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INVERTEBRATE AND FISH ASSEMBLAGE RELATIONS TO DISSOLVED OXYGEN MINIMA IN LOWLAND STREAMS OF SOUTHWESTERN LOUISIANA

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ABSTRACT

Dissolved oxygen (DO) concentrations in lowland streams are naturally lower than those in upland streams; however, in some regions where monitoring data are lacking, DO criteria originally established for upland streams have been applied to lowland streams. This study investigated the DO concentrations at which fish and invertebrate assemblages at 35 sites located on lowland streams in southwestern Louisiana began to demonstrate biological thresholds.

Average threshold values for taxa richness, diversity and abundance metrics were 2.6 and 2.3 mg/L for the invertebrate and fish assemblages, respectively. These thresholds are approximately twice the DO concentration that some native fish species are capable of tolerating and are comparable with DO criteria that have been recently applied to some coastal streams in Louisiana and Texas. DO minima >2.5 mg/L were favoured for all but extremely tolerant taxa. Extremely tolerant taxa had respiratory adaptations that gave them a competitive advantage, and their success when DO minima were <2 mg/L could be related more to reductions in competition or predation than to DO concentration directly.

DO generally had an inverse relation to the amount of agriculture in the buffer area; however, DO concentrations at sites with both low and high amounts of agriculture (including three least-disturbed sites) declined to <2.5 mg/L. Thus, although DO fell below a concentration that was identified as an approximate biological threshold, sources of this condition were sometimes natural (allochthonous material) and had little relation to anthropogenic activity. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS: dissolved oxygen; threshold response; tolerance; metric; intermediate disturbance; lowland streams; Louisiana

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INTRODUCTION

In their most natural condition, streams in coastal areas of southwestern Louisiana are black water bayous that may have little flow but are often deep and meander through dense stands of hardwood and cypress/tupelo gum swamps. The dense vegetation characteristic of the most natural of these bayous is a source of shade (that reduces light and photosynthetic processes) and substantial amounts of allochthonous organic material. As a consequence of substantial decomposition and low aeration and flushing rates under the best of summer conditions, DO concentrations in lowland streams are naturally lower than those in upland streams, and native biota often have respiratory or physical adaptations that enable them to cope with inherently harsh conditions (Eriksen *et al*., 1996; Val *et al*., 1998)

Natural differences in water quality and biology of lowland and upland streams may have led some to perceive that lowland streams are not worthy of the same level of protection as upland streams, and the same level of monitoring resources has not been allocated for the protection of high-quality waters in upland and lowland regions. Consequently, as the need for establishing criteria in lowland streams has increased, criteria originally established for upland streams have often been applied to lowland streams. As monitoring efforts have increased, however, it has become apparent that DO criteria for upland streams are not applicable to lowland streams. Consequently, some lowland bodies of water that are listed as being impaired because of DO concentrations are minimally affected by anthropogenic activities (Weaver, 2004; Todd *et al*., 2009).

Currently, more Louisiana streams and rivers are reported to be impaired by DO than any other source of impairment (Louisiana Department of Environmental Quality, 2010). The minimum DO concentration (henceforth, minimum DO) criteria that have been applied most frequently to coastal lowland streams in Louisiana and lowland streams in surrounding states has varied from 4 to 5 mg/L (Louisiana Department of Environmental Quality, 2005; Arkansas Department of Environmental Quality, 2007; Mississippi Department of Environmental Quality, 2007). Louisiana and Texas, however, have recently applied lower DO criteria to some coastal streams (Texas Commission on Environmental Quality, 2007; Louisiana Department of Environmental Quality, 2009).

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There may be no other characteristic that is more critical to aquatic biota than DO, and relations between biological assemblages and DO concentrations need to be well understood before DO criteria are established. Information regarding biological thresholds (i.e. the DO concentration at which conditions seem to become favourable or unfavourable) can be essential for criteria development (Brenden *et al*., 2008), but aquatic assemblage data are also valuable for indicating antecedent DO conditions for periods equivalent to the length of their aquatic life cycle (i.e. for months or years before biological sampling).

Most field studies that have investigated biological relations to DO have focussed on tolerances and adaptations for fish in harsh environments such as headwater streams (Tramer, 1977; Smale and Rabeni, 1995; Ostrand and Wilde, 2001), prairie streams (Gee *et al*., 1978; Matthews, 1987; Koehle and Adelman, 2007) and wetlands (Schofield, 2007). However, a literature search revealed no studies that had investigated relations between aquatic assemblages and DO minima in coastal streams or that had compared relations of the two assemblages to DO minima. Regarding species-specific tolerances, DO tolerances have been determined in the laboratory for many Canadian invertebrate and fish species (Davis, 1975), but most of the efforts for determining DO tolerances in the United States have focussed on fish (Moore, 1942; Doudoroff and Shumway, 1970).

The objectives of this study were i) to compare the degree that DO minima and other measured habitat variables influenced invertebrate and fish assemblages and ii) to evaluate relations of the two biological assemblages to DO. Results of this study, which was coordinated in cooperation with the US Environmental Protection Agency (USEPA) Region 6, should facilitate a better understanding of the natural biological setting of lowland streams in southwestern Louisiana, indicate the value of the two assemblages for investigating the ecological consequences of DO minima and provide information that can be used to help establish DO criteria for streams in southwestern Louisiana and other areas with coastal plains and large alluvial plains.

Description of the study area

The study area in southwestern Louisiana (Figure 1) is bounded by the southern part of the South Central Coastal Plain ecoregion to the north, the Atchafalaya River Basin to the east, the Gulf of Mexico to the south and the State of Texas to the west. The 35 stream sites were divided among three Level III ecoregions (Omernik, 1987); 17 were located in the Western Gulf Coastal Plain (WGCP) ecoregion, 14 were located in the South Central Plains (SCP) ecoregion and 4 were located in the Mississippi Alluvial Plain (MAP) ecoregion.

Streams that were in the SCP ecoregion generally had more gradient than streams in the WGCP and MAP ecoregions and had surrounding forests of mixed pines, whereas forested sites located within the WGCP and MAP ecoregions were buffered by cypress/tupelo gum swamps. Timber production and commercial nurseries were the two major forms of land use in the SCP ecoregion (Bentley *et al*., 2005; National Agriculture Statistical Service, 2009), row crop agriculture (primarily rice farming) and crayfish and cattle production dominated land use in the parts of the WGCP and MAP ecoregions that were sampled (National Agriculture Statistical Service, 2009).

METHODS

Site selection

All 35 sampling sites (Table I) were located on perennial streams and were selected by staff from USEPA Region 6. Twenty-six of the sites were test sites that were selected using an unequal probability selection approach and grid design (Herlihy *et al*., 2000), but nine sites were sampled because of their potential for being least-disturbed sites for three stream size classes—small, medium and large (i.e. all small streams were wadeable and all large streams required a boat to sample, but some medium streams were wadeable and some were not). The nine "potential least-disturbed sites" were selected using a ranking approach that evaluated land-use metrics identified by Hughes *et al*. (1986) and Braden and Webber (1992). Land-use metrics included the amount riparian vegetation near the stream channel, distance to the nearest wastewater discharge, road density and proximity to populated areas. All nine potential least-disturbed sites were located in the SCP ecoregion and on seven streams that had been sampled previously by the Louisiana Department of Environmental Quality (Dewalt, 1997).

Field observations made during biological sampling indicated that some physical characteristics that could influence DO concentration (e.g. gradient, velocity, water clarity and substrate particle size) of the nine potential least-disturbed sites located in the SCP ecoregion were very different (i.e. usually resulting in better DO conditions) from that of even the most natural sites in the MAP and WGCP ecoregions where most of the 26 test sites were located. Although the ecological quality of reference sites (or least-disturbed sites) would generally be expected to exceed that of test sites, a basic assumption associated with least-disturbed stream selection is that least-disturbed streams in one ecoregion often are not suitable for assessing the condition of streams in a different ecoregion (Hughes *et al*., 1986). As a way of determining the suitability of data from the nine potential least-disturbed sites as reference data, a non-parametric multivariate method—multidimensional scaling

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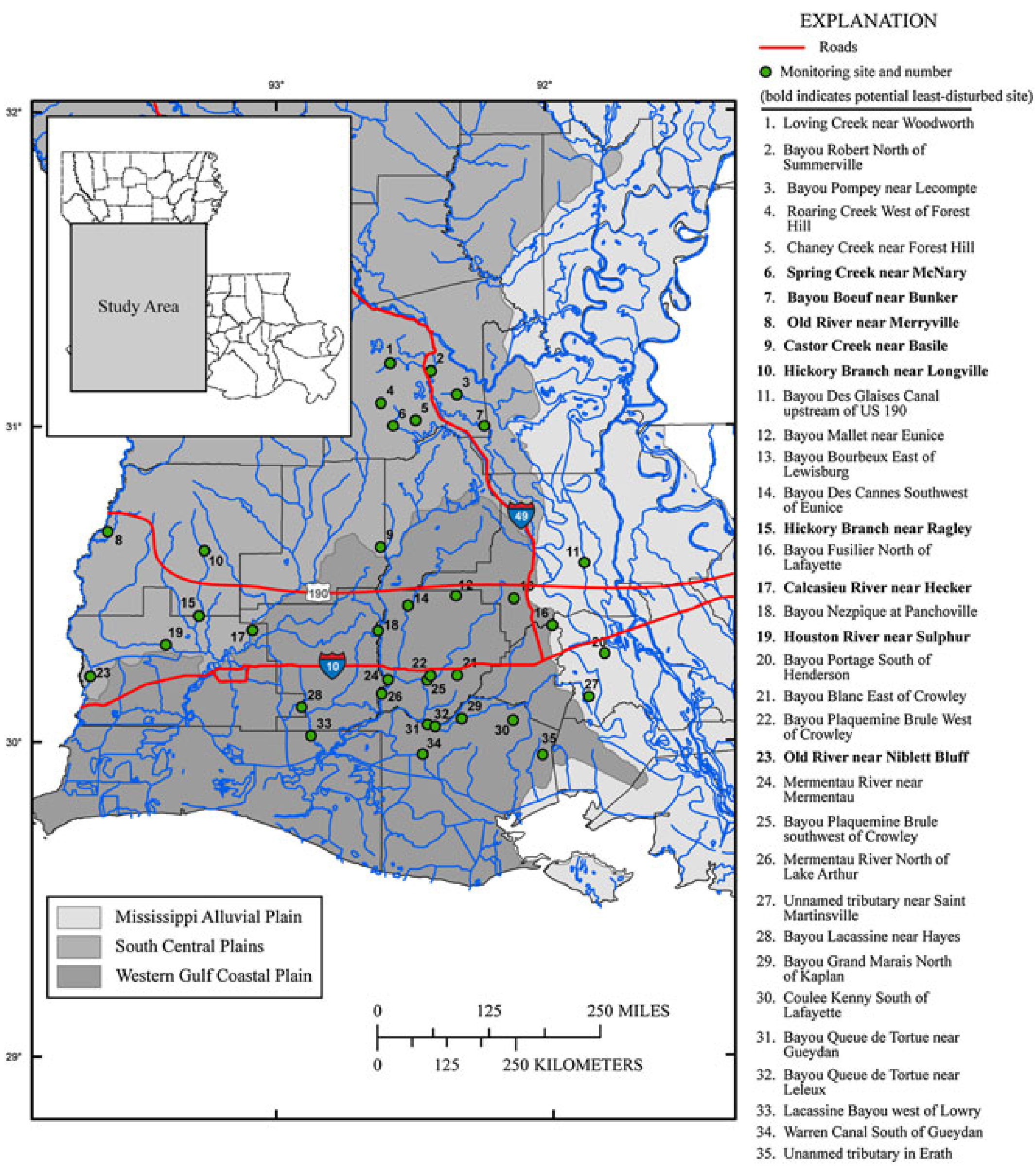

