Macroinvertebrate and diatom metrics as indicators of water-quality conditions in connected depression wetlands in the Mississippi Alluvial Plain

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Abstract: Methods for assessing wetland conditions must be established so wetlands can be monitored and ecological services can be protected. We evaluated biological indices compiled from macroinvertebrate and diatom metrics developed primarily for streams to assess their ability to indicate water quality in connected depression wetlands. We collected water-quality and biological samples at 24 connected depressions dominated by water tupelo (*Nyssa aquatica*) or bald cypress (*Taxodium distichum*) (water depths = 0.5–1.0 m). Water quality of the least-disturbed connected depressions was characteristic of swamps in the southeastern USA, which tend to have low specific conductance, nutrient concentrations, and pH. We compared 162 macroinvertebrate metrics and 123 diatom metrics with a water-quality disturbance gradient. For most metrics, we evaluated richness, % richness, abundance, and % relative abundance values. Three of the 4 macroinvertebrate metrics that were most beneficial for identifying disturbance in connected depressions decreased along the disturbance gradient even though they normally increase relative to stream disturbance. The negative relationship to disturbance of some taxa (e.g., dipterans, mollusks, and crustaceans) that are considered tolerant in streams suggests that the tolerance scale for some macroinvertebrates can differ markedly between streams and wetlands. Three of the 4 metrics chosen for the diatom index reflected published tolerances or fit the usual perception of metric response to disturbance. Both biological indices may be useful in connected depressions elsewhere in the Mississippi Alluvial Plain Ecoregion and could have application in other wetland types. Given the paradoxical relationship of some macroinvertebrate metrics to dissolved O_2 (DO), we suggest that the diatom metrics may be easier to interpret and defend for wetlands with low DO concentrations in least-disturbed conditions.
Key words: dissolved oxygen, specific conductivity, biological indices, Cache River Watershed, rice, irrigation, Arkansas

Wetlands have important functions, such as nutrient and sediment retention, flood regulation, groundwater recharge, nutrient cycling, and C sequestration (Mitsch and Gosselink 2007). They also provide habitat for many native vertebrates, macroinvertebrates, algae, and plants. In Europe and North America, up to 90% of wetlands (including flood plains) are altered by cultivation (Tockner and Stanford 2002). In the Mississippi Alluvial Plain (MAP) Ecoregion in the southeastern USA, wetlands that once covered ~9.7 million ha have been reduced by 80% primarily because of conversion to row-crop agriculture (Cowardin and Golet 1995). Thus, methods for assessing wetland condition must be established so that these important ecological resources can be monitored and protected.

Hydrogeomorphic classification methods have been established to describe wetland function in the MAP (Smith et al. 1995), but the regulatory communities in many states are becoming increasingly interested in biological assessment methods. With the exception of initial efforts in states including Florida, Maine, Maryland, Massachusetts, Michigan, Minnesota, Montana, Ohio, and Oregon (USEPA 2003), few biological assessment methods have been established

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to provide guidance for water-quality-based wetland evaluations. Moreover, biological indices compiled from macroinvertebrate and diatom metrics developed primarily for streams have not been evaluated for use in wetlands even though streams and wetlands have inherent biological, chemical, and physical differences. Our goal was to assess the ability of biological metrics to indicate water-quality conditions in connected depression wetlands.

We chose to study connected depressions because of their large spatial extent and their more integral connectedness to the riverine ecosystem compared with 4 other wetland classes (flats, slopes, riverine, and fringe wetlands) identified in the MAP by the US Environmental Protection Agency (EPA) and the Arkansas Multi-Agency Wetland Planning Team (MAWPT). Connected depressions are river scars situated within a 5-y floodplain and are supplied with water primarily by overbank flow (Klimas et al. 2004).

After the 1972 Clean Water Act was passed, much emphasis was placed on developing biological methods for assessing the health of upland streams, for which the fundamental relationships among dissolved O_2 (DO) concentration, organic pollution, and biological integrity are widely recognized. Consequently, many biological metrics with utility in stream indices include taxa that require DO concentrations (e.g., >6 mg/L) typical of aerated, pristine stream conditions (e.g., the insect orders Ephemeroptera, Plecoptera, and Trichoptera). However, even under reference conditions (Justus et al. 2012), DO concentrations in wetlands naturally can be very low (e.g., <2.0 mg/L; Mitsch and Gosselink 2007) because of a lack of aeration and the degree of decomposition associated with vast amounts of organic matter from allochthonous vegetation in lowland streams and wetlands. Low DO could explain why biological assessments based on macroinvertebrate (Gernes and Helgen 2002, Chipps et al. 2006, Suren et al. 2011) and diatom (Wang et al. 2005, Chipps et al. 2006, Lane and Brown 2007, Reiss et al. 2010) indices have been only moderately successful in wetlands.

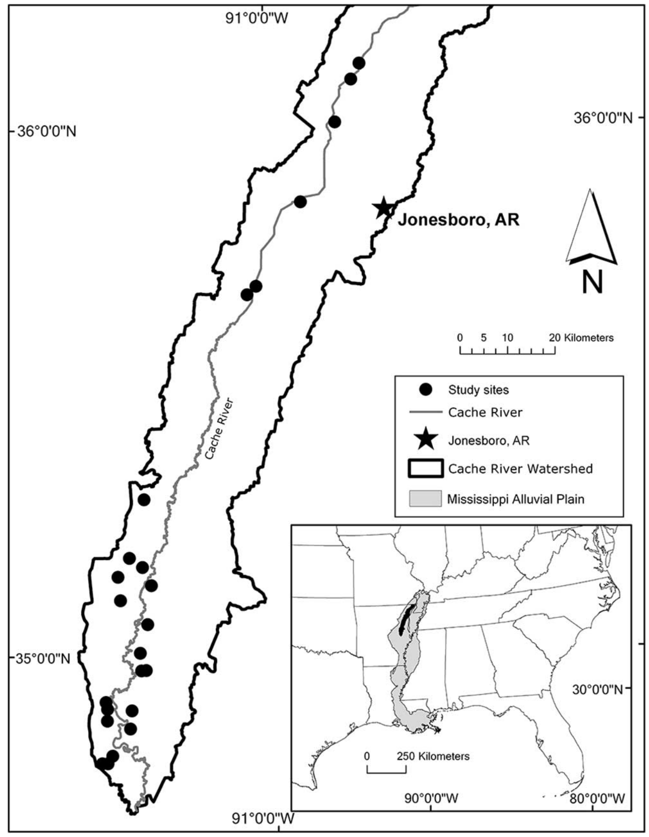

Figure 1. Study site locations for 24 connected depression wetlands in the Cache River Watershed, Arkansas (AR).

