Chin. Geogra. Sci. 2019 Vol. 29 No. 6 pp. 974–984
https://doi.org/10.1007/s11769-019-1083-6

Springer Science Press
www.springerlink.com/content/1002-0063

A Macroinvertebrate Multimetric Index for the Bioassessment of Wetlands Adjacent to Agriculture Fields in the Sanjiang Plain, China

WU Haitao[1], LU Kangle[1, 2], LYU Xianguo[1], XUE Zhenshan[1]

(1. *Key Laboratory of Wetland Ecology and Environment*, *Northeast Institute of Geography and Agroecology*, *Chinese Academy of Sciences*, *Changchun* 130102, *China*; 2. *University of Chinese Academy of Sciences*, *Beijing* 100049, *China*)

Abstract: Adjacent intensive agriculture disturbs the natural condition of wetlands. However, to assess the effect of this agriculture on wetlands, few studies have used indices based on aquatic invertebrates. Multi-metric indices (MMIs) have been successfully used to assess freshwater ecosystems worldwide and are an important management tool, but little is known about their applicability in the Sanjiang Plain, Northeast China. In this study, we developed a MMIs for aquatic invertebrates to assess freshwater wetlands in this region. The aquatic invertebrate assemblages were sampled in 27 wetlands in the Sanjiang Plain that included those in natural reserves and those affected by adjacent, intensive agriculture. Twenty-four candidate metrics were initially reviewed and screened before four core metrics were selected: total number of taxa, number of Hemiptera taxa, proportion of Gastropoda, and proportion of predators. Mann-Whitney *U* tests, Box and Whisker plots, correlation analyses, and redundant metric tests were used to assess the ability of metrics to distinguish among reference and impaired wetlands. Four ordinal rating categories for wetland were defined: poor, fair, good, and excellent. Of the impaired freshwater wetlands, 76.2% were in poor or fair categories. The MMIs was robust in discriminating reference wetlands from impaired wetlands and therefore have potential as a biomonitoring tool to assess the condition and to guide the restoration efforts of freshwater wetlands in Northeast China.

Keywords: bio-assessment; Hemiptera; Mollusca; multi-metric indices (MIS); bioassessment of wetland; the Sanjiang Plain

Citation: WU Haitao, LU Kangle, LYU Xianguo, XUE Zhenshan, 2019. A Macroinvertebrate Multimetric Index for the Bioassessment of Wetlands Adjacent to Agriculture Fields in the Sanjiang Plain, China. *Chinese Geographical Science*, 29(6): 974–984. https://doi.org/10.1007/s11769-019-1083-6

1 Introduction

Wetlands are widely distributed, covering 5%−8% of the land surface of the Earth (Mitsch and Gosselink, 2007; Rydin and Jeglum, 2013). Wetlands provide a diversity of services, including non-replaceable storage of carbon and nitrogen, regulation of climate, supply of water, and conservation of biodiversity (Bullock and Acreman, 2003; Verhoeven et al., 2006; Mitsch and Gosselink, 2007; Wu et al., 2009). Human activities have caused persistent losses of wetlands for centuries, substantially altering the landscape and compromising the function of natural ecosystems. The Sanjiang Plain in Northeast China is one of the most important temperate freshwater wetland regions in the world and plays an irreplaceable role in maintaining regional ecological security. From the 1950s, total wetland area in the Sanjiang Plain has decreased from 35 270 km^2 to 8100 km^2, and most remaining wetlands have been impacted by humans to varying degrees, although small areas of relatively pris-

Received date: 2018-12-06; accepted date: 2019-03-12

Foundation item: Under the auspices of National Key Research and Development Project of China (No. 2016YFC0500408), National Natural Science Foundation of China (No. 41871099, 41671260), Science and Technology Development Program of Jilin Province (No. 20180101080JC)

Corresponding author: WU Haitao. E-mail: wuhaitao@iga.ac.cn

tine wetlands persist in several nature reserves (Wang et al., 2011; Song et al., 2014). Therefore, the development of scientific tools to assess biological conditions of freshwater wetlands is important for wetland management (Weilhoefer, 2011; Riatoet al., 2018).

In recent years, numerous methods have been developed to assess the ecological condition of freshwater ecosystems. Because of the low costs, rapid field deployment, and use of various metrics at different levels in the biological hierarchy, multi-metric indices (MMIs) have been widely used to assess ecosystem conditions (Baptista et al., 2007; De Bikuña et al., 2015; Ntislidou et al., 2018; Lu et al., 2019).