Figure 1. Location of 35 sites in southwestern Louisiana that were sampled for DO, invertebrates and fish from 2005 to 2007. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

(MDS, Clarke and Warwick, 2001)—was used to evaluate the similarity of biological samples at all 35 sites and for both assemblages.

Data characteristics

Biological and physical (habitat) data and DO data were collected and processed consistently from site to site and, except for a few differences noted below, according to US Geological Survey (USGS) protocols (Fitzpatrick *et al*., 1998; Wagner *et al*., 2000; Moulton *et al*., 2002). Field characteristics (DO, water temperature, specific conductivity and pH) were measured with a water-quality monitor on each day when biological sampling occurred. Observations for variables that could influence DO (e.g. “green” colour and velocity) as well as antecedent conditions (e.g. degree of cloud cover and amount of precipitation in recent days) were recorded. Transparency was measured using a Secchi disc reading at the time of fish sampling.

DO monitoring

An underlying assumption of the study design was that biological assemblages (recruitment) should reflect temporal variability in DO condition. Water-quality monitors were deployed at the nine potential least-disturbed sites in 2005

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Table I. Selected information for sites sampled for DO, invertebrates and fish in coastal streams of southwestern Louisiana, 2005–2007

Site name	Site no. (Figure 1)	Abbreviated name	DO monitoring year	Level IV ecoregion	Latitude	Longitude	Stream size	Wadeability	Channel characteristic
Loving Creek near Woodworth	**1**	**Loving**	2005–2007	SCP	31.2031	92.5781	Small	W	Natural
Bayou Robert North of Summerville	2	Robert	2007	SCP	31.1781	92.4278	Medium	NW	Canal
Bayou Pompey near Lecompte	3	Pompey	2007	SCP	31.1029	92.3322	Small	W	Natural
Roaring Creek West of Forest Hill	4	Roar	2007	SCP	31.0770	92.6126	Medium	W	Natural
Chaney Creek near Forest Hill	5	Chaney	2007	SCP	31.0207	92.4856	Small	W	Natural
Spring Creek near McNary	**6**	**Spring**	2005–2007	SCP	31.0028	92.5683	Medium	NW	Natural
Bayou Boeuf near Bunker	**7**	**Boeuf**	2005–2007	SCP	31.0022	92.2313	Medium	NW	Natural
Old River near Merryville	**8**	**SabU**	2005–2007	SCP	30.6664	93.6178	Medium	NW	Natural
Castor Creek near Basile	**9**	**Castor**	2005–2007	SCP	30.6190	92.6157	Medium	W	Natural
Hickory Branch near Longville	**10**	**HickU**	2005–2007	SCP	30.6072	93.2619	Small	W	Natural
Bayou Des Glaises Canal upstream of US 190	11	Des G	2007	MAP	30.5657	91.8687	Large	NW	Canal
Bayou Mallet near Eunice	12	Mallet	2007	WGCP	30.4628	92.3406	Medium	NW	Natural
Bayou Bourbeux East of Lewisburg	13	Bourbo	2007	WGCP	30.4530	92.1284	Small	W	Canal
Bayou Des Cannes Southwest of Eunice	14	Des C	2007	WGCP	30.4331	92.5161	Medium	NW	Natural
Hickory Branch near Ragley	**15**	**HickL**	2005–2007	SCP	30.4027	93.2829	Medium	W	Natural
Bayou Fusilier North of Lafayette	16	Fuse	2007	MAP	30.3699	91.9880	Medium	NW	Natural
Calcasieu River near Hecker	**17**	**Calcas**	2005–2007	SCP	30.3567	93.0878	Large	NW	Natural
Bayou Nezpique at Panchoville	18	Nez	2007	WGCP	30.3558	92.6253	Medium	NW	Natural
Houston River near Sulfur	**19**	**Houstn**	2005–2007	SCP	30.3104	93.4033	Medium	NW	Natural
Bayou Portage South of Henderson	20	Portag	2007	MAP	30.2802	91.7972	Large	NW	Canal
Bayou Blanc East of Crowley	21	Blanc	2007	WGCP	30.2130	92.3375	Small	W	Canal
Bayou Plaquemine Brule West of Crowley	22	PBrulU	2007	WGCP	30.2123	92.4354	Large	NW	Natural
Old River near Niblett Bluff	23	SabL	2007	SCP	30.2085	93.6802	Large	NW	Natural
Mermentau River near Mermentau	24	MermU	2007	WGCP	30.1999	92.5896	Large	NW	Natural
Bayou Plaquemine Brule southwest of Crowley	25	PBrulL	2007	WGCP	30.1973	92.4471	Large	NW	Natural
Mermentau River North of Lake Arthur	26	MermL	2007	WGCP	30.1556	92.6130	Large	NW	Natural
Unnamed tributary near Saint Martinsville	27	StMart	2007	MAP	30.1411	91.8568	Medium	NW	Natural
Bayou Lacassine near Hayes	28	LacU	2007	WGCP	30.1123	92.9063	Large	NW	Natural
Bayou Grand Marais North of Kaplan	29	GMar	2007	WGCP	30.0753	92.3213	Medium	W	Canal
Coulee Kenny South of Lafayette	30	Ckenny	2007	WGCP	30.0687	92.1338	Small	W	Canal
Bayou Queue de Tortue near Gueydan	31	TortL	2007	WGCP	30.0569	92.4463	Medium	NW	Natural
Bayou Queue de Tortue near Leleux	32	TortU	2007	WGCP	30.0509	92.4180	Medium	NW	Natural
Lacassine Bayou west of Lowry	33	LacL	2007	WGCP	30.0223	92.8713	Large	NW	Natural
Warren Canal South of Gueydan	34	WarC	2007	WGCP	29.9632	92.4658	Large	NW	Canal
Unanmed tributary in Erath	35	Erath	2007	WGCP	29.9591	92.0264	Small	W	Canal

Items in boldface denote nine potential least-disturbed sites; DO, dissolved oxygen; macroinvertebrates and fish were sampled in 2006 and 2007, respectively; SCP, South Central Plains; WGCP, West Gulf Coastal Plain; MAP, Mississippi Alluvial Plain; W, wadeable; NW, nonwadeable; canal, a channelized stream.

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and 2006 from late August to early September. DO-related stress is typically highest for aquatic biological assemblages during this late summer period because water temperatures are highest and DO saturation rates are lowest for the year. DO data were collected every 15 min, at a depth of approximately 0.5 m, and a mechanical stirrer was used to mix water near the probe. DO data were collected continuously for a total of 11–20 days of record.

A partial day of continuous DO data were collected again at the nine potential least-disturbed sites, and also at 26 test sites as fish were sampled from late August through early October in 2007. DO data were collected at the 35 sites for an average time of 5.4 h per site and for the 26 test sites; these were the only DO data available for analysis. A mechanical stirrer was not used while DO was monitored in 2007 because of the abbreviated monitoring period. When possible, WQMs were deployed upstream from the sampling reach to avoid disturbance as fish were sampled.

Physical habitat and biological sampling

All biological sampling was conducted within a stream reach with a length that was approximately 20 times the mean stream width. Invertebrates were sampled in March and April 2006. Fish samples were collected from mid-August through early October 2007. Habitat measures (e.g. stream width and depth, flow velocity, canopy cover and percent in-stream cover) were recorded at the same time invertebrates were sampled.

Aquatic invertebrate samples were collected from woody snags because snags often represent the taxonomically richest-targeted habitat in lowland streams (Moulton *et al.*, 2002). Snags were collected from five different locations throughout the reach and generally ranged from 2 to 10 cm in diameter and from 30 to 40 cm in length. Invertebrates were handpicked or brushed from the snags as loose bark and wood were removed. Before disposal, snags were allowed to dry and were inspected for additional organisms. The invertebrate sample was preserved in 50% ethanol, and rare and large organisms were preserved separately. Invertebrates were identified and enumerated by EcoAnalysts (Moscow, Idaho) using a subsample of 300 organisms per site. Invertebrate counts were adjusted to account for subsampling.