METHODS

Study area

Within the MAP, the Cache River Watershed in northeastern Arkansas (Fig. 1) includes ~5066 km^2 of wetlands (Arkansaswater.org 2012). The upstream part of the watershed has been extensively altered by agricultural activity (Arkansas Natural Heritage Commission 2010), but the downstream part contains extensive bottomland hardwood swamps in 2 public holdings: the Rex Hancock Black Swamp Wildlife Management Area (Arkansas Game and Fish Commission, 2600 ha) and the Cache River National Refuge (US Fish and Wildlife Service, 22,600 ha).

Site selection

We used geographic information system (GIS) data provided by the MAWPT to locate connected depressions for potential sampling. MAWPT provided an ArcGIS (version 10.3; Environmental Systems Research Institute, Redlands, California) map layer of connected depressions, as defined by a hydrogeomorphic model (Smith et al. 1995), which was compared to Google Earth® aerial imagery to identify possible study sites. We conducted reconnaissance from October 2011 to May 2012 and visited ~85 sites. We noted nearby land use (e.g., amount of agriculture, proximity to roads and ditches), physical habitat (e.g., dominant vegetation, wetted area, and range of depths), and hydrology. We selected 38 sites for water-quality reconnaissance evaluation that we suspected of being along a water-quality gradient and were isolated from the Cache River at low stage. All sites selected for water-quality reconnaissance were dominated by water tupelo (*Nyssa aquatica*) or bald cypress (*Taxodium distichum*) and had closed canopies and water depths of 0.5 to 1.0 m. We used data from the water-quality reconnaissance conducted in early May 2012 to select 24 study sites along a water-quality and landuse gradient (least- to most-disturbed condition). We used surrounding land use, indications of altered hydrology, and water-quality data to indicate levels of disturbance.

We focused primarily on nutrient-related constituents because nutrients are the leading cause of waterbody impairment across the nation (USEPA 2002) and are a common reason for waterbody impairment in Arkansas (Arkansas Department of Environmental Quality 2008). However, the amount of row-crop agriculture in the study area was another consideration. Studies conducted in the 1990s during the US Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Program demonstrated that high concentrations of N and P were common in streams draining agricultural areas (Fuhrer et al. 1999).

Water-quality sampling

We collected reconnaissance and routine water-quality samples near the deepest point of each connected depression with a long-handled dipper (Danielson 2004). We preserved water samples appropriately and transported them to the Ecotoxicology Research Facility (ERF) at Arkansas State University, Jonesboro, Arkansas (A-State), within specified holding times. Samples were analyzed for 6 constituents—dissolved PO_4^{3-}, total P, dissolved NH_3-N, dissolved NO_2^--N, dissolved NO_3^--N, and chlorophyll *a* (Chl *a*) (Table S1). Standard quality-assurance procedures were conducted to evaluate data quality and adequacy of field and laboratory analyses (Cobb 2013). We measured DO, temperature, specific conductance, pH, and turbidity onsite on each sampling occasion with a Thermo Scientific Orion Star A329 multiparameter meter (Thermo Scientific, Waltham, Massachusetts) and a HACH 2100P Turbidimeter (Hach, Loveland, Colorado).

We conducted water-quality sampling under stable hydrological conditions in late May, June, and July 2012. Connected depressions tend to fill during the winter and spring and dry very slowly in late summer (Klimas et al. 2004). However, relatively extreme drought conditions in late spring and early summer 2012 prevented sampling at 3 of the original 24 sites in June. We discontinued sampling at those 3 sites. Because some of the original 38 sites also were dry, we conducted additional reconnaissance to identify 3 replacement sites that were needed in June (and were resampled for water quality in July). We completed a 3rd and final water-quality sampling event at 21 of the 24 study sites during late July 2012, but 3 sites were dry. Thus, 18 of the 24 sites were sampled on 3 occasions, and 6 sites were sampled twice. We calculated mean concentrations for all nutrient constituents and field variables (except pH, which was calculated as a median) that were compared with biological metrics.

We conducted a 72-h DO study in early July 2012. We deployed water-quality monitors (Hydrolab MS 5; Hach) floating almost parallel to the water surface but with the DO probe submerged just beneath the surface. DO, temperature, and specific conductance were measured at 30-min intervals. Continuous DO data could not be collected at 5 of the 24 sites because of drought conditions (i.e., shallow depths < 8 cm). At those 5 sites, DO estimates were obtained by substituting values from sites that had similar conditions. Similar sites were identified by comparing instantaneous DO values among all sites obtained at a similar time of day (±1 h) during the May, June, and July water-quality sampling events. The site with the lowest mean absolute difference in DO from the dry site was used as a substitute site for estimating DO at the dry site.

Biological sampling for macroinvertebrates and diatoms

We used biological sampling methods identified in other states and regions (Lane and Brown 2007, Carlisle et al. 2013) and national protocols that have a known application (Barbour et al. 1999, Moulton et al. 2002) as the basis for methods to collect and process biological samples (Cobb 2013, Burge 2014). We conducted biological sampling in June, a time typically near baseflow conditions, in an effort to reduce variability in the quality of the biological samples. The dominant benthic habitat consisted of submerged bases of water tupelo and bald cypress, 2 species of trees that are extremely tolerant of flooding. We collected diatoms and macroinvertebrates from this substrate.