Aquatic invertebrate assemblages have been widely used as indicators of ecosystem conditions. These communities encompass a diverse group with a wide range of life-history requirements and can provide information about the current conditions of a freshwater wetland, as well as the effects of past, cumulative stressors (Milošević et al., 2016, O'Brien et al., 2016; Odountan et al., 2019). The advantages of using aquatic invertebrates for MMIs include 1) aquatic life stages that respond to a broad range of environmental conditions; 2) being relatively immobile and 3) living in close contact with bottom sediments and the water column (Bonada et al., 2006; Mereta et al., 2013). Thus, aquatic invertebrates have been widely used in MMIs, because they meet many of the criteria that characterize the efficacy of this bio-monitoring tool (De Bikuña et al., 2015; Ntislidou et al., 2018; Fierro et al., 2018). However, most studies advocating the use of aquatic invertebrates in MMIs have primarily focused on streams (Leung and Dudgeon, 2011; Huang et al., 2015; Fierro et al., 2018), lakes (Šidagytė et al., 2013; Ntislidou et al., 2018) and estuaries (Williams et al., 2010). Few studies have applied an aquatic invertebrate-based MMIs approach to freshwater wetlands, particularly in China.

In this study, the aquatic invertebrate assemblages were sampled in 27 wetlands across a range of conditions, from reference wetlands in Natural Reserves to those affected by adjacent, intensive agriculture in the Sanjiang Plain, China. The primary goal was to develop an aquatic invertebrate-based MMIs that was sensitive to the effects of human activity and therefore could be used to evaluate the biological condition of freshwater wetlands in the Sanjiang Plain, Northeast China.

2 Materials and Methods

2.1 Study area

The Sanjiang Plain, formed by the Heilong, Songhua, and Wusuli rivers, has a total area of 10.9 million ha. The plain is in Heilongjiang Province, Northeast China (45°01′N–48°28′N, 130°13′E–135°05′E) (Liu and Ma, 2002), and supports one of the largest freshwater wetlands in China. The wetland areas are strongly affected by agricultural drainage and reclamation efforts. Over the past 60 years, the wetlands have been extensively drained for agriculture production, and 77% of the original wetland area has been lost (Wang et al., 2011). Human activities have also led to fragmentation of the current wetlands.

The study area lies in a seasonally frozen zone with a frost-free period of 125 d and experiences a temperate moist monsoon climate with an annual mean temperature of 2.7℃ and mean annual precipitation of 550 mm. More than 60% of the annual precipitation falls between July and September. The altitude of the study area averages 55 m. Freshwater sedge marsh is the major type of original wetlands in the Sanjiang Plain. Plant species composition varies with water depth, and species includes *Carex pseudocuraica* F. Schmidt., *C. lasiocarpa* Ehrh, *C. meyeriana* Kunth, *C. appendiculata* (Trautv.) Kük, and *C. schmidtii* Meinsh (Wang et al., 2010; Wu et al., 2010; 2017).

2.2 Field sampling and laboratory processing

We sampled 27 wetlands in the Sanjiang Plain (Fig. 1 and Table 1) during March and October of 2013, and March, July, and October of 2017. Aquatic invertebrates were sampled using a D-shaped sweep net (35-cm diameter, 1-mm mesh). Four 1-m horizontal sweeps were performed at randomly selected locations in each wetland. As the nets sieved the water column, scraped the bottom, and swept submersed and emergent plant surfaces, a fairly complete sample of the invertebrate community was collected (Batzeret al., 2001). The four 0.35 m × 1.00 m sub-samples per wetland per sampling date were deposited into labeled plastic bags and preserved in 95% alcohol. In the laboratory, the samples were hand sorted and invertebrates were identified and counted. Aquatic invertebrates were identified to the lowest taxonomic level practical using standard references (Clifford, 1991; Morse et al., 1994). Aquatic invertebrate data were expressed as means that were calculated for each wetland.