Fish were sampled using electrofishing as a primary method and seining as a secondary method at all sites, but the amount of effort varied by stream size to a small degree. Nonwadeable sites (23) were sampled using a boat-mounted 5.0 GPP (Smith Root, Seattle, WA), whereas wadeable sites (12) were sampled using backpack-mounted electrofishing gear (Model 12B; Smith Root). At nonwadeable sites, boat electrofishing passes progressed from the upstream boundary of the sampling reach to the downstream boundary, but at wadeable sites, electrofishing was conducted from downstream to upstream. Both banks of the reach were electrofished at all sites except the largest site, the downstream site on Lacassine Bayou (LacL), where only one bank was sampled. Three to six seine hauls (relative to stream size) were made at each site, in unique habitats when available, and after electrofishing was completed. At some sites where water depth or other factors prevented seining by wading, a seine haul consisted of sweeping the seine beneath floating vegetation and scooping the material into a boat. Fish collected were identified, enumerated and released outside the sampling reach to prevent recapture. Fish were recorded on separate field sheets by sampling method, but all fish collected at a site were combined into one sample before analysis.

Fish that could not be identified in the field were preserved and identified to the lowest possible taxon (usually species) in a laboratory at the USGS Arkansas Water Science Center. Fish identified in the USGS laboratory were transferred to the Mississippi Museum of Natural History in Jackson, Mississippi, where taxonomy was verified and specimens were permanently vouchered with locality information. Fish were identified using keys for Louisiana (Douglas, 1974), Arkansas (Robison and Buchanan, 1988) and Mississippi (Ross, 2001).

DO calculations

As a way of determining the DO concentrations that might constitute a biological threshold for sites in this study, biological metrics were compared with the minimum DO concentration for the day that DO was monitored. After a review of the DO data indicating that concentrations at approximately 0800 h (central standard time) were consistently near the daily minimum, DO concentrations at 0800 h were selected to be a surrogate concentration for minimum DO.

DO minima for 0800 h were obtained for the nine sites where DO concentrations were measured in 2005 and 2006 by averaging DO concentrations that were measured at 0800 h for multiple days; however, for the 26 sites where DO was only measured in 2007 (and during fish sampling), DO minima were estimated using an extrapolation method. That method involved using DO data collected at 15-min intervals during the fish sampling effort to calculate the average change in DO concentration per hour. That rate of change was then used to estimate the DO concentration at 0800 h. The linear rate of change that was applied follows the general limnological concept that DO (concentration) curves between early morning minimums and early evening maximums have less curvature than other periods of the day (e.g. near dawn and after dusk, Wetzel, 2001).

To assess annual variability and variability associated with the process of estimating DO concentrations at 0800 h, DO minima that were measured (in 2005 and 2006) or estimated (in 2007) for the nine potential least-disturbed

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sites were compared with a Wilcoxon rank sum test. Averages for DO minima at the nine sites that were continuously monitored in 2005 and 2006 were compared, by year, to the average DO minimum for the 11–20 days that were monitored in both years and to the DO minima that were estimated for 2007.

Analysis

As a way of addressing the first objective—to compare the degree that DO minima and other habitat variables influenced invertebrate and fish assemblages—canonical correspondence analysis (CCA; ter Braak, 1986), a constrained ordination technique was used to compare the strength of relations of DO minima and 13 stream-habitat and water-quality variables (Table S1 in Supplementary material) to invertebrate and fish abundance data. CCA was conducted using the statistical software package, PC-ORD (McCune and Mefford, 2006). Abundance data were left untransformed because there was little difference in plots when data were transformed and untransformed. PC-ORD also was used to construct CCA biplots depicting relations of the habitat or water-quality variables with the highest correlations to the first two axes for both assemblages. PC-ORD inserts vectors into the biplot, and the length and angle of those vectors to the ordination axes represent the degree that the habitat or water-quality variables are related to the two axes.

The response of the two biological assemblages to DO minima was evaluated (the second but primary study objective) using an approach that combined multimetric and regression techniques. The primary benefit of the multimetric evaluation was that it facilitated observations of the response of multiple species of varying tolerance from two assemblages to DO concentrations (i.e. was holistic in nature) to identify possible thresholds. This approach is consistent with a basic ecological principle—as less tolerant organisms become stressed (by DO, in this case), competition is reduced and tolerant organisms increase (Hynes, 1960). Metrics associated with intolerant taxa were not used in this analysis because our experience has been that even reference lowland streams (bayous that have little anthropogenic disturbance but are heavily forested and poorly flushed) can be expected to have DO conditions that are naturally limiting to almost all sensitive taxa (e.g. plecopterans). Consequently, because there are few truly intolerant species (not to be confused with rare species) that are associated with lowland streams, intolerant metrics are not robust.

As a first step to address the second objective, the relative abundance (RA) percentage of three invertebrate taxa and three fish taxa representing three (organic pollution) tolerance classifications—moderately tolerant, tolerant and extremely tolerant—were compared with DO minima. Second, three metrics that were common to both assemblages and had little dependence on stream size—taxa richness, Brillouins diversity and total abundance—were also compared with DO minima. Scatterplots were used to compare the six metrics calculated for each assemblage to DO minima, and a locally weighted scatterplot smoothing (LOWESS) line was constructed for each scatterplot (using SigmaPlot Version 11; Systat Software, 2008). LOWESS lines were constructed using a sampling proportion of 0.5 and a polynomial regression degree of 1. LOWESS lines were used to generally indicate when metrics changed dramatically in response to DO minima and those break points were considered as potential DO thresholds.

Although considerable effort has been spent identifying species-specific DO thresholds for both assemblages (Moore, 1942; Doudoroff and Shumway, 1970; Davis, 1975), there is little DO tolerance information in the literature for most invertebrate and fish species collected in this study. However, because of the strong relation between some aspects of organic pollution (particularly eutrophication and decomposition) and DO concentrations (Hynes, 1960; Wetzel, 2001), species-specific tolerances to organic pollution (TOPs) were assumed to have relevance to DO tolerance, particularly for invertebrate taxa.

The two pair of invertebrate and fish taxa selected to represent the moderately tolerant and tolerant classifications were selected because of knowledge regarding their TOPs. Both extremely tolerant taxa, however, were selected because of respiratory adaptations that enable them to tolerate hypoxic conditions. TOPs have been fairly well documented for a large number of invertebrates (Hilsenhoff, 1987; Lenat, 1993), but guidance for fish is much more general. Lenat (1993) applied a TOP scale ranging from 0 to 10 for invertebrates in the southeastern United States and applied TOP values of approximately 6.5, 8 and 8.5, respectively, to the three invertebrate taxa that we selected to represent the moderately tolerant, tolerant and extremely tolerant classifications. Barbour *et al.* (1999) summarized tolerances for several fish species; however, tolerances were not associated with specific stressors (e.g. nutrients or DO). Also, the scale used to classify fish by Barbour *et al.* (1999) is less robust than for invertebrates, and rather than numeric categories, fish are divided into narrative categories (e.g. intolerant, moderately tolerant and tolerant). Barbour *et al.* (1999) list the fish taxa that we associate with the moderately tolerant classification as an intolerant, and they list the two species that we associate with the tolerant and extremely tolerant classifications as being moderately tolerant.

The invertebrate and fish taxa selected to represent the moderately tolerant classification included a genus collectively called green midges (*Tanytarsus* spp.) and the longear sunfish (*Lepomis megalotis*). The two taxa selected to represent the tolerant classification were a genus of side swimmers (*Crangonyx* spp.) and the smallmouth buffalo (*Ictiobus bubalus*).

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For the extremely tolerant classification, the two taxa included a genus of bloodworm (*Glyptotendipes* spp.) and the spotted gar (*Lepisosteus oculatus*).

Piecewise regression, a nonlinear regression technique that is sometimes used for identifying ecological thresholds (Toms and Lesperance, 2003; Brenden *et al*., 2008), was used to identify (DO minima) break points for the same three metrics across both assemblages—taxa richness, Brillouins diversity and total abundance. Before conducting piecewise regression, general locations of potential thresholds were identified with LOESS curves. DO minima in the general “threshold” area were evaluated with a two-segmented regression and by manually testing DO concentrations (in 0.25-mg/L increments) to determine which concentrations were break points that were statistically significant or, in two of six cases, most statistically significant. The most statistically significant (lowest *p*-value) break points identified for taxa richness, diversity and total abundance metrics were averaged to obtain an average mean threshold value for each assemblage. Piecewise regressions also were performed using SigmaPlot Version 11 (Systat Software, 2008).

Land-use comparisons

Land use was determined at three different scales (that included the sampling reach): a 1000-m reach, the entire stream and the entire watershed. A buffer width of 500 m was used with all three scales because many sites had floodplains that were less than 500 m in width. Preliminary analysis indicated there were only slight differences between results of the three scales, and land-use data for the 500-m buffer area of the entire length of each stream were selected for comparison to DO minima.

Land-use percentages were determined using GIS software and geospatial data sets for vegetation that were obtained from Geographic Approach to Planning Projects in Louisiana (USGS, 2005a) and Texas (USGS, 2005b). Only land-use types that composed at least 10% of the land use at one or more sites were used in the analysis (Table S1 in Supplementary material).

CCA (ter Braak, 1986) was used (a second time) to compare the strength of relations for DO minima and selected land-use variables to invertebrate and fish assemblage data and to determine how variables with strongest relations compared for the two assemblages. Agricultural land-use data also were plotted against DO minima to evaluate relations between agricultural practices and DO minima.

RESULTS

For most of the six metrics that were calculated for each of the two assemblages, a wedge-shaped scatterplot was apparent when abundance data were compared with DO minima. Wedge-shaped plots often result when sites that have similar concentrations of an independent test variable (DO in this case) are associated with low (unfavourable) and high biological metric scores. This variability in metric scores often occurs because variables other than the independent test variable are negatively influencing metric scores. Terrel *et al*. (1996) demonstrated that the 90th regression quantile is often the most valuable quantile for identifying habitat variables that influence biological assemblages. Consistent with that finding, the highest regression quantiles (i.e. > 0.50) were crucial for determining when changes in metric data occurred for this study (Figure 2).

The MDS analysis revealed that the biological composition of the 35 sampling sites could be coarsely divided by Level IV ecoregion (Figure 3) and indicated that differences in ecological conditions between the potential least-disturbed sites and the test sites could be the result of spatial (i.e. physical habitat) variability. Consequently, little analytical emphasis was placed on comparing test sites to the least-disturbed sites, and only the three potential least-disturbed sites that were nearest to the test sites (both in terms of biological composition and physical location)—Castor Creek near Basile (Castor), Houston River near Sulfur (Houstn) and Hickory Branch near Ragley (HickL)—were considered to have value as least-disturbed (reference) streams. Data from the six remaining potential least-disturbed sites were valuable to other aspects of the analysis, however, because they extended the DO range beyond what would have been possible had only data from the remaining 29 sites been used.