We followed a revised method based on EPA protocol (Barbour et al. 1999) to collect composited benthic macroinvertebrate (BMI) samples from the dominant habitat. At each study site, we collected BMI samples from the bases of 5 trees within a 1-ha area that included the site of water-quality sampling. We selected trees that were standing in at least 0.3-m-deep water. We used a D-frame dip net (500-μm mesh) to sweep a 0.3-m (linear) area 5 times. Sweeping was done upward from the base of the tree and at an angle to prevent the loss of specimens. We combined sampled material from all 5 trees in a 19-L bucket, filtered it through a 500-μm-mesh sieve, and elutriated it several times (Moulton et al. 2002) until it was reduced to ~250 mL. We examined coarse material remaining after elutriation for BMIs and then discarded it. We preserved all BMI samples in 75% ethanol and returned them to the ERF for identification.

We removed and identified all BMIs except oligochaetes and chironomids, which we subsampled. Subsamples of 100 oligochaetes/sample were identified by personnel at the Aquatic Resources Center (Nashville, Tennessee). We separated Chironomidae into subsamples consisting of the larger of 10% of the total number collected or 50 individuals and chose random subsamples for identification according to Campbell et al. (2009). We mounted each specimen in Euparal (ANSCO Laboratories, Manchester, UK), removed the head, and pressed the coverslip firmly to expose the mouthparts (Epler 2001).

We identified BMIs with the aid of appropriate taxonomic keys (Klemm 1995, Epler 2001, 2006, 2010, Smith 2001, Richardson 2003, 2010, Merritt et al. 2008). We identified all BMIs to the lowest possible taxonomic level, usually genus (Cobb 2013). For quality assurance, 10% of the macroinvertebrate identifications were confirmed by EnviroScience (Stow, Ohio). All specimens collected are labeled and stored at the ERF.

We collected epidendritic diatom samples from 5 trees at each site and composited the samples. Samples were collected from bald cypress at 22 sites and water tupelo at 2 sites that lacked bald cypress (Burge 2014). We used a 3.8-cm wood chisel to remove a strip of bark ~5 cm long from each tree. We processed samples at the ERF where a toothbrush and a razor blade were used to dislodge diatoms from a 3.5-cm-diameter circle (9.6 cm^2) on each of the 5 pieces of bark. We placed all samples in 50-mL centrifuge tubes and immersed them in 3% glutaraldehyde for preservation. We cleaned and mounted diatoms by the protocol published by Stoermer et al. (1995). We identified diatoms to the lowest possible taxonomic level, usually species (Burge 2014), with the aid of keys published by Patrick and Reimer (1966, 1975), Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Spaulding et al. (2010), Krammer (2011), Lange-Bertalot et al. (2011), and Burge et al. (2015). To ensure accurate estimates of relative abundance, we used 600 valves/site to estimate biological metrics and excluded samples with abundances <300 valves/site (Stevenson and Bahls 1999). Identification and counting were conducted using light microscopy under oil immersion at 1000× magnification on an Axio Lab A1 (Zeiss, Oberkochen, Germany). Labeled slides and cleaned material used for counting are stored in the A-State Herbarium (acronym STAR, as indexed in *Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff*).

Disturbance-gradient analysis

We created a water-quality disturbance gradient to facilitate comparison of the relative importance of the candidate biological metrics. We used nonmetric multidimensional scaling (NMDS) in PC-ORD (version 6; MjM Software Design, Gleneden Beach, Oregon) to explore the relationships among BMI and diatom assemblages and 11 water-quality (Table 1) and 2 landuse variables (amount of agriculture and forest buffer within 100 m of the wetland). We minimized stress in the ordinations per McCune and Grace (2002). The 3 water-quality variables with the strongest correlations with ordination scores for both assemblages and with agricultural land use in the 100-m buffer were selected for use in the disturbance gradient. We calculated scores for the disturbance gradient with the same centering method (Justus et al. 2010) used to score biological indices (described below) except that values for the 3 water-quality variables with strongest correlations with biological variation in both biological assemblages were combined. Thus, sites with the highest disturbance-gradient scores had the most-disturbed conditions.

Table 1. Selected water-quality and physical variables measured at 24 connected depressions sampled in the Cache River Watershed and evaluated with biological assemblages in nonmetric multidimensional scaling ordination plots.

Variable	Median	Range
Temperature (°C)	24.58	22.2–27.43
pH	6.66	5.59–7.37
Dissolved O_2 (mg/L)	1.66	0.05–7.65
Specific conductance (μS/cm)	147	71.00–834.00
Turbidity (NTU)	17.26	8.05–320.95
NH_3^+-N (mg/L)	0.31	0.08–13.03
NO_3^--N (mg/L)	0.04	0.01–0.30
NO_2^--N (mg/L)	0.01	0.00–0.05
Total P (mg/L)	0.24	0.10–3.93
PO_4^{3-} (mg/L)	0.06	0.02–2.08
Chlorophyll *a* (μg/L)	4.67	0.17–100.38

Metric sources

We used 2 USGS software programs—the Macroinvertebrate Data Analysis System (IDAS; Cuffney 2003) and the Algal Data Analysis System (ADAS; a derivative of the IDAS program)—as the primary methods for calculating macroinvertebrate and diatom metrics. Both programs process multiple levels of taxonomic resolution, resolve taxonomic ambiguities, and use attribute files to calculate assemblage and tolerance metrics common to the literature (Barbour et al. 1999, Porter 2008). For most metrics, we evaluated richness, % richness, abundance, and % relative abundance values.

We calculated 162 BMI metrics with pollution tolerance data specific to the southeastern (Lenat 1993, Barbour et al. 1999) and midwestern (Hilsenhoff 1987) USA (Table S2). We also evaluated BMI metrics used in other wetland studies, including chironomid abundance; BMI diversity (Chipps et al. 2006); chironomid taxa; Ephemeroptera, Trichoptera, Sphaeriidae, and dragonflies (ETSD); and Odonata metrics (Gernes and Helgen 2002).