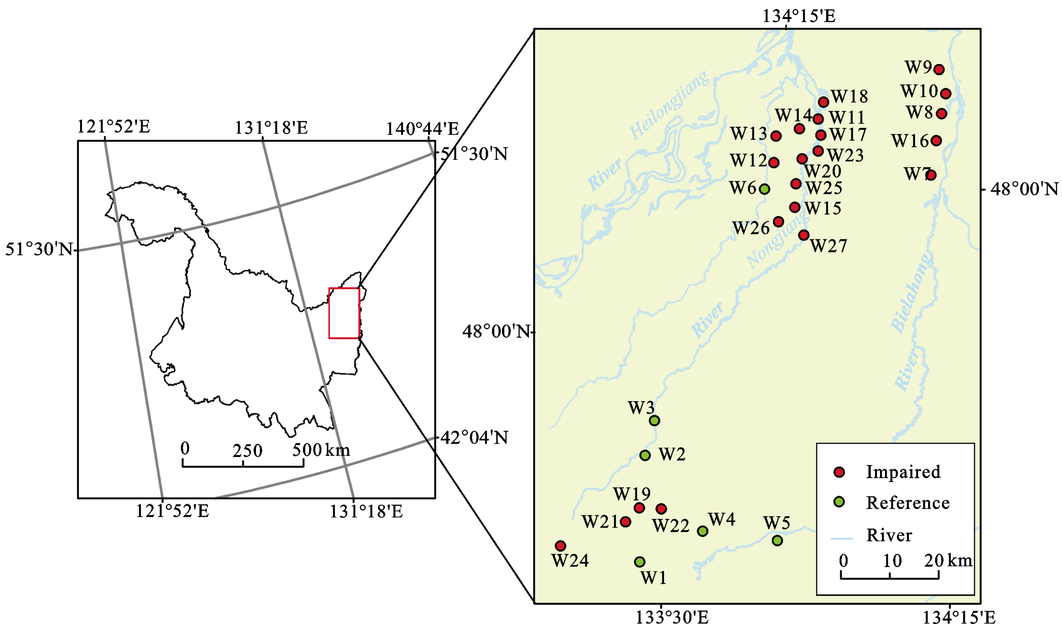

Fig. 1 The locations of the 27 wetlands sampled in the Sanjiang Plain, Northeast China. The wetlands are defined by the number labels (green circle, reference wetland; red circle, impaired wetland) in Table 1

Table 1 The 27 study wetlands locations and general characteristics of the Sanjiang Plain in Northeastern China

No.	Wetland Name	Latitude/Longitude	Anthropogenic impacts	Hydrology	Category
W1	Sanjiang Station marsh	47°35'10"N, 133°30'03"E	Minimal, long term research site	Perennially flooded	Reference
W2	Honghe Reserve marsh 1	47°45'07"N, 133°35'11"E	Minimal, natural reserve	Perennially flooded	Reference
W3	Honghe Reserve marsh 2	47°47'42"N, 133°38'02"E	Minimal, natural reserve	Perennially flooded	Reference
W4	Honghe 3 marsh	47°35'27"N, 133°38'38"E	Protected long-term research site	Perennially flooded	Reference
W5	Bielahong foodplain	47°31'59"N, 133°52'57"E	Natural floodplain	Seasonally flooded	Reference
W6	No. 1 marsh	48°08'14"N, 134°05'21"E	Closing hills and afforestation areas	Perennially flooded	Reference
W7	Zongheshifan marsh	48°04'02"N, 134°33'02"E	Ditched after agricultural reclamation	Seasonally flooded	Impaired
W8	Bagai marsh	48°09'35"N, 134°37'06"E	Ditched, adjacent paved road, isolated fragment	Perennially flooded	Impaired
W9	Yaoliang marsh	48°12'44"N, 134°39'07"E	Ditched after agricultural reclamation, isolated fragment	Perennially flooded	Impaired
W10	Northzhuaji marsh	48°10'59"N, 134°39'01"E	Ditched after agricultural reclamation, isolated fragment	Perennially flooded	Impaired
W11	Nongjiang marsh	48°12'16"N, 134°16'44"E	Adjacent paved road, adjacent rice agriculture	Perennially flooded	Impaired
W12	Qingshui marsh	48°10'17"N, 134°7'46"E	Adjacent paved road, adjacent rice agriculture	Perennially flooded	Impaired
W13	Zhenjiang marsh	48°12'19"N, 134°09'04"E	Adjacent rice agriculture, isolated fragment	Perennially flooded	Impaired
W14	Tongfu marsh	48°12'18"N, 134°13'48"E	Adjacent paved road, adjacent rice agriculture	Perennially flooded	Impaired
W15	Pingyuandian marsh	48°04'35"N, 134°08'53"E	Adjacent paved road, adjacent rice agriculture	Perennially flooded	Impaired
W16	Donghe marsh	48°07'47"N, 134°34'20"E	Adjacent paved road, grazed	Perennially flooded	Impaired

Continuing Table

No.	Wetland Name	Latitude/Longitude	Anthropogenic impacts	Hydrology	Category
W17	Yanan marsh	48°10'44"N, 134°15'08"E	Ditched after agricultural reclamation, adjacent paved road	Seasonally flooded	Impaired
W18	No. 101 marsh	48°13'21"N, 134°18'44"E	Adjacent paved road, adjacent rice agriculture	Perennially flooded	Impaired
W19	Honghe 7 marsh	47°38'03"N, 133°29'52"E	Adjacent rice agriculture, ditched, isolated fragment	Seasonally flooded	Impaired
W20	No. 203 marsh	48°09'02"N, 134°10'40"E	Adjacent soybean agriculture	Perennially flooded	Impaired
W21	Honghe 8-1 marsh	47°37'52"N, 133°27'12"E	Adjacent rice agriculture, ditched, isolated fragment	Seasonally flooded	Impaired
W22	Honghe 8-2 marsh	47°37'42"N, 133°32'28"E	Adjacent rice agriculture, ditched, isolated fragment	Seasonally flooded	Impaired
W23	Luqing marsh	48°09'37"N, 134°13'22"E	Ditched, adjacent paved road	Perennially flooded	Impaired
W24	Qianfeng marsh	47°38'44"N, 133°17'13"E	Ditched, grazed, adjacent paved road	Seasonally flooded	Impaired
W25	Jiugongli marsh	48°06'59"N, 134°08'50"E	Adjacent soybean agriculture, isolated fragment	Perennially flooded	Impaired
W26	Shi marsh	48°04'02"N, 134°05'09"E	Ditched, isolated fragment, adjacent rice agriculture	Perennially flooded	Impaired
W27	Nongqiao marsh	48°01'35"N, 134°10'12"E	Adjacent rice agriculture and paved road	Seasonally flooded	Impaired

