive taxa
Normalized total abundance	NumberPer100m-Tolerant	The abundance of fish per 100 m minus that of tolerant taxa
Relative abundance	DomTwoPct	The percentage of total abundance of the two most dominant taxa
	FishDELTpct	The percentage of individuals with deformities, lesions, or tumors
	OmnivorePct	The percentage of total abundance of the omnivore taxa
	PiscivorePct	The percentage of total abundance of the piscivore taxa
	SlithoPct	The percentage of total abundance of the lithophilic taxa
	TolerantPct	The percentage of total abundance of the tolerant taxa

**Table 2**

The scoring metrics used in each type of fish index of biotic integrity (F-IBI) calculation in Minnesota. See Table 1 for description of the metrics.

Drainage basin	Drainage area (DA, km <sup>2</sup> )	Ecoregion	Metrics used
Saint Croix	DA < 52		CountofTaxa, Headwater-Tolerant, Minnows-Tolerant, TolerantPct, DomTwoPct, Insect-Tolerant, SLithopPct, NumberPer100m-Tolerant, FishDELTpct
	52 ≤ DA < 140		CountofTaxa, Sensitive, Minnows-Tolerant, TolerantPct, DomTwoPct, BenInsect, SLithopPct, NumberPer100m-Tolerant, FishDELTpct
	140 ≤ DA ≤ 699		CountofTaxa, Darter, Sensitive, TolerantPct, BenInsect, Omnivore, PiscivorePct, SLithopPct, NumberPer100m-Tolerant, FishDELTpct
	DA > 699	Northern Lakes and Forests	CountofTaxa, Darter, Sensitive, TolerantPct, BenInsect, Omnivore, PiscivorePct, SLithopPct, NumberPer100m-Tolerant, FishDELTpct
	DA > 699	North Central Hardwood Forest	CountofTaxa, Darter, Sensitive, TolerantPct, BenInsect, Omnivore, PiscivorePct, SLithopPct, NumberPer100m-Tolerant, FishDELTpct
Upper Mississippi	DA < 13		CountofTaxa, Wetland-Tolerant, TolerantPct, DomTwoPct, Insect-Tolerant, NumberPer100m-Tolerant, FishDELTpct
	13 ≤ DA < 91		CountofTaxa, Wetland-Tolerant, Minnows-Tolerant, Sensitive, TolerantPercent, DomTwoPct, Insect-Tolerant, SLithopPercent, NumberPer100m-Tolerant, FishDELTpct
	91 ≤ DA ≤ 518		CountofTaxa, DarterSculpNot, Wetland-Tolerant, Sensitive, TolerantPct, SLithopPct, Piscivore, SLithopPct, NumberPer100m-Tolerant, FishDELTpct
	DA > 518		CountofTaxa, DarterSculpNot, Sensitive, TolerantPct, LithopPct, Piscivore, SLithopPercent, NumberPer100m-Tolerant, FishDELTpct, OmnivorePct

an indication of the similarity between the original metric scores and the resampled metric scores. The coefficient of determination ( $R^2$ ) is a dimensionless measure of goodness-of-fit of the regression line between the two sets of IBI scores. The slope and intercept demonstrated whether the scores increased or decreased after rare taxa were removed, and are therefore important in interpreting the regression results.

Finally, we sought to understand how the impacts of rare taxa on IBI scores might be affected by the drainage area of stream site. We used box plots to demonstrate how taxa richness, total abundance, and nRMSEs for the three rare taxa removal procedures varied with drainage area across the following five drainage area classes (km<sup>2</sup>): <5, 5–15, 15–30, 30–115, and >115, respectively.

### 3. Results

#### 3.1. Single rare taxa removal

The removal of single rare taxa generally resulted in a slight reduction in IBI metric scores. Slopes of regression lines and  $R^2$

values were close to one for most metrics (mean ± SE  $R^2$ : 0.93 ± 0.05, Table 3). The lowest  $R^2$  (0.82 and 0.84) and slope (0.90 and 0.89) values were observed for the *Wetland-Tolerant* and *Headwater-Tolerant* metrics, respectively. Resampled metric scores based on richness were more likely to deviate from the original scores (mean ± SE  $R^2$ : 0.90 ± 0.04) relative to resampled metric scores based on relative abundance, which corresponded closely to the original scores (mean ± SE  $R^2$ : 0.99 ± 0.01). Regression intercept values for the regression analysis were generally close to zero, except for the *Omnivore* and *CountofTaxa* metrics (0.59 and −0.36, respectively). A positive intercept indicated an increase in the resampled IBI metric score for, at least, small original IBI metric scores.