Minimum DO characteristics

DO minima estimated for the 35 sites using data collected on the day of fish sampling in 2007 ranged from 0.2 to 7.9 mg/L. Averages of the daily DO minima for seven and six of the nine sites (respectively) that were continuously monitored for multiple days in 2005 and 2006 were lower than DO minima estimated for 2007. DO minima that were averaged for three of the nine sites—Castor, Houstn and HickU—were much lower in 2005 than that in 2006 (Table II).

Wilcoxon ranked sum tests indicate that averages of DO minima for the nine sites sampled in 2005 for multiple days were significantly different from DO minima that were estimated with data collected on the day of fish sampling in 2007, but averages of DO minima (for the same nine sites) in 2006 were not significantly different from DO minima estimated in 2007 (Table II). Consequently, because data averaged from multiple days would be expected to be less variable than data collected on one day, average DO minima for the nine potential least impaired sites that were sampled in 2006 were substituted for minima values that

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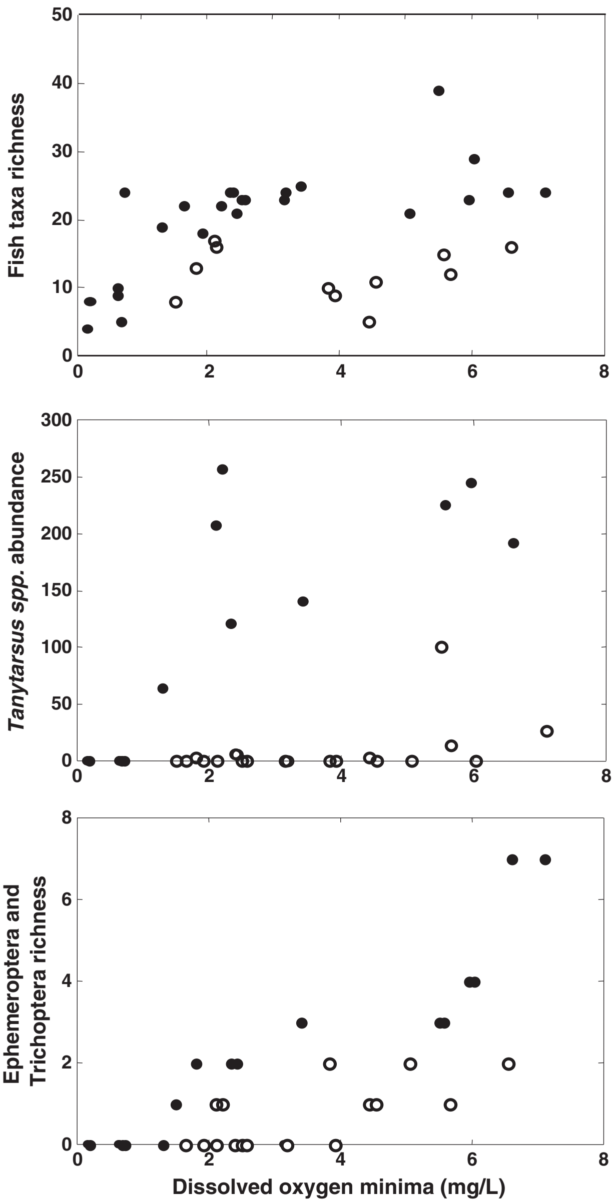

Figure 2. Examples of selected taxa and their response to DO minima demonstrate how high regression quantiles can be used to identify effects of known variables from other unknown or unmeasured variables. Metric values at sites depicted with unfilled circles were much lower than values at sites with lower or comparable DO minima (filled circles) and are presumed to be responding to other variables

were estimated with DO data collected as fish were sampled in 2007. Thus, the range of DO minima that was ultimately used for all statistical analysis was 0.2 to 7.1 mg/L (Table III).

Influence of DO minima and other habitat variables on biological assemblages

Of the 14 stream-habitat and water-quality variables that were compared with invertebrate and fish assemblage data with CCA (Table S1 in Supplementary material), DO minima was one of three variables with the strongest relations to the first two axes for both ordinations. For the invertebrate assemblage, canopy cover ($r = -0.78$) and DO minima ($r = -0.73$) had the highest correlations to axis one, whereas median turbidity had the highest correlation ($r = -0.95$) to axis 2 (Figure 4). For the fish assemblage, wetted width ($r = -0.811$) and percent canopy cover had the highest correlations to axis 1 ($r = 0.73$) and DO minima had the highest correlation to axis 2 ($r = 0.56$, Figure 4). The 14 variables explained almost the same amount of variance for the first two axes for each set of assemblage data; 23.0% of the variance associated with the invertebrate data and 22.6% of the variance associated with the fish data.

Biological assemblage response to DO minima

Relations between RA percentage and DO minima were fairly distinct for the three pairs of invertebrate and fish taxa that were selected to represent the moderately tolerant, tolerant and extremely tolerant classifications for both assemblages. The three patterns had in common that there were modest to dramatic fluctuations in RA percentage when DO minima declined to approximately 2.5 mg/L (Figure 5); the general descriptions of the three patterns were as follows.

For the two moderately tolerant taxa (green midges and longear sunfish), the highest RA percentages were typically associated with DO minima that were between 2.3 and 7 mg/L. Where DO minima were <2.3 mg/L, green midge RA percentage was consistently near 0 at all sites and RA percentage for longear sunfish did not exceed 17%. The abundance pattern typical of the two tolerant taxa, side swimmers and smallmouth buffalo, was unimodal in shape. The highest RA percentages were observed at sites having DO minima between approximately 2.5 and 4 mg/L, but sites with the lowest and highest DO minima generally had low RA percentages. For the two extremely tolerant taxa, bloodworm and spotted gar, RA percentages were inversely related to DO minima and were highest when DO minima were ≤ 2 mg/L. Several other invertebrate and fish taxa also had abundance distributions that generally fit the three tolerance patterns described above (and in Figure 5, Table IV).

Scatterplots (and LOESS lines) comparing DO minima to invertebrate and fish taxa richness, diversity and abundance indicate that most favourable (highest, in this case) metric values generally were associated with DO minima that were 1.75 mg/L or higher (Figure 6). The one exception, invertebrate abundance, was inversely related to DO, and the highest invertebrate abundances occurred at sites where DO minima were less than approximately 3 mg/L.

Thresholds identified with piecewise regression for taxa richness, diversity and abundance metrics ranged from 1.5 to 3.5 mg/L (Figure 6). The average threshold concentration

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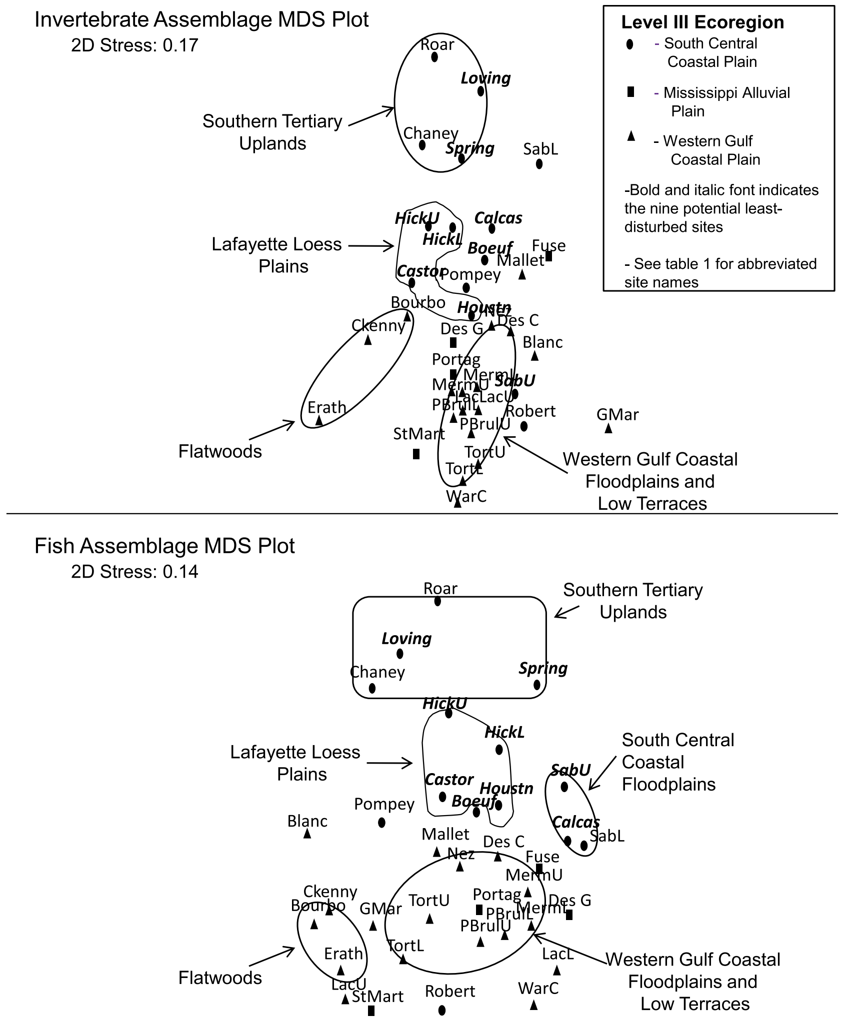

Figure 3. MDS plots indicate how invertebrate and fish assemblage samples collected at 35 sites in southwestern Louisiana correspond to Level IV ecoregion divisions (Daigle *et al.*, 2006). To demonstrate the proximity of the sites within specific ecoregion classifications to each other, ellipses are drawn around sites within selected Level IV ecoregions, and Level III ecoregions are denoted with symbols. The stress value, a measure of the extent that scatter points deviate from the regression line, is relatively small for both assemblages and indicates that the sample relationships were easily compressed into two dimensions

for the three metrics was 2.6 and 2.3 mg/L for the invertebrate and fish assemblage, respectively. All thresholds identified for the fish assemblage were statistically significant ($p < 0.05$); however, of the three invertebrate metrics, only the threshold identified for invertebrate diversity was statistically significant. Thresholds identified for invertebrate taxa richness and abundance had p-values of 0.169 (about a DO minimum of 1.75 mg/L) and 0.083 (about a DO minimum of 2.75 mg/L). Although these thresholds are not statistically significant, they do indicate the probabilities that changes that occurred for these two metrics at these concentrations were approximately 83% and 92%, respectively.