2.3 Reference wetlands

To develop MMIs as a part of a bio-assessment, reference wetlands must first be established (Barbour et al., 1996). Four of the reference wetlands were established in a protected, long-term research site (W1 and W4, Table 1) and the Honghe National Natural Reserve (W2 and W3). One reference wetland was selected in a protected river floodplain (W5), which is an area that experiences regular natural flooding, and one (W6) was in an environment under strict state and local protection.

2.4 Multi-metric indices construction

2.4.1 Candidate metrics

To establish the MMIs, 24 candidate metrics were selected based on the abundance, structure, and function of aquatic invertebrate communities in the reference and impaired wetlands. Metrics targeted various aspects of aquatic invertebrate communities, including measures of richness, abundance, biodiversity, and functional feeding group, in order to reflect diverse responses and increase the amount of information contained in the index (Kerans and Karr, 1994; Barbour et al., 1995) (Table 2).

2.4.2 Metric screening

Metrics were selected based on their capacity to discriminate between the reference and impaired wetlands. This was tested using a non-parametric Mann-Whitney *U* test (The SPSS package version 20.0 was used for the calculations, $P < 0.05$), and the degree of inter-quartile (IQ) overlap in Box and Whiskerplots. When the IQ values did not overlap, the sensitivity score was 3; when some overlap occurred, but both medians were outside the IQ range overlap, the score was 2; when moderate overlap occurred in the IQ range, but one median was outside the IQ range overlap, the score was 1. A score of 0a reflected that one IQ range completely overlapped the other, but one median was outside the IQ range overlap; and a score of 0b reflected that both medians were inside the IQ range overlap (Barbour et al., 1996). A metric with $P < 0.05$ from the Mann-Whitney *U* test and a sensitivity score of ≥ 2 was considered to be a strong discriminator between reference and impaired wetlands, and selected as a metric for final MMIs development. We evaluated metrics for redundancy using Pearson correlations and metrics were considered redundant if the Spearman correlation coefficient was > 0.70 and the *P* value was < 0.05 (Shi et al., 2017). Redundant metrics were then eliminated from further analyses, only one metric per category should remain according to Stoddard et al. (2008). The final core metrics for the MMIs were determined in this way.

2.4.3 Development of the final MMIs

The upper and lower anchors mark the indicative range of a metric, i.e., the values that are empirically set and defined as upper anchor and lower anchor, respectively, to normalize a metric's result (Heringet al., 2006). The upper anchor corresponds to the upper limit of the metric's

Table 2 Twenty-four candidate metrics for the multi-metric indices and their predicted responses to impairment. The metrics were screened on the basis of the differences in aquatic invertebrate communities between reference and impaired wetlands in the Sanjiang Plain, Northeast China

Categories	No.	Metric	Response to disturbance
Richness	M1	Total number of taxa	Decrease
	M2	Number of Hemiptera taxa	Decrease
	M3	Number of Coleoptera taxa	Decrease
	M4	Number of Diptera taxa	Decrease
	M5	Number of Gastropoda taxa	Decrease
Abundance	M6	Proportion of Lymnaeidae	Decrease
	M7	Proportion of Planorbidae	Decrease
	M8	Proportion of Valvatidae	Decrease
	M9	Proportion of Odonata	Decrease
	M10	Proportion of Ephemeroptera	Decrease
	M11	Proportion of Corixidae	Increase
	M12	Proportion of Dytiscidae	Decrease
	M13	Proportion of Hydrophilidae	Decrease
	M14	Proportion of Gastropoda	Decrease
	M15	Proportion of Chironomidae	Increase
Biodiversity	M16	Shannon-Wiener index	Decrease
	M17	Margalef's index	Decrease
	M18	Pielou's index	Decrease
	M19	Simpson's index	Decrease
Functional feeding group	M20	Proportion of Collector-Filterers	Increase
	M21	Proportion of Scrapers	Decrease
	M22	Proportion of Collector-Gatherers	Increase
	M23	Proportion of Predators	Decrease
	M24	Proportion of Shredders	Decrease

value under reference sites. If data on reference sites are available, the upper anchor should be set as a percentile of all the metric values of the reference sites (e.g., 95%, 75% or the median, depending on the quality of the reference sites). If few data (e.g., up to 5–10 samples) are available for reference sites, and the site classification is to some extent uncertain, the highest observed value can also be considered (excluding abundance metrics). The lower anchor corresponds to the lower limit of the metric's value under the worst attainable conditions. If data on sites of bad ecological quality are available, the lower anchor should be set as a percentile (e.g., 5% or 25%) of all metric values of the bad ecological quality sites, or at the lowest value obtained or obtainable.