A summary of the impact of the removal of single rare taxa on the IBI total score is shown in Fig. 2A. Changes in the resampled scores relative to original scores ranged from a 7-point increase to a 17-point decrease. The median change in IBI total score was approximately 0. Finally, the nRMSE between original and resampled IBI metric scores reflected a relatively minor impact of the removal of single rare taxa. The nRMSE values did not appear to be related to the number of taxa in the sample and the total abundance in the sample (Fig. 3A).

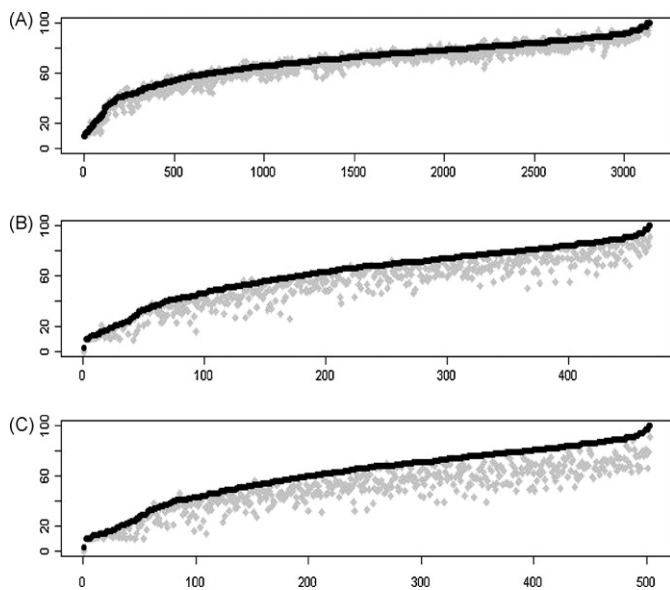
**Table 3**

Fit ( $R^2$ ) between original IBI metric scores and resampled IBI metric scores. The three scenarios examined were: (1) single taxa were removed one at a time from the sample, (2) all singletons and doubletons were removed from the sample, and (3) all rare taxa within the lower 5% of the rank abundance curve were removed from the sample.  $N$  is the number of samples where a metric was employed.

Metric	Single taxon				Singletons and doubletons				All rare taxa			
	$N$	$R^2$	Slope	Intercept	$N$	$R^2$	Slope	Intercept	$N$	$R^2$	Slope	Intercept
BenInsect	1691	0.88	0.97	-0.18	214	0.54	0.70	-0.05	216	0.38	0.51	0.09
CountofTaxa	3101	0.90	0.99	-0.36	467	0.62	0.77	-1.24	503	0.37	0.43	-0.46
Darter	1248	0.93	0.97	-0.08	130	0.54	0.73	-0.07	130	0.39	0.58	-0.08
DarterSculpNot	870	0.92	0.97	-0.07	114	0.57	0.71	-0.05	115	0.40	0.49	0.58
Insect-Tolerant	1410	0.88	0.93	0.00	253	0.65	0.68	-0.13	287	0.52	0.53	0.02
Headwater-Tolerant	242	0.84	0.89	0.16	62	0.70	0.80	-0.03	77	0.60	0.64	0.21
Minnows-Tolerant	948	0.92	0.93	-0.04	206	0.75	0.80	-0.29	233	0.64	0.60	0.01
Omnivore	1248	0.95	0.94	0.59	130	0.57	0.52	4.89	130	0.32	0.29	7.11
Piscivore	870	0.89	0.94	-0.06	114	0.45	0.52	0.00	115	0.16	0.26	0.25
Sensitive	2824	0.94	0.98	-0.11	388	0.68	0.74	-0.10	401	0.49	0.55	0.03
Wetland-Tolerant	643	0.82	0.90	0.19	133	0.53	0.70	0.10	152	0.41	0.55	0.06
NumberPer100m-Tolerant	3101	0.93	1.00	0.00	467	0.88	0.99	0.00	503	0.81	0.97	0.00
DomTwoPct	983	0.97	1.00	-0.11	223	0.92	0.98	-0.37	258	0.91	0.95	-0.31
FishDELTpct	3101	0.98	0.98	0.19	467	0.92	0.93	0.73	503	0.99	1.00	0.00
OmnivorePct	525	1.00	1.00	0.00	58	0.99	1.01	-0.08	58	0.99	1.01	-0.08
PiscivorePct	1248	0.99	1.00	0.00	130	0.98	1.00	-0.11	130	0.95	1.01	-0.37
SLithopPct	3066	0.99	1.00	0.04	450	0.98	1.01	0.02	478	0.97	1.01	0.02
TolerantPct	3101	0.99	1.00	0.00	467	0.98	1.00	-0.09	503	0.98	1.00	-0.10