The 123 metrics calculated for diatoms were primarily indicative of trophic preferences (van Dam et al. 1994), water-chemistry tolerances (Potapova and Charles 2003, 2007), and pollution tolerance (Lange-Bertalot 1979, Bahls 1993; Table S3). In other wetland studies (Wang et al. 2006, Lane 2007, Lane and Brown 2007), the genera *Eunotia* and *Pinnularia* declined with disturbance, whereas *Navicula* and *Nitzschia* increased with disturbance. Therefore, we also evaluated metrics associated with these 4 genera for the diatom index.

Multimetric biological indices

We used primarily correlation analysis and scatterplots to evaluate relationships between biological metrics and the disturbance gradient. This process is summarized in a decision tree (Fig. 2). We used Spearman rank correlations to identify and eliminate redundant metrics. When 2 metrics involving the same taxa were strongly correlated ($\rho > |0.70|$), we removed 1 of the pair to prevent index bias and error. We used scatterplots to detect strong relationships,

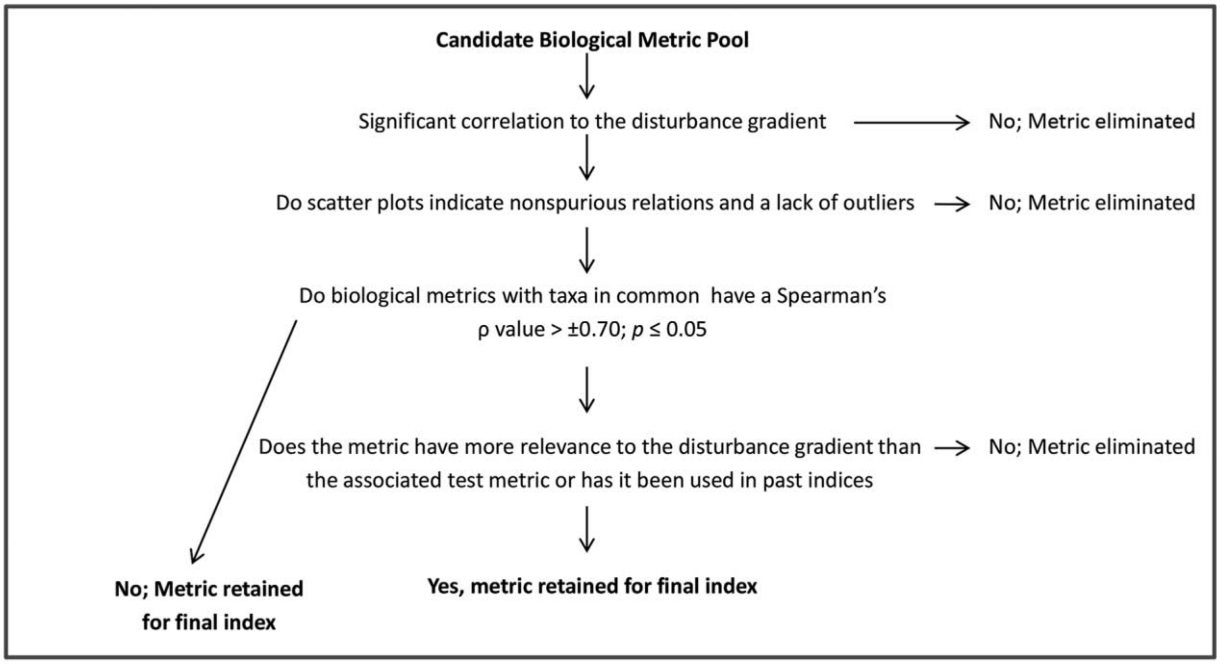

Figure 2. Decision tree indicating considerations in a metric-selection process evaluating relationships between macroinvertebrate and diatom metrics and wetland disturbance.

outlying values, and spurious correlations. Metric relevance to the disturbance gradient (i.e., decreasing or increasing abundance or richness) was the primary consideration used to decide which of the redundant metrics was retained for further analysis. We also considered ecological relevance and past use in published indices before selecting the final 4 metrics composing each biological index.

We calculated scores for the 2 biological indices by combining values for the 4 metrics on the basis of a centering method (Justus et al. 2010). The centering method is more robust than most other scoring methods (e.g., scores range from 0–100 rather than belong to preassigned metric classes of 1, 3, or 5), but it does not facilitate comparison with sites from other data sets because metric scores are based on the range of sampling conditions at sampled sites, which might not include least- or most-disturbed sites. The scoring procedure used in the centering method depends on whether high or low metric values represent least-disturbed conditions. If a high metric value indicates least-disturbed conditions, the metric value is first divided by the maximum metric value (for all 24 sites), and the quotient is multiplied by 100 to obtain a score. If a low metric value indicates least-disturbed conditions, the metric value is divided by the maximum metric value, but the resulting quotient is subtracted from 1 before being multiplied by 100. We averaged the scores for the 4 metrics to obtain an index score. Sites having the highest biological index scores had the least-disturbed conditions. Last, we evaluated overall relationships between the final 2 biological indices and the disturbance gradient with correlations and scatterplots.

RESULTS

Relationships between biological assemblages and water-quality variables

Statistics for the NMDS ordinations indicated that both assemblage models were adequate (McCune and Grace 2002). Less variance was explained for the BMI than for the diatom assemblage (79 vs 91%, respectively), and stress on the ordination was slightly higher for the BMI than for the diatom assemblage (14.3 and 10.4, respectively).

BMI and diatom assemblages had strong relationships with 3 water-quality variables—specific conductance, pH, and NO_3^--N (Figs 3A, B, 4A–C). Water in the least-disturbed connected depressions had low specific conductance, low pH, and the lowest NO_3^--N concentrations (25th percentile of mean values = 105 μS/cm, 6.35, and 0.023 mg/L, respectively), and the 3 variables were combined to form the disturbance gradient.