The results of the various core metrics selected for contributing to a MMIs may vary with different ranges of values. For example, whileas the total number of taxa can range from 0 to n, the proportion of Gastropoda can vary from 0 to 50. To combine these individual measures into an integrated MMIs, it is essential to normalize the core metrics via transformation to unitless scores. We used the 3rd and 1st quartiles as the upper and lower anchors for standardizing scores, thereby avoiding the inclusion of extreme values that are not representative of the bulk of the data. In practice, each metric result must be translated into a value between 0 and 1 (Heringet al., 2006; Stoddard et al., 2008).

For metrics that decreased as impairment increased, the upper and lower anchors corresponded to the 75th percentile of the reference wetlands (R75) and the 25th percentile of the impaired wetlands (I25), respectively.

$$MS = (Mi-I25)/(R75-I25) \quad (1)$$

where *MS* is the metric score that is used in scoring the MMIs, *Mi* is each core metric value from the raw field data, *I*25 is the 25th percentile of the impaired wetlands, and *R*75 is the 75th percentile of the reference wetlands.

For metrics that increased as impairment increased, the upper and lower anchors corresponded to the 75th percentile of the impaired wetlands (*I*75) and the 25th percentile of the reference wetlands (*R*25), respectively.

$$MS = (Mi - I75)/(R25 - I75) \quad (2)$$

The final MMIs were generated as the sum of the scores from each core metric. The threshold for excellent condition was set using the 25th percentile value of the reference wetlands. The range of values under the excellent condition threshold was divided into three equal-sized groups to determine the thresholds for the remaining good, fair and poor categories (De Bikuñaet al., 2015).

3 Results

3.1 Selection of core metrics

Of the 24 candidate metrics, 9 metrics had sufficient discriminating power to separate reference and impaired wetlands, on the basis of their Mann-Whitney *U* test ($P < 0.05$, Table 3). According to sensitivity testing (Box and Whisker plots, Fig. 2; Table 3), 10 metrics were highly sensitive (score ≥ 2). The metrics with both a significant Mann-Whitney *U* test and a sensitivity score of ≥ 2 were retained for redundancy testing and final metric selection. Six metrics were selected: total number of taxa, number of Hemiptera taxa, number of Coleoptera taxa, number of Diptera taxa, proportion of Gastropoda, and proportion of Predators.

Table 3 Selection criteria and response of 24 candidate metrics to wetland impairment in the Sanjiang Plain, Northeast China

Metrics	Response to disturbance	*P*	Sensitivity score*	Meets the test criteria
Total number of taxa	Decrease	0.001	3	Yes
Number of Hemiptera taxa	Decrease	0.002	3	Yes
Number of Coleoptera taxa	Decrease	0.002	3	Yes
Number of Diptera taxa	Decrease	0.001	3	Yes
Number of Gastropoda taxa	Decrease	0.085	3	No
Proportion of Lymnaeidae	Decrease	0.712	0b	No
Proportion of Planorbidae	Decrease	0.065	2	No
Proportion of Valvatidae	Decrease	0.195	1	No
Proportion of Odonata	Decrease	0.125	1	No
Proportion of Ephemeroptera	Decrease	0.441	0b	No
Proportion of Corixidae	Increase	0.441	0b	No
Proportion of Dytiscidae	Decrease	0.376	0a	No
Proportion of Hydrophilidae	Decrease	0.065	3	No
Proportion of Gastropoda	Decrease	0.010	2	Yes
Proportion of Chironomidae	Increase	0.085	2	No
Shannon-Wiener index	Decrease	0.712	0b	No
Margalef’s index	Decrease	0.049	1	No
Pielou’s index	Decrease	0.110	1	No
Simpson’s index	Decrease	0.755	0b	No
Proportion of Collector-Filterers	Increase	0.887	0b	No
Proportion of Scrapers	Decrease	0.010	1	No
Proportion of Collector-Gatherers	Increase	0.042	1	No
Proportion of Predators	Increase	0.022	2	Yes
Proportion of Shredders	Decrease	0.408	0b	No

Notes: * A sensitivity score of 3 was given if there was no overlap in the interquartile range (IQ) from Box and Whisker plots of the metric values; a score of 2 reflects someoverlap but both medians were outside the IQ rangeoverlap; a score of 1 was given if there was moderate overlap in the IQ range but one median was outside the IQ rangeoverlap; a score of 0a reflects that one range was completely overlapping the other IQ range but one median was outside the IQ range overlap; and a score of 0b reflects thatboth medians were inside IQ range overlap