### 3.2. Removal of all singletons and doubletons

Singletons and doubletons typically comprised less than 2% of total sample abundance. Nevertheless, the removal of all singletons and doubletons caused resampled metric scores based on taxa richness to deviate from the original scores, indicated by relatively low  $R^2$  values (mean  $\pm$  SE  $R^2$ :  $0.60 \pm 0.09$ , Table 3). The greatest change was observed for the *Piscivore* metric, indicated by a relatively poor fit between the original and resampled scores ( $R^2 = 0.45$ ). Alternatively, the *Minnows-Tolerant* metric was relatively insensitive to the removal of singletons and doubletons ( $R^2 = 0.75$ ). As opposed to the taxa richness metrics, the resampled scores of relative abundance metrics corresponded closely to the original scores (mean  $\pm$  SE  $R^2$ :  $0.96 \pm 0.03$ ). The *OmnivorePct*, *PiscivorePct*, *SlithoPct*, and *TolerantPct* metrics were particularly insensitive to the removal of singleton and doubletons ( $R^2 = 0.99, 0.98, 0.98$  and  $0.98$ , respectively).



**Fig. 2.** Original IBI total score (black solid circles) and resampled IBI total score (grey solid diamonds) after the removal of (A) a single rare taxon, (B) removal of all singletons and doubletons, and (C) removal of all rare taxa. The x-axis denotes the ranks of the original scores and the y-axis denotes the score values.

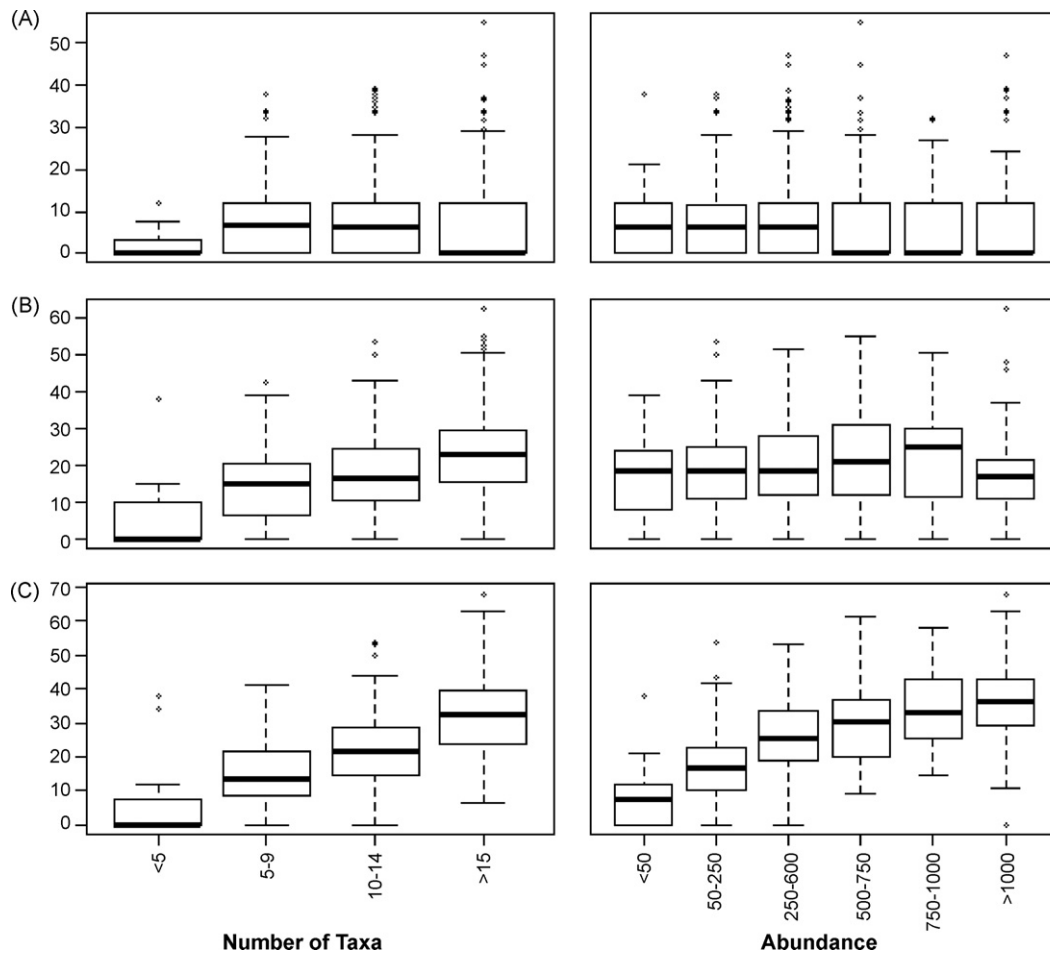
The slopes indicated similar patterns to the  $R^2$  values (Table 3). The slopes for the taxa richness metrics were much less than one (mean  $\pm$  SE slope:  $0.70 \pm 0.10$ ), with the lowest slope values observed for the *Piscivore* and *Omnivore* metrics (0.52 and 0.52, respectively). The slopes for all relative abundance metrics were approximately one (mean  $\pm$  SE slope:  $0.99 \pm 0.03$ ). As for the removal of single taxa, the *Omnivore* and *CountofTaxa* metrics had the largest absolute intercepts (4.89 and -1.24, respectively).