DO concentration (measured in the 72-h study) was a 4th water-quality variable that helped explain variability in the macroinvertebrate data (Fig. 3A). DO was positively correlated with specific conductance ($\rho = 0.58$, $p = 0.003$) and NO_3^--N ($\rho = 0.67$, $p < 0.001$), which were used in the disturbance gradient. The positive relationship between DO and disturbance was in contrast to expectations for most other aquatic systems (e.g., streams and lakes). Therefore, we did not include it the disturbance gradient.

The disturbance gradient calculated with pH, specific conductance, and NO_3^--N ranged from 1.11 to 79.7, and relationships between the disturbance gradient and the 3 water-quality variables were similar (Fig. 4A–C). The dis-

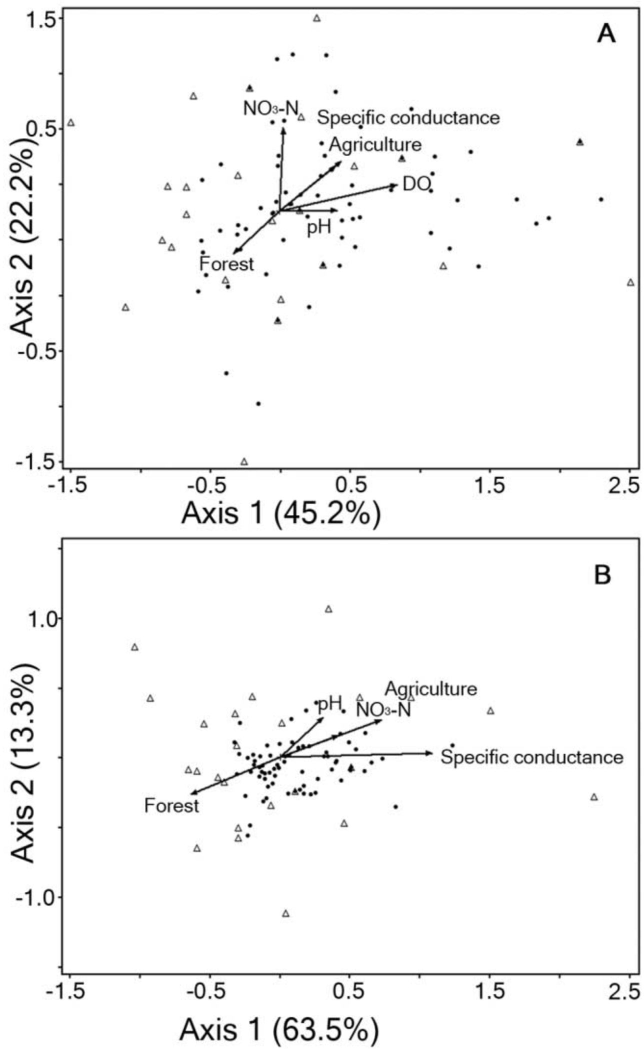

Figure 3. Nonmetric multidimensional scaling ordination plots of epidendritic macroinvertebrate (A) and diatom (B) assemblages with joint biplots of significantly correlated environmental variables at 24 connected depression wetlands sampled in the Cache River Watershed, Arkansas. Triangles and circles represent sampling sites and species, respectively. Length and direction of arrows indicate the degree of correlation with the ordination axes (% variation accounted for by each axis in parentheses). DO = dissolved O_2.

turbance gradient was significantly correlated ($p < 0.05$) with axis 1 of the macroinvertebrate and diatom ordinations ($\rho = -0.44$ and -0.50, respectively).

Biological metrics and index performance

The 4 metrics selected for the macroinvertebrate index included 3 that decreased with disturbance and 1 that increased with disturbance (Table 2, Fig. 5A–D). The 3 metrics that decreased with disturbance were % relative abundance of one of the most common chironomids collected, *Kiefferulus* (*Kiefferulus*RA) (Fig. 5A), % relative abundance of Diptera (DipteraRA) (Fig. 5B), and % richness of mollusks and crustaceans (MOLCRU) (Fig. 5D). Oligochaeta richness (OligoR) increased with increasing disturbance (Fig. 5C). DipteraRA, *Kiefferulus*RA, and OligoR were significantly correlated ($\rho = -0.49$, -0.58, and 0.43, respectively, all $p < 0.05$) with the disturbance gradient (Fig. 5), whereas MOLCRU was not ($\rho = -0.31$, $p = 0.14$). The BMI index ranged from 20.8 to 80.4 and decreased in relation