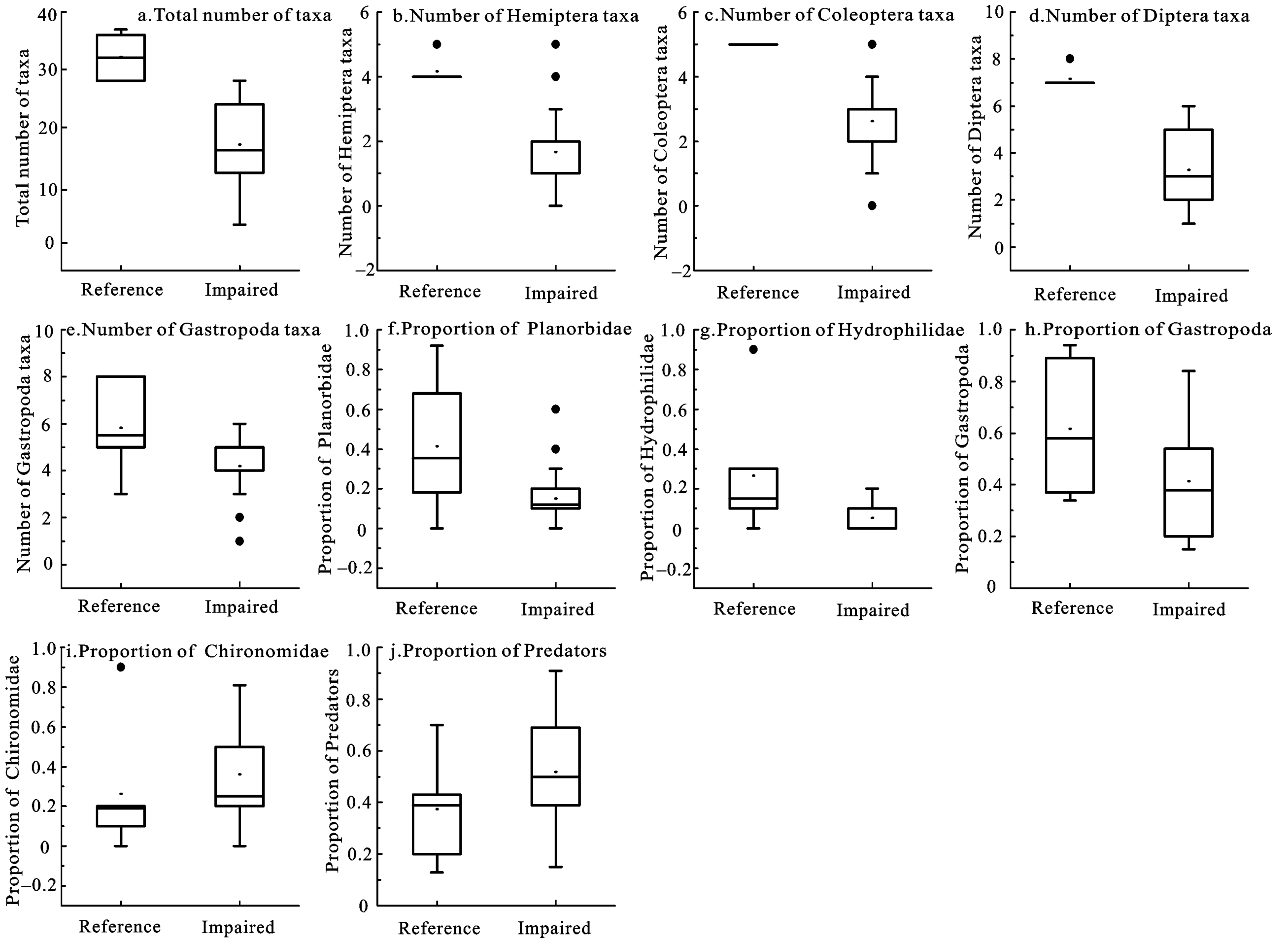

Fig. 2 Box and Whisker plots of the ten selected metrics used to discriminate between reference and impaired wetlands in the Sanjiang Plain of Northeastern China. Horizontal solid lines at the bottom and top of the boxes indicate the minimum and maximum of non-outliers; boxes represent interquartile ranges (25^{th}–75^{th} percentiles). The mean (squares), median (horizontal solid lines in boxes), and outliers (black point) are shown in the box plots

In redundancy tests, the total number of taxa was highly correlated with both number of Coleoptera taxa and number of Diptera taxa (R = 0.732, R = 0.758, respectively, P < 0.01; Table 4). The total number of taxa was retained, rather than number of Coleoptera taxa or number of Diptera taxa, because it is widely used as a biodiversity metric. Although the number of Hemiptera taxa was correlated with number of Coleoptera taxa and number of Diptera taxa (R = 0.760, R = −0.809, respectively; P < 0.01), it was not significantly correlated with total number of taxa (Table 4). Therefore, number of Hemiptera taxa was selected. The proportion of Gastropoda and the proportion of Predators were not significantly correlated with any other parameters and therefor were retained (Table 4). Thus, total number of taxa, number of Hemiptera taxa, proportion of Gastropoda, and proportion of Predators were the final selected metrics used to generate our MMIs.