Fish and invertebrate abundance had a weaker relation to DO minima than did richness and diversity. The thresholds identified using invertebrate and fish abundance had the lowest R^2 values of all six metrics evaluated with piecewise regression, and also invertebrate abundance was one of the two metrics where the most significant threshold had a p-value that was slightly > 0.05.

Of the five land-use variables that were compared with invertebrate and fish assemblage data (Table S1 in Supplementary material), the percentage of upland vegetation and the percentage of agriculture were the two variables most related to the two biological assemblages (Figure 7). Eigenvalues for the two axes representing the invertebrate assemblage were 0.58 and 0.26. The first two axes explained 17.7% of the variance in the invertebrate species data. Eigenvalues for the two axes representing the fish assemblage were 0.45 and 0.42. The first two axes explained 20.2% of the variance in the fish species data.

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Table II. Summary information comparing minimum DO concentrations measured, averaged or estimated for nine least-disturbed sites sampled in 2005–2007

Abbreviated name (Table I)	Averaged DO minima, 2005	Averaged DO minima, 2006	Averaged DO minima, 2005 and 2006	Estimated DO minima, 2007	Difference			
					2005 and 2006	2005 and 2007	2006 and 2007	2005–2006 average and 2007
Boeuf	3.1	3.7	3.4	3.1	0.6	0.0	−0.5	−0.3
Calcas	5.4	5.6	5.5	6.5	0.2	1.2	0.9	1.1
Castor	0.8	2.8	1.8	3.7	2.0	2.9	0.9	1.9
HickL	2.5	1.7	2.1	2.9	−0.7	0.4	1.2	0.8
HickU	0.5	3.9	2.2	2.1	3.4	1.6	−1.8	−0.1
Houstn	0.2	2.3	1.3	3.4	2.1	3.2	1.1	2.2
Loving	7.4	5.7	6.6	7.9	−1.7	0.4	2.1	1.3
SabU	2.7	2.3	2.5	7.8	−0.3	5.1	5.4	5.3
Spring	7.1	7.1	7.1	6.9	0.0	−0.2	−0.2	−0.2
Statistical difference	**A**	AB	AB	**B**				

Sampling years that were statistically different (as indicated by the Wilcoxon ranked sum test, $p \leq 0.05$) are signified with different and bold letters.

DO minima generally had a positive relation to upland vegetation and a negative relation to agriculture (Figure 7). However, although a high percentage of agriculture in the watershed was generally associated with a low DO minimum, some sites with low percentages of agriculture also had DO minima < 2.5 mg/L (Figure 8). Habitat and land-use surveys indicated several sites had floodplains with deep-water swamps that remained hydraulically connected to the streams even at low stage.

DISCUSSION

Variability of DO minima

A comparison of data collected at the nine sites that were monitored for the 3-year period indicates that the process used to estimate DO minima at the 26 test sites did not result in overly protective (i.e. erroneously low) DO threshold concentrations. For six of the nine sites where DO data were collected at 0800 h for multiple days in 2005 and 2006, the average daily DO minima were lower than the DO minima that were estimated with DO data collected in 2007 on the day of fish sampling (Table III), and at the three remaining sites, the average daily DO minima were only slightly higher than estimated DO minima. Low streamflows resulting from a drought in 2005 (and associated low aeration rates) may explain why DO minima measured or estimated at the nine sites from 2005 to 2007 typically were much lower in 2005 compared with the other 2 years.

Influence of DO minima and other habitat variables on biological assemblages

The strong relation between the DO minima and the first two axes for both CCA ordinations is an indication that both assemblages were constrained by DO concentrations to some degree; however, there is also some indication that the relation between DO minima and invertebrate assemblage was much stronger than the relation between DO minima and fish assemblage (Figure 4). Although taxa from both assemblages may be constrained by DO, for some fish taxa, the importance of DO may be overshadowed by habitat variables related to stream size (i.e. canopy cover and stream width). The sampling design could have influenced this aspect of the analysis because fish were sampled from all habitats within the designated sampling reach while invertebrates were sampled only from woody snags. Large river fish species (e.g. alligator gar, *Atractosteus spatula*; freshwater drum, *Aplodinotus grunniens*; and blue catfish, *Ictalurus furcatus*) were not collected from small streams, but many invertebrates that prefer wood were collected in small and large streams.

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Table III. Averaged or estimated DO minima for sites located on lowland streams in southwestern Louisiana

Abbreviated name (Table I)	Date	Estimated DO minima, 2007 (mg/L)	Average DO minima, 2005–2006 (mg/L)	Average DO minima, 2006 (mg/L)	Combined DO minima, 2006–2007 (mg/L)
Blanc	9/20/2007	4.4		—	4.4
Boeuf	8/23/2007	3.1	3.4 (14)	3.7 (9)	3.7
Bourbo	10/3/2007	0.2	—	—	0.2
Calcas	10/2/2007	6.5	5.5 (18)	5.6 (12)	5.6
Castor	9/18/2007	3.7	1.8 (13)	2.8 (7)	2.8
Chaney	8/20/2007	5.6	—	—	5.6
Ckenny	8/30/2007	3.9	—	—	3.9
Des C	9/19/2007	2.3	—	—	2.3
Des G	8/31/2007	6.5	—	—	6.5
Erath	8/30/2007	0.2	—	—	0.2
Fuse	8/29/2007	5.1	—	—	5.1
GMar	9/21/2007	3.8	—	—	3.8
HickL	9/24/2007	2.9	2.1 (20)	1.7 (14)	1.7
HickU	10/1/2007	2.1	2.2 (19)	3.9 (14)	3.9
Houstn	10/3/2007	3.4	1.3 (18)	2.3 (13)	2.3
LacL	9/23/2007	3.2	—	—	3.2
LacU	9/23/2007	0.7	—	—	0.7
Loving	8/21/2007	7.9	6.6 (17)	5.7 (12)	5.7
Mallet	9/19/2007	2.1	—	—	2.1
MermL	9/12/2007	3.1	—	—	3.1
MermU	9/11/2007	2.4	—	—	2.4
Nez	9/20/2007	2.4	—	—	2.4
PBrulL	9/12/2007	0.7	—	—	0.7
PBrulU	9/13/2007	2.6	—	—	2.6
Pompey	8/24/2007	5.7	—	—	5.7
Portag	8/28/2007	1.6	—	—	1.6
Roar	8/22/2007	6.0	—	—	6.0
Robert	8/22/2007	4.5	—	—	4.5
SabL	10/4/2007	6.0	—	—	6.0
SabU	10/3/2007	7.8	2.5 (19)	2.3 (14)	2.3
Spring	8/21/2007	6.9	7.1 (11)	7.1 (6)	7.1
StMart	9/10/2007	1.5		—	1.5
TortL	9/22/2007	0.6		—	0.6
TortU	9/22/2007	0.6		—	0.6
WarC	9/21/2007	1.9		—	1.9

Estimates for 2007 were obtained by determining the average hourly change in DO as fish were sampled (for a period averaging 5.4 h) and using that rate of change to estimate the DO concentration (usually back in time) to 0800 h. DO minima that were measured continuously for multiple days at nine sites sampled in 2005 and 2006 are averaged for both years and for 2006 (when invertebrates were sampled) separately. Before analysis, average DO minima from the nine sites that were sampled in 2006 were substituted for minima values that were estimated with DO data collected as fish were sampled in 2007 because data averaged from multiple days would be expected to be less variable than data collected on one day. DO minima, DO measured or estimated at 0800 h; 3.4 (14), the average DO minima for 14 days.

Biological relations to DO minima

A problem inherent to threshold analysis is that it is rare in nature that one independent variable will influence biological assemblages to the degree that there are obvious changes in assemblage data that can be attributed to that variable. However, if viewed with an understanding of the direct and indirect influences that DO can have on species (abundance) with different tolerances (Hynes, 1960), the analysis of tolerance information for both assemblages can be used for indicating a general threshold.

Distributions for the three invertebrate and fish taxa of varying tolerance indicated that a DO threshold could be near 2.5 mg/L (Figure 5). Although the highest RA percentages for the three pair of taxa representing the three tolerance classifications were observed at different locations along the gradient of DO minima, RA percentage seemed to fluctuate dramatically at sites when DO minima declined to approximately 2.5 mg/L. More specifically, conditions >2.5 mg/L were much more favourable than conditions <2.5 mg/L for all but the extremely tolerant species.