Removal of all singletons and doubletons from the sample indicated a general decrease in the IBI total score (Fig. 2B). Changes in the resampled scores ranged from a 5-point increase to a 33-point decrease. The median decrease in IBI total score was 8. The nRMSE between original and resampled IBI metric scores increased in response to the taxa richness of the sample; the nRMSE had slight trend of increasing for the first five abundance classes, indicated either by the lower quantile, higher quantile or median on the boxplot (Fig. 3B).

### 3.3. Removal of all rare taxa

The removal of all rare taxa caused the largest change in resampled metric scores relative to original metric scores (Table 3). Metrics based on taxa richness exhibited a greater lack of fit between the original and resampled IBI scores (mean  $\pm$  SE  $R^2$ :  $0.43 \pm 0.13$ ). The resampled *Piscivore* scores deviated most from the original scores ( $R^2 = 0.16$ ). As with the other resampling procedures, the metrics based on relative abundance did not deviate significantly from the original values when all rare taxa were excluded (mean  $\pm$  SE  $R^2$ :  $0.97 \pm 0.03$ ). The slopes indicated similar patterns as  $R^2$  values. The slopes for the taxa richness metrics were much less than one (mean  $\pm$  SE slope:  $0.49 \pm 0.12$ ) with the lowest slope values observed for the *Piscivore* and *Omnivore* (0.16 and 0.32, respectively). The slopes for all relative abundance metrics were close to one (mean  $\pm$  SE slope:  $1.00 \pm 0.02$ ). The *Omnivore* had the largest absolute intercept (7.11).

Removal of all rare taxa from the sample caused a general decrease in the IBI total score (Fig. 2C). The changes in the score ranged from a 5-point increase in the resampled score to a 38-point decrease. The median decrease in IBI total score was 11. When all rare taxa were removed, the nRMSE between original and resampled metric scores responded strongly to the taxa richness and total abundance (Fig. 3C). The nRMSE increased as the number of taxa and total sample abundance increased.



**Fig. 3.** Box plots of normalized RMSE and number of taxa and abundance for (A) removal of single rare taxa, (B) removal of all singletons and doubletons, and (C) removal of all rare taxa. The y-axis denotes nRMSE values. The x-axis of the left panel denotes the richness classes: <5, 5–9, 10–14, and >15 taxa in a sample, respectively; the x-axis of the right panel denotes the abundance classes: <50, 50–250, 250–500, 500–750, 750–1000, and >1000 individuals in a sample, respectively. The lower and upper boundaries of a box are placed at the first and third quartiles of the nRMSE values for each class, and the thick line within the box is the median nRMSE value for each class.