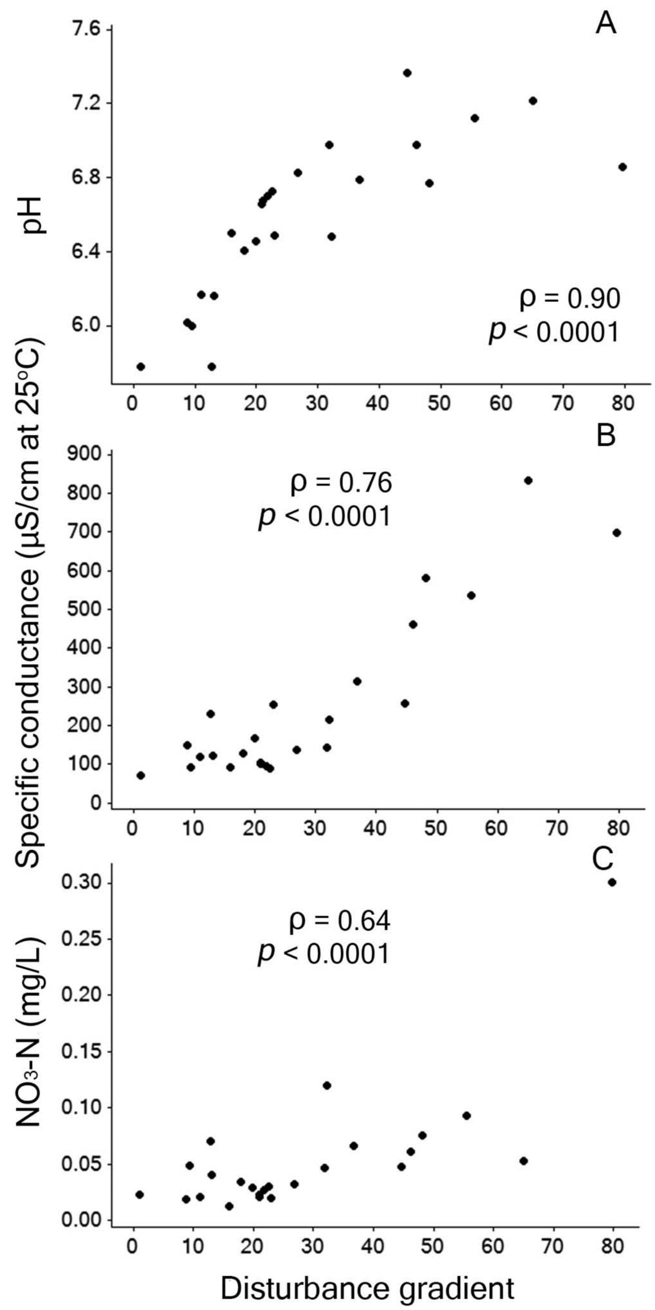

Figure 4. Scatterplots and correlations for pH (A), specific conductance (B), and NO_3^--N (C) along a disturbance gradient at 24 connected depression wetlands sampled in the Cache River Watershed.

Table 2. Macroinvertebrate and diatom metrics selected for 2 indices with the expected response to stream disturbance and correlation with a wetland disturbance gradient (developed using pH, specific conductance, and NO_3^--N data). *Kiefferulus*RA = % relative abundance of *Kiefferulus*, DipteraRA = % relative abundance of Diptera, OligoR = Oligochaeta richness, MOLCRU = % richness of mollusks and crustaceans, Eunotia = % relative abundance of *Eunotia*, NFac = high organic N facultative diatoms, low TP = diatoms in Eastern Highland streams with an affinity for low total P, MesoSap = % relative abundance of meso/polysaprobic diatoms, BMI = benthic macroinvertebrate.

Assemblage	Metric description	Expected response to stream disturbance	ρ
BMI	*Kiefferulus*RA	↑ Lenat 1993, Barbour et al. 1999, Merritt et al. 2008	−0.58
BMI	DipteraRA	↑ Lenat 1993, Barbour et al. 1999, Merritt et al. 2008	−0.49
BMI	OligoR	↑ Lenat 1993, Barbour et al. 1999, Merritt et al. 2008	0.43
BMI	MOLCRU	↑ Lenat 1993, Barbour et al. 1999, Merritt et al. 2008	−0.31
Diatom	Eunotia	↓ Lane and Brown 2007	−0.58
Diatom	Nfac	↑ van Dam et al. 1994	0.47
Diatom	low TP	↓ Potapova and Charles 2007	−0.43
Diatom	Meso/Sap	↑ van Dam et al. 1994	0.36

to the disturbance-gradient scores ($\rho = -0.71$, $p < 0.001$; Fig. 5E).

The 4 metrics selected for the diatom index included 2 that decreased with disturbance and 2 that increased with disturbance (Table 2, Fig. 5F–I)). Percent relative abundance of *Eunotia* (*Eunotia*) (Fig. 5F) and diatoms in Eastern Highland streams with an affinity for low total P (low TP) (Fig. 5H) decreased with disturbance, whereas the high organic N facultative (NFac) (Fig. 5G) and % relative abundance of meso/polysaprobic diatoms (MesoSap) (Fig. 5I) increased with disturbance (Table 2). Three diatom metrics, *Eunotia*, low TP, and NFac, were significantly correlated with the disturbance gradient ($\rho = -0.58$, -0.43, and 0.47, respectively, all $p < 0.05$), whereas MesoSap was not ($\rho = 0.36$, $p = 0.08$). The diatom index ranged from 10.8 to 79.8 and decreased in relation to the disturbance-gradient scores ($\rho = -0.64$, $p < 0.001$; Fig. 5J).

Index comparison

Based on performance of the 2 indices relative to the disturbance gradient, both assemblages seem to be good candidates for biological monitoring of connected depressions. The BMI index had a slightly higher correlation with the disturbance gradient than the diatom index ($\rho = -0.71$ and -0.64, respectively). Correlations of the BMI and diatom metrics with the disturbance gradient ranged from $|0.31|$ to $|0.58|$ and $|0.36|$ to $|0.58|$, respectively, and both indices used 1 metric that was not significantly correlated with disturbance. The diatom index had a higher correlation with specific conductance than did the BMI index ($\rho = -0.70$ and -0.53, respectively), but the 2 indices were similarly correlated with pH and NO_3^--N (ρ range from -0.55 to -0.61). Correlations and scatterplots indicated that relationships between the 2 biological indices and the disturbance gradient were much stronger than relationships between individual biological metrics and the disturbance gradient.

DISCUSSION

Significance of the disturbance gradient and relationship of DO to wetland disturbance

The water quality of least-disturbed connected depressions was characteristic of inland backwater swamps in the southeastern USA, which generally have low specific conductance, nutrient concentrations, and pH (Mitsch and Gosselink 1993). Mitsch and Gosselink (1993) reported that NO_3-N concentrations in bald cypress–water tupelo swamps in southern Illinois were often <0.01 mg/L and pH values near 6.0 could be expected. Specific conductance, pH, and NO_3^--N concentrations were higher at connected depressions sampled in the extreme northern part of our study area (where adjacent agricultural land use was substantially greater) than at connected depressions in the southernmost part of the study area. Thus, our use of specific conductance, pH, and NO_3^--N in the disturbance gradient can be justified because they have recognized relationships to disturbance in wetlands.