3.2 MMIs development

The MMIs were calculated by aggregating the scores of each of the four metrics as mentioned above. Each coremetric value was rescaled for comparison using the 25^{th} and 75^{th} percentiles across reference and impaired wetlands (Table 5). We standardized scores using the third and first quartiles (75^{th} and 25^{th} percentiles), thereby restricting the inclusion of extreme values. The upper and lower anchors marked the range of a given metric and any transformed values that were > 1 were set to 1, while values < 0 were set to 0. Four ordinal rating categories for wetland were defined: 1) poor (0–0.93), moderately severe impairment; 2) fair (0.94–1.87),

Table 4 Pearson's correlation coefficients verifying redundancy ($R > 0.70$) in the metrics selected

	Number of Hemiptera taxa	Number of Coleoptera taxa	Number of Diptera taxa	Proportion of Gastropoda	Proportion of Predators
Total number of taxa	0.557^{**}	0.732^{**}	0.758^{**}	0.432^{*}	−0.162
Number of Hemiptera taxa		0.760^{**}	0.809^{**}	0.403^{*}	−0.128
Number of Coleoptera taxa			0.886^{**}	0.238	−0.294
Number of Diptera taxa				0.358	−0.282
Proportion of Gastropoda					−0.125

Notes: Significantly different values: $^{*}P < 0.05$, $^{**}P < 0.01$

Table 5 Quartiles of core aquatic invertebrate community metrics for reference and impaired wetlands in the Sanjiang Plain of Northeast China

No.	Metric	Reference		Impaired	
		25^{th} percentile	75^{th} percentile	25^{th} percentile	75^{th} percentile
M1	Total number of taxa	28.75	35.25	12	24
M2	Number of Hemiptera taxa	4	4	1	2
M14	Proportion of Gastropoda	0.39	0.85	0.2	0.54
M23	Proportion of Predators	0.24	0.43	0.39	0.69

moderate impairment; 3) good (1.88–2.80), mildly impaired biological conditions; and 4) excellent (2.81–3.80), conditions equal to the reference wetlands. All wetlands in the study area were evaluated (Fig. 3).

Based on the samples collected from 21 impaired wetlands and the evaluation criteria, 9 habitats (42.9%) were classified as poor, 7 (33.3%) were classified as fair, and 5 (23.8%) were classified as good according. For the 6 reference wetlands, 2 habitats (33.3%) were classified as good and four (66.7%) were classified as excellent. The results of the MMIs showed that 76.2% of the impaired wetlands werein poor or fair. Box plots of the MMIs based on aquatic invertebrates scores show that the MMIs was able to discriminate well (IQ=3) between the reference and impaired wetlands (Fig. 4).

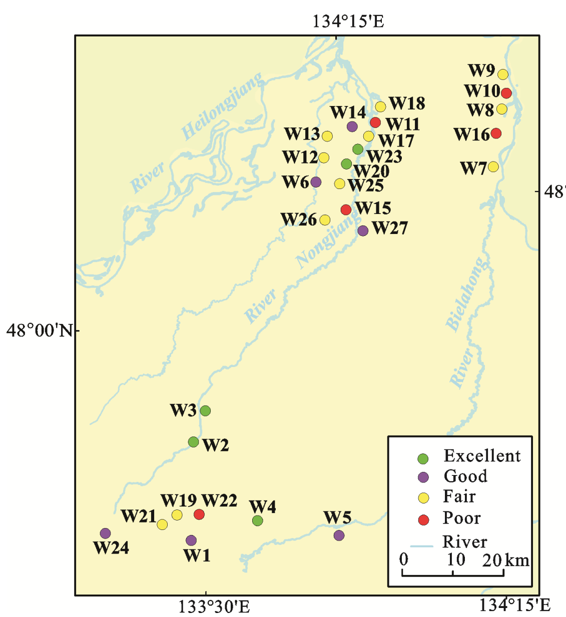

Fig. 3 Evaluated results of the 27 wetlands in the Sanjiang Plain of Northeast China

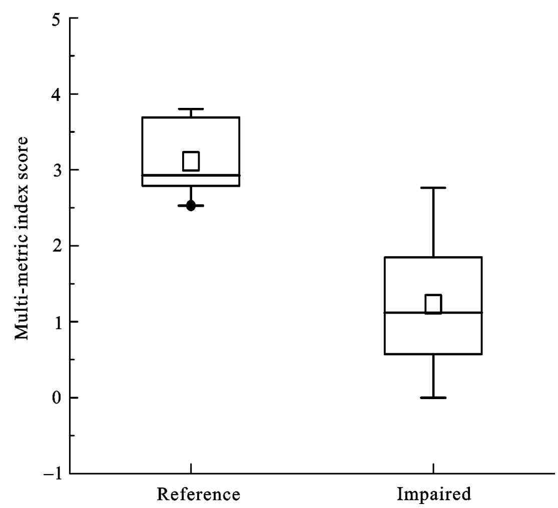

Fig. 4 Box plots of total multi-metric index scores of reference and impaired wetlands in the Sanjiang Plain of Northeastern China. Horizontal solid lines at the bottom and top of boxes indicate the minimum and maximum of non-outliers; boxes represent interquartile ranges (25^{th}–75^{th} percentiles). The mean (squares), median (horizontal solid lines in boxes), and outliers (black point) are shown in the box plots

4 Discussion

The MMIs developed to assess freshwater wetlands in Northeast China demonstrated that aquatic invertebrates are suitable indicators for classifying wetland conditions and provided proof-of-concept for the application of biomonitoring protocols to freshwater wetland habitats in China.