DO concentrations <2 mg/L seemed to be inhibiting the two taxa selected to represent the moderately tolerant classification and also, but to a slightly lesser degree, the two taxa representing the tolerant classification. There was a fairly dramatic fluctuation in the RA percentage for both

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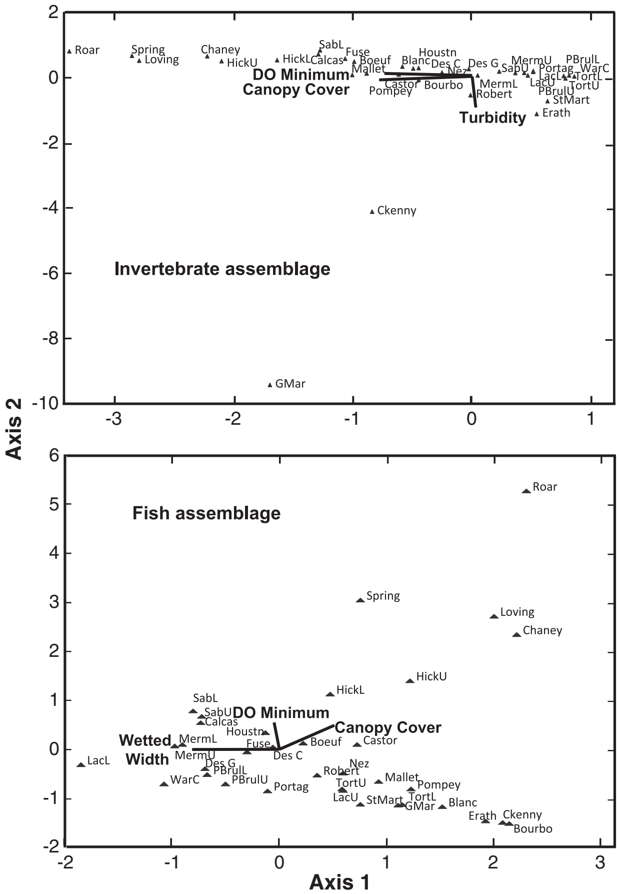

Figure 4. CCA biplots that compare the three habitat variables having strongest relations (of 14 considered) to invertebrate and fish assemblage data at 35 stream sites in southwestern Louisiana (see Table I for abbreviated site names and Table IV for the 11 other variables that were evaluated). The length of the vector reflects the degree that the variable was correlated to axis scores for the assemblage data

moderately tolerant taxa when DO minima were <2 mg/L. The pattern associated with the RA percentage of the two tolerant taxa, side swimmers and smallmouth buffalo, is typical of biological distributions that are subjected to intermediate disturbances (Connell, 1978). In theory, diversity and other measures of biological condition can be highest in response to moderately disturbed conditions (i.e. when DO minima were between 2.0 and 3.0 mg/L in this situation) as tolerant taxa begin to compete with other less tolerant taxa (Ward *et al*., 1983; Petraitis *et al*., 1989).

The pattern typical of the two extremely tolerant taxa, bloodworms and spotted gar, was most different of the three tolerance groups in that highest RA percentages for both taxa occurred at sites when DO minima were <2 mg/L (Figure 5). Extremely tolerant taxa that use atmospheric oxygen [e.g. mosquito larvae, (culicids)], store atmospheric oxygen [e.g. gar, bowfin (*Amia calva*), diving beetles (dytiscids) and back swimmers (notonectids)] or are capable of anaerobic respiration (e.g. tubificid worms and bloodworms) are often found in high abundances in hypoxic waters when other less tolerant invertebrate and fish taxa that compete with or predate on them are absent (Hynes, 1960; Gaufin, 1974; Justus and Harp, 1992). Related to the latter adaptation in particular, dominance by what is usually a small number of extremely tolerant taxa can result in exceedingly high numbers of individuals, especially in the case of invertebrates (Del Rosario *et al*., 2002; Ortiz and Puig, 2007).

Similar to plots for the three pair of taxa selected to represent the tolerance classifications, LOESS lines within scatterplots comparing taxa richness, diversity and total abundance metrics to DO minima also indicate that changes occurred in biological conditions when DO minima declined to approximately 2.5 mg/L (Figure 6). Detailed analysis of the scatterplot data associated to taxa richness, diversity and abundance with piecewise regression indicates that in most cases, statistically significance thresholds were found when DO minima were approximately 2.6 and 2.3 for invertebrates and fish, respectively.

For both assemblages, relations between DO minima and total abundance were weaker than relations between DO minima and taxa richness and diversity. Although, a few fish taxa have behavioural (e.g. positioning near the surface) or physiological (e.g. swim bladders that can function as lungs) adaptations that enable them to withstand low DO conditions, compared with invertebrates, there are relatively few fish taxa with adaptations that enable them to withstand hypoxic conditions. Our data indicate that fish abundance can be low or high when DO concentrations are near the estimated biological threshold.

The three metrics that were evaluated with piecewise regression—taxa richness, diversity and total abundance—were some of the first metrics used to describe biological assemblages (Gaufin and Tarzwell, 1956) and are precursors to many of the metrics that have been used for indices of biological integrity (Karr, 1981; Davis and Simon, 1995). It should be noted, however, that some overlap probably exists in the analyses with particular regard to diversity and taxa richness. Diversity is calculated with taxa richness and total abundance data, and it is certain that the three metrics will sometimes be correlated (e.g. for both assemblages, the Spearman rho correlation between taxa richness and diversity was approximately 0.80, and for diversity and total abundance was < 0.26). That being stated, the ability of the metrics for demonstrating the response of the two assemblages to DO minima was considered to exceed the negative aspect associated with a small part of the analyses being redundant.

DO thresholds would be expected to be below DO criteria commonly established for the protection of aquatic life but well above the minimum DO concentration that is lethal to species native to lowland streams. The average DO

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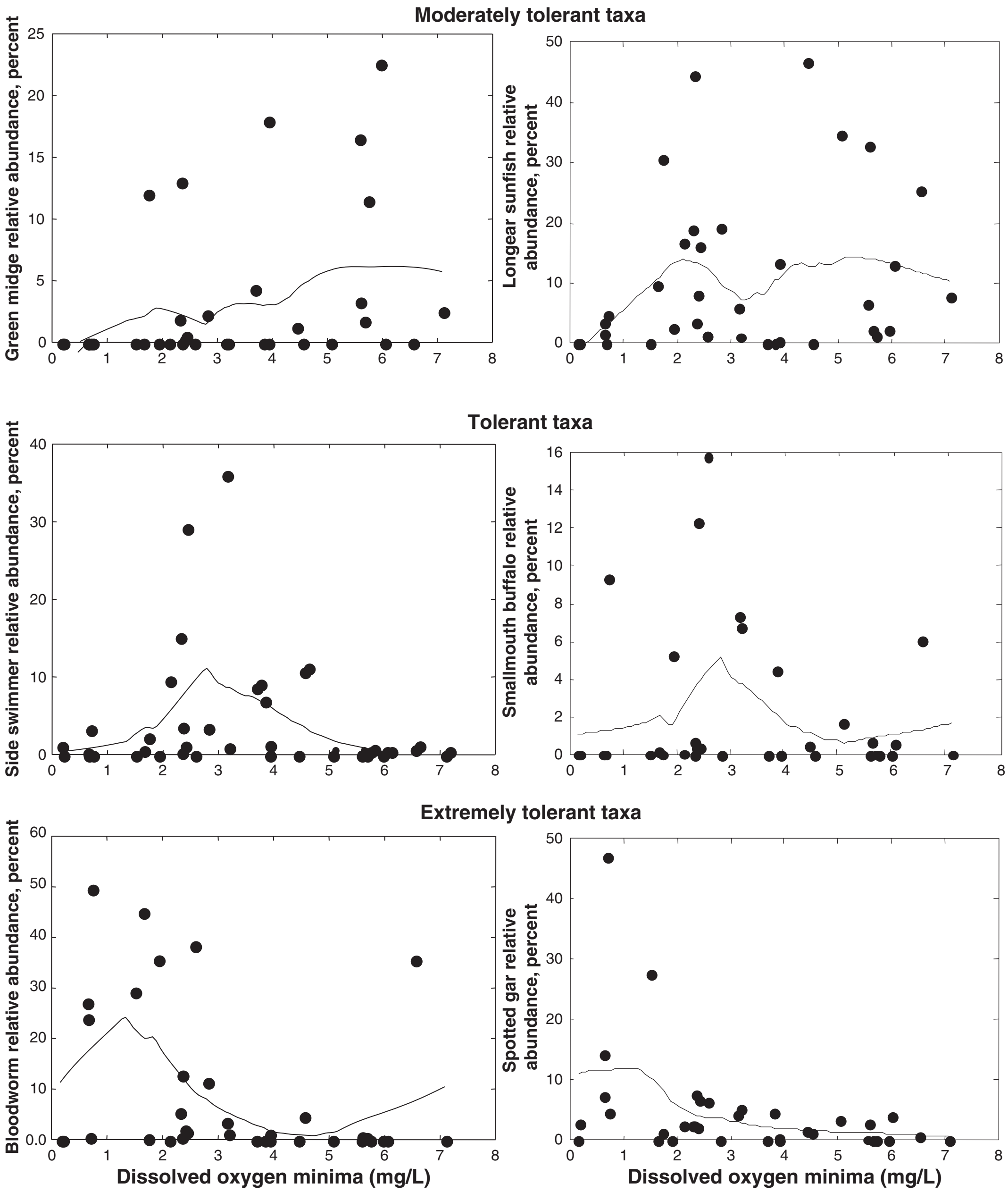

Figure 5. Scatterplots comparing RAs for selected invertebrate and fish taxa that are moderately tolerant, tolerant and extremely tolerant to DO minima at 35 stream sites in southwestern Louisiana. Locally weighted scatterplot smoothing (LOWESS) lines were used to generally indicate when metrics change dramatically in response to DO minima and those break points were considered as potential thresholds

thresholds determined for the invertebrate and fish assemblage (2.6 and 2.3 mg/L) slightly exceed DO criteria that are being applied to some coastal streams in Louisiana and Texas. Louisiana has recently applied a minimum DO criterion of 2 mg/L for some coastal streams (Louisiana Department of Environmental Quality, 2009), and the Texas criterion for the daily minimum DO concentrations for some coastal streams is 2 mg/L (Texas Commission on Environmental Quality, 2007).

There are numerous references indicating that a large number of invertebrate and fish species are capable of tolerating DO concentrations of 1 mg/L (Moore, 1942; Doudoroff and Shumway, 1970; Davis, 1975; Kilgore and Hoover, 2001), which is slightly less than half of the average thresholds for the two assemblages. The time that fish can withstand low DO concentrations may depend on several factors (e.g. fish size, water temperature and behaviour). Doudoroff and Shumway (1970) reported that some species

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Table IV. Examples of taxa that had abundance distributions with similar relations to DO minima as three invertebrate and three fish taxa that were chosen to depict moderately tolerant, tolerant and extremely tolerance classifications

Invertebrate taxa/metric	Moderately tolerant	Tolerant	Extremely tolerant
Polypedilum spp.	X		
Elmidae[a]	X		
Ephemeroptera[a,b]	X		
Trichoptera[a,b]	X		
Endochironomus spp.		X	
Bravislava undentata spp.		X	
Chironomus spp.		X	
Physidae		X	
Lirceus spp.		X	
Micromenetus spp.			X
Glyptotendipes spp.			X
Dero digitata			X
Ancylidae			X
Erpobdellidae			X
Planorbidae			X
Naiidae			X
Hyalella spp.			X
Fish taxa/metric			
Micropterus spp.	X		
Aplodinotus grunniens	X		
Notemigonus crysoleucas	X		
Cyprinus carpio		X	
Opsopoeodus emiliae		X	
Aphredoderus sayanus		X	
Lepomis miniatus		X	
Ictalurus punctatus		X	
Lepomis macrochirus		X	
Dorosoma spp.		X	
Pomoxis spp.		X	
Amia calva			X
Gambusia affinis			X
Lepomis gulosus			X

[a]Scatterplots comparing Elmidae, Ephemeroptera and Trichoptera metrics to DO minima indicate these taxa might be more intolerant than other taxa listed as moderately tolerant.
[b]Ephemeroptera and Trichoptera were calculated with taxa richness data rather than abundance data.