The 3 variables included in the disturbance gradient, and particularly specific conductance, also have a probable connection to agricultural irrigation. The complex drainage system in the low-gradient MAP often facilitates hydrologic connectivity between wetlands and agricultural fields where irrigated crops, such as corn, milo, rice, and soybeans, are rotated. Most crops are irrigated to some degree, but rice fields are flooded with several centimeters of water for most of the growing season. Rice is the most common crop grown in the Cache River Watershed (National Agriculture Statistics Service 2015).

In the Cache River Watershed and other areas of the MAP that are not near large rivers, groundwater is used

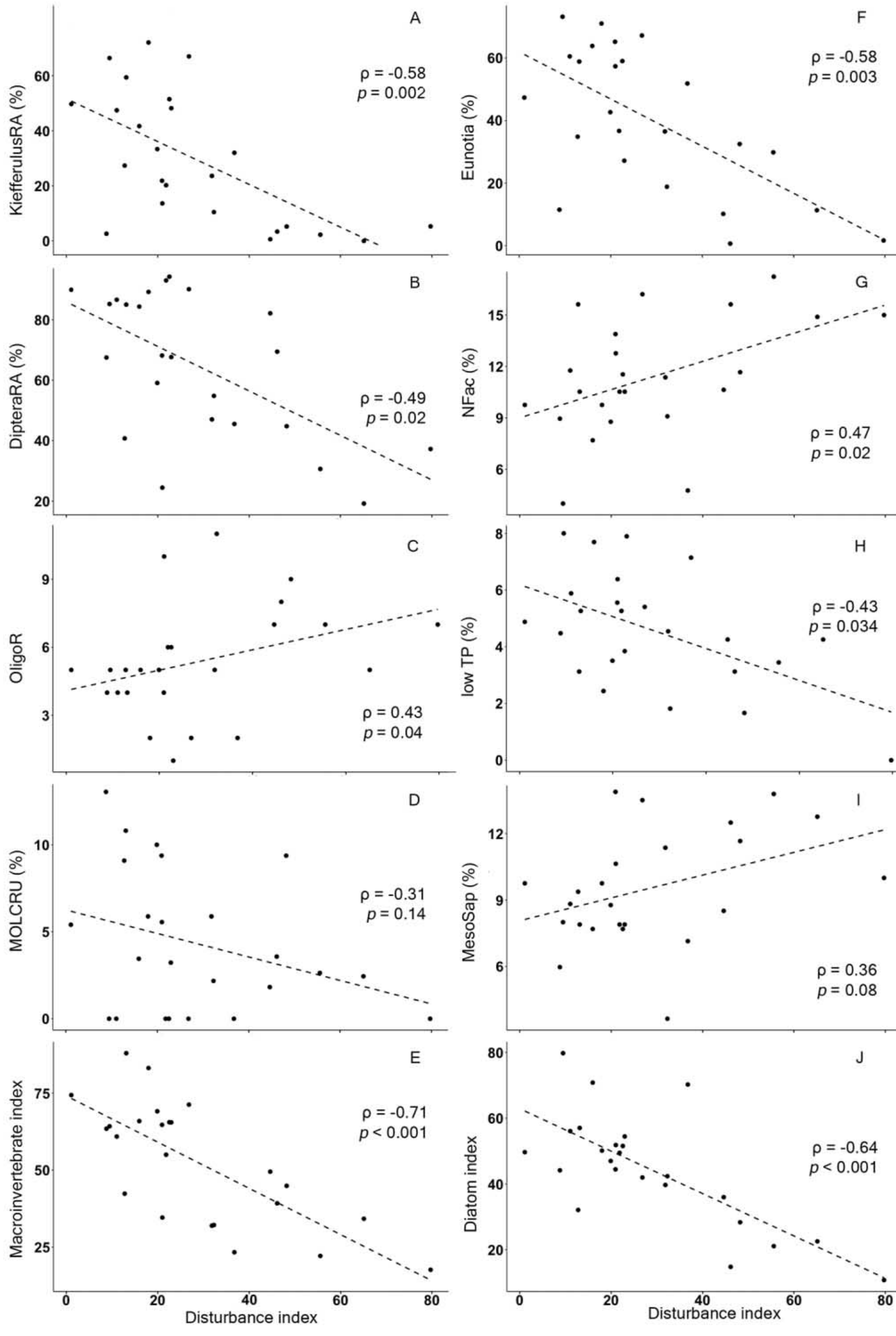

Figure 5. Scatterplots and correlations for % relative abundance of *Kiefferulus* (*Kiefferulus*RA) (A), % relative abundance of Diptera (DipteraRA) (B), Oligochaeta richness (OligoR) (C), % richness of mollusks and crustaceans (MOLCRU) (D), the macroinvertebrate index (E), % relative abundance of *Eunotia* (Eunotia) (F), high organic N facultative diatoms (NFac) (G), diatoms in Eastern Highland streams with an affinity for low total P (low TP) (H), % relative abundance of meso/polysaprobic diatoms (MesoSap) (I), and the diatom index (J) along a disturbance gradient index (developed using pH, specific conductance, and NO_3^--N) at 24 connected depression wetlands sampled in the Cache River Watershed, Arkansas.

more often than surface water for irrigation. Groundwater from the Mississippi River Valley alluvial aquifer has much higher specific conductance than does surface water (Boswell et al. 1968), and streams generally would be expected to have higher specific conductance than wetlands because of the degree of groundwater influence on streams during baseflow conditions (Payn et al. 2012). Data from the USGS National Water Information System database (USGS 2001) were used to calculate median specific conductance for 2 large surface-water and groundwater data sets. The median specific conductance of 220 measurements made at 38 streams in the MAP from 1996 to 1998 by the USGS NAWQA Program was 334 μS/cm. However, the median specific conductance for 2938 measurements from the Mississippi River Valley alluvial aquifer made by the USGS was 593 μS/cm, which was ~77% higher than surface-water measurements of specific conductance (Kresse et al. 2014). The highest specific conductance measurements recorded in connected depressions in our study were an indication of runoff associated with groundwater irrigation. NO_3^--N concentrations and pH of irrigation runoff from agricultural fields also would be higher than expected in water from least-impaired wetlands.