4.1 Selection of reference wetland

The Development of MMIs are a stepwise process that begins with the definition of reference wetlands. The MMIs are expected to be able to distinguish impaired from reference wetlands through the responses of assemblages (Stoddard et al., 2006; Ligeiro et al., 2013). The reference conditions should describe wetlands minimally affected by anthropogenic activities and represent natural patterns across a region (Stoddard et al., 2006; Whittier et al., 2007; Ligeiro et al., 2013). However, minimally disturbed wetlands are rarely found in regions with long-term and widespread human activities (Whittier et al., 2007). In this study, reference samples were obtained from national nature reserves and other areas protected at the state and local level.

To develop robust MMIs, appropriate biological information should be included (Vander Laan, 2012), and spatial and temporal variability in assemblages must be considered. The selection of appropriate metrics for inclusion in MMIs is critical to producing an accurate and ecologically meaningful index (Stoddard et al., 2008; Vander Laan, 2012). Ecoregions are used in several countries as a fundamental classification unit for aquatic bio-assessment programs (Hughes, 1995; Omernik, 1995) to help managers identify natural variability in aquatic biota (Hawkins et al., 2000). The habitats in this study were all occurred within the same ecoregion, the Agricultural Wetland Ecological Area in the Sanjiang Plain (Fu et al., 2001). Therefore, we hypothesized that they should have similar aquatic invertebrate structure and composition, allowing for the application of an MMIs developed for our region (Baptista et al., 2007).

4.2 Selection of core metrics

MMIs should optimally contain at least one metric of each type (e.g., richness/diversity, sensitivity/tolerance, and functional) in order to reflect the complexity of biological communities (Hering et al., 2006; Stoddard et al., 2008). In this study, 24 candidate metrics were tested for inclusion in the MMIs, with most previously recognized as being sensitive to a range of anthropogenic stressors (Ferreira et al., 2011; Couceiro et al., 2012), and used in the bio-assessment of wetlands. However, most of the candidate metrics were eliminated, because they did not adequately discriminate between reference and impaired wetlands. Four core metrics were selected to develop the MMIs, each reflecting different features of aquatic invertebrate assemblages. Total number of taxa is common metric shaping MMIs globally (Baptista et al., 2007; Couceiro et al., 2012; Lu et al., 2019), demonstrating its capability to respond to diverse ecological alterations. Snails (Mollusca: Gastropoda) are widely distributed in freshwater wetlands and are good indicator of environmental conditions in other Chinese wetlands (Wu et al., 2017; Lu et al., 2019).

4.3 Developmentof multi-assemblage index

Samples from multiple seasons and years collected at unique sites were used to construct the MMIs in this study, which differed from the suggestions of Leung and Dudgeon (2011), who suggest sampling only during the dry season to avoid potentially confounding seasonal effects. Data from reference and impaired wetlands across broader temporal scales (multiple seasons and years) encompasses greater natural variability and should improve the precision and accuracy of the MMIs. In addition, indicators that incorporate multiple groups can be more robust and useful tools in assessment. For example, Hughes et al. (2004) developed robust and inclusive stream MMIs by combining amphibians with fish. Therefore, it may be productive to develop a multi-assemblage index through future study.

Wetland habitats are crucial components of the Sanjiang Plain. An evaluation system that can be used effectively to determine the condition of freshwater wetlands in the Sanjiang Plain and to help regulators evaluate current wetland mitigation and restoration projects is clearly valuable. Further assessment of these freshwater wetlands could help to delineate the valuable ecological services that they provide.

5 Conclusions

In this paper, MMIs based on aquatic invertebrates were developed to evaluate wetlands adjacent agricultural fields in Northeast China. Four core indicators were selected to build the MMIs: total number of taxa, number of Hemiptera taxa, Proportion of Gastropoda, and Proportion of Predators. Four ordinal rating categories for the wetland condition were defined: poor, fair, good, and excellent. Of the impaired wetlands, the condition of 76.2% was rated as poor or fair. The MMIs were robust in discriminating reference wetlands from impaired wetlands, demonstrating potential as a biomonitoring tool to assess freshwater wetlands and restoration efforts in northeastern China.

Acknowledgments

The staff of the Sanjiang Mire Wetland Experimental Station and Honghe National Natural Reserve provided support. We thank Dr. Darold P Batzer for a helpful review of the earlier version of this paper.

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