### 3.4. Impacts of drainage area

Median taxa richness at the smallest drainage areas (<5 km<sup>2</sup>) was 7 and increased gradually to 24 at the largest drainage areas (>115 km<sup>2</sup>) (Fig. 4A). Median taxa abundance at the smallest drainage areas was 184 and increased gradually to 484 at the fourth (30 km<sup>2</sup> < area < 115 km<sup>2</sup>) drainage area class, with a similar median value of 474 at the largest drainage areas (Fig. 4B). Median nRMSE for the single rare taxa removal procedure did not indicate a trend as drainage area increased (Fig. 4C). Median nRMSE for the removal procedure of all singletons and doubletons increased gradually from 15.12 at the smallest drainage areas to 24.29 at the largest drainage areas (Fig. 4D). Median nRMSE for the removal procedure of all rare taxa within the lower 5% of rank abundance curve increased from 17.95 at the smallest drainage areas to 31.62 at the largest drainage areas, with similar values of 22.71 and 21.45 at the second and third drainage area classes (5 km<sup>2</sup> < area < 15 km<sup>2</sup>, 15 km<sup>2</sup> < area < 30 km<sup>2</sup>), respectively (Fig. 4E).

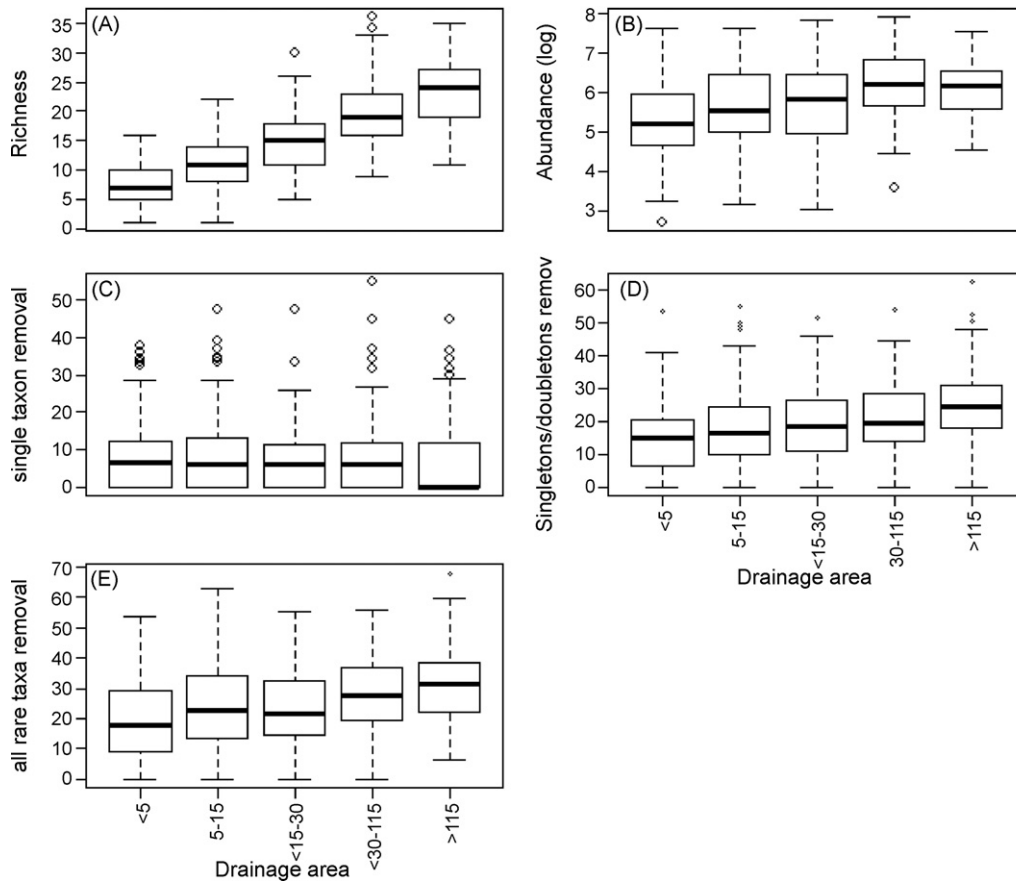
## 4. Discussion

### 4.1. Metric sensitivity

Evaluation of metric sensitivity is an essential process in developing sound bioassessment tools (Hughes et al., 1998). Ideally, managers should seek indicators of stream health that