DO concentrations in the connected depressions also indicate that agricultural irrigation may be influencing water quality, albeit in a manner that would be considered favorable for a stream study. Isolated wetlands in the MAP characteristically have low DO concentrations because of anaerobic conditions, particularly in summer (Mitsch and Gosselink 2007). Thus, in this habitat, low DO concentration is not an indication of poor water quality (Vymazal and Kröpfelová 2008). Overflowing irrigation waters from shallow, ponded rice fields exposed to intense sunlight have DO concentrations much higher (Forés and Comín 1992) than concentrations in least-disturbed connected depressions that have dense canopies, which inhibit light and are sources of copious amounts of organic material.

Metrics selected for the indices and issues regarding ecological relevance

In some cases, BMI metrics used to indicate sources of organic pollution in streams were useful for indicating least-disturbed conditions in connected depressions. This apparent discrepancy probably is associated with differences in DO at least-disturbed connected depressions and least-disturbed streams. The positive relationship between DO and wetland disturbance observed in our study was an important consideration regarding the ecological relevance of all biological metrics. Three of the 4 macroinvertebrate metrics that were most beneficial for identifying disturbance in connected depressions decreased relative to the disturbance gradient even though they generally increase relative to disturbance gradients in streams (Lenat 1993; Table 2). Three examples of macroinvertebrate metrics that performed differently (if not inversely) than would be expected in a stream setting were *Kiefferulus*RA, DipteraRA, and MOLCRU. *Kiefferulus* was often the most common chironomid collected in the 24 connected depressions. Even though *Kiefferulus* has been assigned a tolerance value of 10 (most tolerant) in southeastern streams (Lenat 1993), *Kiefferulus*RA decreased with increasing disturbance in our wetland study. Our results are supported by the findings of Gernes and Helgen (2002) and Chipps et al. (2006), who noted that chironomids generally decrease with increasing wetland disturbance. Like *Kiefferulus*, other dipterans, mollusks, and crustaceans generally are considered to be tolerant in streams (Lenat 1993, Barbour et al. 1999, Merritt et al. 2008), but the relationships of *Kiefferulus*RA, DipteraRA, and MOLCRU to disturbance suggest that for some macroinvertebrate taxa, the organic-pollution "tolerance" responses and, therefore, the tolerance values that should be used, can differ between wetlands and streams. Rather than being an indicator of undesirable, unnatural conditions, the occurrence of taxa tolerant of low DO conditions in wetlands may be described more appropriately as a response to an ecological-niche-based mechanism (Chase and Myers 2011). Essentially, low DO conditions in shallow, connected depressions result in niche space that is limited in other aquatic environments and is limiting to many aquatic species that occur in those environments.

In contrast, relationships between diatom metrics and the disturbance gradient were more typical of the classic response that might be predicted from stream bioassessment literature. At least 3 of the 4 metrics chosen for the diatom index reflect published tolerance values (van Dam et al. 1994, Potapova and Charles 2007) or fit the traditional perception of metric response to wetland disturbance (Lane and Brown 2007, Lougheed et al. 2007). Ortiz-Lerin and Cambra (2007) documented strong relationships between *Eunotia* and specific conductance and pH, in which the relative abundance of *Eunotia* declined as the disturbance gradient increased. Two diatom metrics are related to nutrients—NFac and diatoms with a low TP affinity. The increase of NFac diatoms relative to disturbance was expected because NO_3^--N concentration was identified as an important water-quality variable and was used in the disturbance gradient. The negative relationship between diatoms with a low TP affinity and disturbance also might be expected, although P did not seem to be a good indicator of disturbance in the connected depressions, perhaps because P was naturally limited.

The diatom metric MesoSap performed opposite of what would be expected in a stream. MesoSap diatoms typically are associated with high organic matter and generally increase as O_2 is depleted (Porter 2008). However, values were slightly higher at connected depressions with the highest DO concentrations. Slight (but not significant) increases in the relative abundance of MesoSap diatoms also could be related to nutrients (e.g., increasing NO_3^--N), which were associated with our disturbance gradient.

Our results demonstrate that some metrics performed differently in the wetland setting than they would in a stream setting. Nevertheless, BMI and diatom metrics developed for streams were useful indicators of water-quality conditions in connected depression wetlands. Given the paradoxical relationships of some BMI metrics to DO, however, our results suggest that, compared with macroinvertebrate metrics, the ecological relevance of diatom metrics may be easier to interpret and defend for wetlands that have low DO under least-disturbed conditions.

Limitations and future research

The hydrology of areas dominated by row crops is often complex and highly altered because drainage systems are engineered to remove water from cropland. Our study demonstrated that these drainage systems can be sources of agricultural irrigation runoff and can influence wetland water quality and, ultimately, resident biota. Water-quality conditions in connected depressions and other wetlands are reset seasonally when they are flooded by adjacent rivers or irrigation water, but the degree of river and field connectivity is often subtle and difficult to track. If streamflow gages were proximal to sites suspected of being affected by irrigation, researchers could account better for variability associated with natural stream hydrology and water-quality conditions.

Studies are needed to investigate the specific interactions among wetland disturbances and aquatic biota. Wetland condition assessments are important, and even though the recent, immediate focus has been on streams, more energy is being focused on wetlands in some states. Continued efforts to develop biological indicators for depressional wetlands and other wetland types will inform future work and expand bioassessment capabilities in the MAP Ecoregion and elsewhere.

Diatom data from our study have been contributed to the website, Diatoms of the United States (Spaulding et al. 2010), which is a publicly accessible database collaboratively supported by the US EPA and the USGS, with contributions from numerous academic institutions across North America. Wetlands have been monitored less than most other aquatic environments, and new diatom metrics probably will be developed as diatom taxonomy is expanded to include more taxa that inhabit wetlands.

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