(e.g. bluegill, *Lepomis macrochirus*; orangespotted sunfish, *Lepomis humilus*; warmouth, *Lepomis gulosus*; and plains minnow, *Hybognathus placitus*) could tolerate DO concentrations around 1 mg/L for 18 h or longer when provided access to the surface, but survival was much lower when they could not access the surface.

DO criteria considerations for lowland regions and implications to land use

The impetus for investigating DO thresholds stems not only from the need to establish DO criteria but also because DO can be related to nutrients and other water-quality variables (USEPA, 2000; Robertson *et al.*, 2001). Relatedly, there are efforts in many regions to establish links between DO concentrations and anthropogenic sources of nutrient enrichment (i.e. associated processes related to photosynthesis and decomposition). Much of the guidance for nutrient criteria assumes there is a strong, positive connection between nutrient water quality and the amount of vegetated buffer (USEPA, 2000); however, this association may not always apply to DO in lowland regions. Although DO minima generally had an inverse relation to the amount of agriculture in the buffer area, DO concentrations at three least-disturbed sites with low amounts of agriculture also declined to less than 2.5 mg/L. Ice and Sugden (2003) found that in the summer, almost 60% of the least-impaired or reference streams in forested streams of northern Louisiana had DO concentrations less than 3 mg/L. Thus, indications are that in some lowland settings, the link between DO and degree of aeration and organic decomposition (i.e. flushing, Mallin *et al.*, 2006) will sometimes be stronger than the link between DO and stream–nutrient concentrations. Further, although DO may fall below a concentration known to impair biological assemblages, sources of this impairment will sometimes be related to natural settings.

Future considerations

There are several modifications that could be made to this study design that should increase precision regarding the DO concentrations at which a threshold response occurs for the two assemblages. Precision might be improved if DO was monitored continuously at all sites (thereby eliminating the need for DO extrapolations), if both assemblages were sampled while DO was continuously monitored, if stream size were more uniform and if more sites were sampled that had a DO minima ≤ 2 mg/L. Sample replication across multiple years would facilitate temporal variability, which can be fairly dramatic. Studies seeking to differentiate between agricultural and natural sources of DO minima could potentially benefit from stable isotope analysis that might identify sources of carbon production and oxygen depletion.

CONCLUSIONS

As the need for establishing criteria in lowland streams has increased, criteria originally established for upland streams have often been applied to lowland streams. As monitoring efforts have increased, however, it has become apparent that DO criteria for upland streams are not applicable to lowland streams. Consequently, some lowland bodies of water that are listed as being impaired because of DO concentrations are minimally affected by anthropogenic

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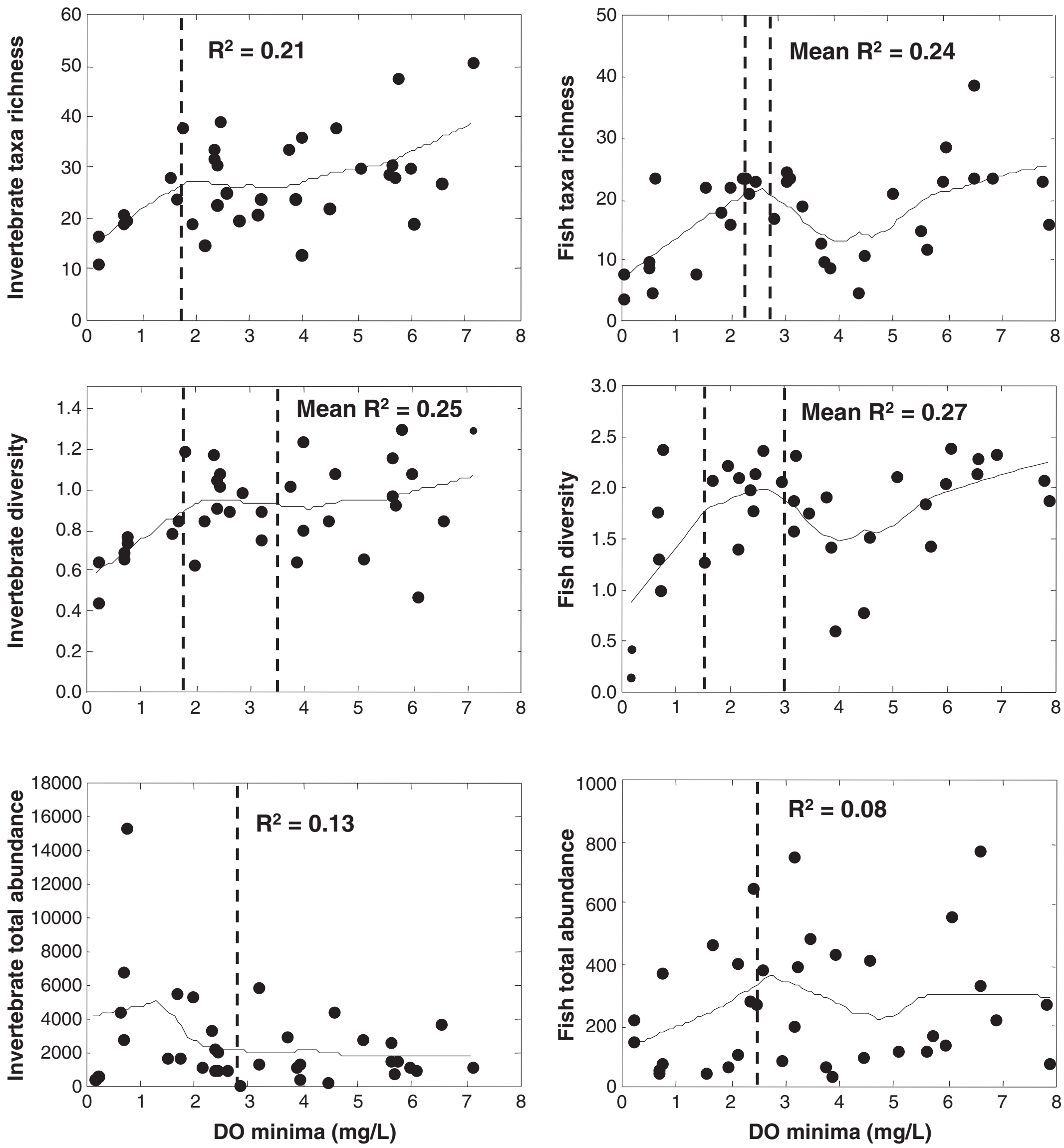

Figure 6. Scatterplots comparing invertebrate and fish assemblage taxa richness, Brillouins diversity and total abundance to DO minima. Horizontal (curved) lines in the scatterplots are LOESS lines, and vertical lines represent or bracket the most statistically significant thresholds that were identified with piecewise regression. Invertebrate taxa richness and total abundance were the only metrics of the six evaluated where identified thresholds did not have a statistically significant (≤0.05) relation to DO minima

activities. Given the tendency of lowland streams for intermittent flow and associated low aeration rates during some part of the year, DO concentrations may naturally influence species composition more than any other variable. Our intent was to evaluate the holistic response (i.e. multiple species of varying tolerance) of both assemblages to DO concentrations and to consider concentrations at which changes seemed to occur for several taxa as possible thresholds. This approach is consistent with the underlying ecological principle that as less tolerant organisms become stressed (by DO, in this case), competition is reduced and tolerant organisms increase.

The relation between DO minima and invertebrate assemblage was much stronger than between DO minima and fish assemblage, probably because most invertebrate species that were collected occurred in small and large streams but some fish species occurred only in large streams. More tolerant and extremely tolerant taxa were collected than moderately tolerant taxa, and more extremely tolerant invertebrate taxa were collected than extremely tolerant fish taxa. All extremely tolerant taxa had respiratory adaptations that gave them a competitive advantage, and their success when DO minima were <2 mg/L is probably related more to a reduction in competition or predation than to DO directly.

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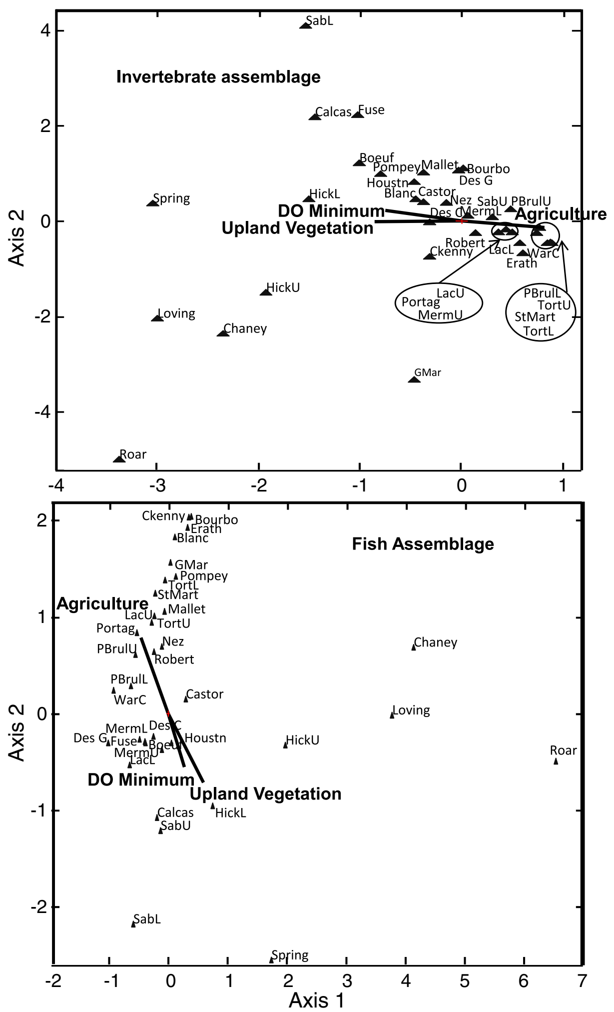