exhibit maximum sensitivity to human disturbance and minimal sensitivity to sampling error and natural variability. In striking this balance, decisions about the emphasis given to rare taxa can be particularly difficult, since these taxa may be both highly sensitive to water quality condition and especially prone to sampling error. At the very least, such decisions should be supported by the knowledge of how sampling errors related to rare taxa could affect overall assessment outcome.

In our study of two watersheds in Minnesota, we sought to understand how rare taxa could affect IBI metric and total scores by comparing scores before and after rare taxa were removed. Our analysis indicated that the sensitivity of fish metrics to rare taxa depends on whether these metrics are based on taxa richness or relative abundance. Taxa richness metrics (i.e., *BenInsect*, *CountofTaxa*, *Darter*, *DarterSculpNot*, *Insect-Tolerant*, *Headwater-Tolerant*, *Minnows-Tolerant*, *Omnivore*, *Piscivore*, *Sensitive*, *WetlandTolerant*, *Minnows-Tolerant*) were more sensitive to the removal of rare taxa than abundance metrics (i.e., *DomTwoPct*, *FishDELTpct*, *OmnivorePct*, *PiscivorePct*, *SlithopPct*, *TolerantPct*), particularly when multiple rare taxa were removed. This outcome is not surprising because taxa richness metrics assign the same weight to all the taxa, regardless of whether they are rare, whereas relative abundance metrics are determined primarily by abundant (non-rare) taxa. For example, singletons and doubletons usually accounted for less than 2% of total abundance of a sample. The removal of these taxa would thus not be expected to strongly affect abundance metrics. The resampled scores of all the metrics tended to be lower than the



**Fig. 4.** Box plots of drainage area impacts. The y-axes denote (A) taxa richness, (B) log abundance, (C) nRMSE for the removal of single taxa, (D) nRMSE for the removal of all singletons and doubletons, and (E) nRMSE for the removal of all rare taxa, respectively. The x-axes denote the drainage area classes (km<sup>2</sup>): <5, 5–15, 15–30, 30–115, and >115, respectively. The lower and upper boundaries of a box are placed at the first and third quartiles of the y-values for each drainage area class, and the thick line within the box is the median values for each class.

original scores, except the *Omnivore* metric, because it was designed to have a higher score for lower abundance. The *Piscivore* metric demonstrated the greatest sensitivity to the removal of multiple taxa, likely because piscivorous species typically occur as only a few individuals in any given sample (i.e., most piscivore species are rare).

#### 4.2. Removal levels

The influence of rare taxa on the Minnesota F-IBI was related to the number of rare taxa excluded from fish samples. Removal of a single taxa from a sample resulted in a relatively small change in IBI total score. When all singletons and doubletons were removed, however, there was a marked influence on IBI total score. This decrease in scores is of practical importance for managers, given that a large percentage of fish samples in our dataset (93%) had at least one taxon that occurred only as a singleton or doubleton, and that failure to capture taxa that occur with such rarity could easily result from sampling error. Changes in IBI scores were greatest following the removal of all taxa within the lower 5% of the rank abundance curves. Although failure to capture all rare taxa at a stream site may not represent a likely outcome, the large change in IBI scores that can occur when rare taxa are excluded illustrates that fish IBI scores in Minnesota tend to greatly weight the importance of rare taxa.

In Minnesota, IBI total scores are used to determine whether stream sites should be placed on the Clean Water Act 303(d) impaired waters list. To make these water quality determinations, the Minnesota Pollution Control Agency (MPCA) has established an

“impairment threshold” or an IBI total score below which streams communities can be considered impaired, for each stream class in Minnesota. The MPCA also designates a confidence limit around the impairment threshold ( $\pm 9$  points for the St. Croix River basin and  $\pm 13$  points for the Upper Mississippi River basin) that is based on variability of IBI total scores at least impacted sites over time (MPCA, 2007). IBI total scores falling above and below this confidence region are considered unimpaired or impaired in relation to the given threshold, respectively. IBI total scores within the confidence region of the threshold are considered potentially impaired, with additional evidence required to verify status. Our analyses indicated that the median decrease in IBI total scores resulting from the exclusion of multiple rare taxa is proximate to the magnitude of the confidence limits around the impairment thresholds used by the MPCA, and the maximum decreases from all three levels of rare taxa removal far exceed that. Thus, failure to capture rare taxa as the result of inadequate sampling (e.g., Reynolds et al., 2003) could alter the outcome of impairment decisions based on F-IBIs.

#### 4.3. Taxa richness, total abundance and drainage area

The influence of rare taxa on metric scores was also related to the taxa richness and total abundance of the sample and the drainage area of the stream site. The amount of error associated with the removal of all rare taxa was higher for samples that had greater fish abundance. This relationship likely reflects the fact that, in large assemblages, there tend to be more rare taxa than a log-normal model predicts (Nee et al., 1999; Magurran and Henderson, 2003). Other studies have also suggested that richness

metrics are sensitive to sample abundance. In a study of macroinvertebrate metrics, Barbour and Gerritsen (1996) found that richness metrics were more affected by sample abundance than relative abundance metrics. A higher proportion of rare taxa in large assemblages relative to small assemblages could cause taxa richness metrics in large samples to vary relatively more in response to the exclusion of rare taxa.

Metric error following taxa removal was also higher for samples that had higher taxa richness, likely because more diverse fish samples are more likely to contain rare taxa. Greater taxa richness tends to indicate less human impact, and presumably rare taxa are more likely to thrive in unimpacted conditions. In contrast, samples with few taxa are likely to be more disturbed and harbor few sensitive rare taxa. For example, Oberdorff and Hughes (1992) described entire families extirpated or threatened in basins with a history of anthropogenic disturbance. An increased number of rare taxa in taxa-rich samples would render metrics and IBI scores for these samples more sensitive to rare taxa removal.

We found an indication that the number of fish taxa and the fish abundance were related to drainage area. Not surprisingly (see Connor and McCoy, 1979), smaller drainage areas had fewer fish taxa than larger drainage areas; there was more than a threefold increase in the number of taxa from the smallest to the largest drainage area classes in our study. As the result, rare taxa account of larger portion of IBI variance at the sites with larger drainage areas. Indeed, in an analysis of F-IBI sensitivity to random sampling error, Dolph et al. (2010) suggested that IBI sensitivity was related to the number of rare taxa in a sample, which was in turn strongly related to stream drainage size. Therefore, we suggest that greater effort and care should be placed on streams with larger drainage areas to effectively sample all the available taxa.

## 5. Conclusions

Our assessment of the effect of rare taxa on F-IBIs using resampling procedures indicated that rare taxa contribute much of the information to fish assemblage metrics based on taxa richness. When managers create multi-metric indices, they may thus want to carefully consider the weight given to metrics that rely on rare taxa. In designing sampling protocols for fish, our analyses point to the need to understand the relationship between sampling effort and the likelihood of capturing one or more additional rare taxa, especially in larger streams. If IBIs include many taxa richness metrics, it may be beneficial to spend additional time capturing rare taxa, especially in more diverse or abundant communities.

Rare taxa are often viewed as indicative of higher biodiversity and a well-functioning ecosystem. Indeed, Davies and Jackson (2006) listed “sensitive-rare taxa” as one of the ten attributes of a stream system that define the Biological Condition Gradient (BCG), which is rapidly becoming the foundation of bioassessment approaches. However, an effective bioassessment tool must operate not only with sensitivity, but also with precision and efficiency. Our study, combined with the existing scientific literature, suggests that each taxonomic group, e.g., macroinvertebrate, periphyton, or fish, may need to be considered differently with regards to these factors. For example, we have shown that the removal of all fish that occur as singletons and doubletons can substantially affect the IBI metric and total scores, despite the fact that these individuals usually account for no more than 2% of sample abundance. In contrast, Lavoie et al. (2009) demonstrated that a bioassessment tool using diatoms was not markedly affected by removing 40% of the taxa, likely because diatoms have much higher biodiversity. We suggest a meta-analysis could be conducted to evaluate how rare taxa might differentially affect bioassessment based on different types of assemblages.

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