Figure 7. A CCA biplot comparing the strength of relations for DO minima and selected land-use variables to invertebrate and fish assemblage data at 35 stream sampling sites in southwestern Louisiana. The length of the vector reflects the degree that the variable was correlated to axis scores for the assemblage data

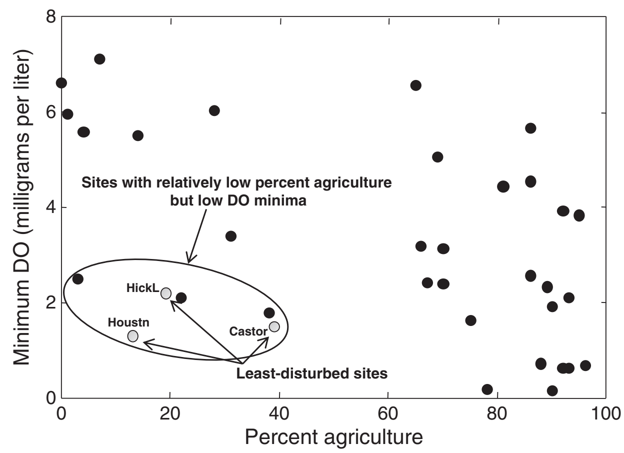

Figure 8. Relations of DO minima and the percentage of agriculture in the basin at 35 stream sampling sites in southwestern Louisiana

Statistically significance thresholds were found when DO minima were approximately 2.5 mg/L; the average threshold values (for taxa richness, diversity and total abundance data) for invertebrates and fish were 2.6 and 2.3 mg/L, respectively. These thresholds are comparable with some DO criteria that are now being applied to some coastal streams in Louisiana and Texas and are twice or more the concentration that some native fish species are capable of tolerating.

Although DO minima generally had an inverse relation to the amount of agriculture in the buffer area, DO minima at sites in the study area with both low and high amounts of agriculture (including the three least-disturbed sites) were ≤2 mg/L. Thus, indications are that in some lowland settings, the link between the DO and the degree of aeration and organic decomposition will sometimes be stronger than the link between DO and stream–nutrient concentrations. Further, although DO may fall below a concentration known to impair biological assemblages, sources of this impairment will sometimes be natural and have little relation to anthropogenic activity.

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REFERENCES

Arkansas Department of Environmental Quality. 2007. *Regulation 2. Regulation establishing water quality standards for surface waters of the State of Arkansas*. Arkansas Department of Environmental Quality: Little Rock, Ark. Available at: http://www.adeq.state.ar.us/regs/files/reg02_final_071125.pdf (accessed 04/19/2010).

Barbour MT, Gerritsen J, Snyder BD, Stribling JB. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Algal, Benthic Macroinvertebrates, and Fish* (2nd edn), EPA 841-B-99-002. US Environmental Protection Agency, Office of Water: Washington, DC; variously paginated.

Bentley JW, Howell M, Johnson TG. 2005. *Louisiana's Timber Industry—An Assessment of Timber Product Output and Use, 2002*. Resource Bulletin. SRS–103. US Department of Agriculture Forest Service, Southern Research Station: Asheville, NC; 44.

ter Braak CJF. 1986. Canonical correspondence analysis—a new eigenvector technique for multivariate direct gradient analysis. *Ecology* **67**: 1167–1179.

DOI: 10.1002/rra

Braden S, Webber S. 1992. *Selecting reference sites within an ecoregion: guidelines*. Louisiana Department of Environmental Quality, Water Quality Management Division: Baton Rouge, LA; variously paginated.

Brenden TO, Wang L, Su Z. 2008. Quantitative identification of disturbance thresholds in support of aquatic resource management. *Environmental Management* **42**: 821–832.

Clarke KR, Warwick RM. 2001. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation* (2nd edn). *PRIMER-E*: Plymouth Marine Laboratory: United Kingdom; variously paginated.

Connell JH. 1978. Diversity in tropical rain forests and coral reefs. *Science* **199**: 1302–1310.

Daigle JJ, Griffith GE, Omernik JM, Faulkner PL, McCulloh RP, Handley LR, Smith LM, Chapman SS. 2006. *Ecoregions of Louisiana (color poster with map, descriptive text, summary tables, and photographs)*. US Geological Survey: Reston, VA. (map scale 1:1,000,000).

Davis JC. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: A review. *Journal of the Fisheries Research Board of Canada* **32**: 2295–2332.

Davis WS, Simon TP. 1995. *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making*. CRC Press, Inc.: Boca Raton, FL; 415.

Del Rosario RB, Betts EA, Resh VH. 2002. Cow manure in headwater streams: tracing aquatic insect responses to organic enrichment. *Journal of the North American Benthological Society* **21**: 278–289.

Dewalt RE. 1997. *Fish and macroinvertebrate taxonomic richness, habitat quality, and in situ water chemistry of ecoregion reference streams in the Western Gulf Coastal Plains and Terrace Uplands Ecoregions of southern Louisiana*. Prepared by Illinois Natural History Survey for the Louisiana Department of Environmental Quality: Baton Rouge, LA; 72.

Doudoroff P, Shumway DL. 1970. Dissolved oxygen requirements of freshwater fishes. Fisheries Technical Paper # 86, Food and Agriculture Organization of the United Nations; 291.

Douglas NH. 1974. *Freshwater Fishes of Louisiana*. Claitor's Publishing Division: Baton Rouge, LA; 443.

US Environmental Protection Agency. 2000. Nutrient Criteria Technical Guidance Manual, Rivers and Streams EPA-822-B-00-002; 150. Available at: http://www.epa.gov/waterscience/criteria/nutrient/guidance/rivers/rivers-streams-full.pdf (accessed 04/19/2010).

Eriksen CH, Resh VH, Lamberti BA. 1996. Aquatic Insect Respiration. In *An Introduction to the Aquatic Insects of North America*, Merritt RW, Cummins KW (eds). Kendall/Hunt Publishing Co.: Dubuque, Iowa; 29–40.

Fitzpatrick FA, Waite IR, D'Arconte PJ, Meador MR, Maupin MA, Gurtz ME. 1998. Revised methods for characterizing stream habitat in the National Water-Quality Assessment Program. US Geological Survey Water-Resources Investigations Report 98-4052; 67.

Gaufin AR. 1974. *Use of aquatic invertebrates in the assessment of water quality [Biological methods for the assessment of water quality]. Amer. Soc. Test. Material Publ. Issue STP 528*: Philadelphia, PA; 96–116.

Gaufin AR, Tarzwell CM. 1956. Aquatic macroinvertebrate communities as indicators of organic pollution in Lytle Creek. *Water Environment and Technology* **28**: 906–924.

Gee JH, Tallman RF, Stone HJ. 1978. Reactions of some Great Plains fishes to progressive hypoxia. *Canadian Journal of Zoology* **56**: 1962–1966. Ecol. Indicators 8: 599-613.

Herlihy AT, Larsen DP, Paulsen SG, Urquhart NS, Rosenbaum BJ. 2000. Designing a spatially balanced, randomized site selection process for regional stream surveys: the EMAP mid-Atlantic pilot study. *Environmental Monitoring and Assessment* **63**: 95–113.

Hilsenhoff WL. 1987. An improved biotic index of organic stream pollution. *Great Lakes Entomologist* **20**: 31–39.

Hughes RM, Larsen DP, Omernik JM. 1986. Regional reference sites: a method for assessing stream potentials. *Environmental Management* **10**: 629–635.

Hynes HBN. 1960. *The Biology of Polluted Waters*. Liverpool University Press: Liverpool; 202.

Ice G, Sugden B. 2003. Summer dissolved oxygen concentrations in forested streams of northern Louisiana. *Southern Journal of Applied Forestry* **27**: 92–99.

Justus BG, Harp GL. 1992. Boat Clarifier Effectiveness, *Water Environment and Technology* **4**(3): 5.

Karr JR. 1981. Assessment of biotic integrity using fish communities. *Fisheries* **6**: 21–27.

Kilgore KJ, Hoover JJ. 2001. Effects of hypoxia on fish assemblages in a vegetated waterbody. *Journal of Aquatic Plant Management* **39**: 40–44.

Koehle JJ, Adelman IR. 2007. The effects of temperature, dissolved oxygen, and Asian tapeworm infection on growth and survival of the Topeka shiner. Trans.of the Amer. Fish. *Soc* **136**: 1607–1613.

Lenat DR. 1993. A biotic index for the southeastern United States: Derivation and list of tolerance values, with criteria for assigning water quality ratings. *Journal of the North American Benthological Society* **12**: 279–290.

Louisiana Department of Environmental Quality. 2005. Title 33 Environmental Regulatory Code. Available at: http://doa.louisiana.gov/osr/lac/33v09/33v09.pdf (accessed 10/23/2009).

Louisiana Department of Environmental Quality. 2009. Title 33 Environmental Regulatory Code. Available at: http://www.deq.louisiana.gov/portal/Default.aspx?tabid=1674 (accessed 09/11/2009).

Louisiana Department of Environmental Quality. 2010. Louisiana Water Quality Assessment Report. Available at: http://iaspub.epa.gov/waters10/attains_index.control?p_area=LA (accessed 10/18/2011).

Mallin MA, Johnson VL, Ensign SH, MacPherson TA. 2006. Factors contributing to hypoxia in rivers, lakes, and streams. *Limnology and Oceanography* **51**: 690–701.

Matthews WJ. 1987. Physicochemical tolerance and selectivity of stream fishes as related to their geographic ranges and local distributions. In *Community and Evolutionary Ecology of North American Stream Fishes*, Matthews WJ, Heins DC (eds.). Univ. of Oklahoma Press: Norman, Oklahoma; 111–120.

McCune B, Mefford MJ. 2006. *PC-ORD*. Multivariate Analysis of Ecological Data. Version 5.10, MjM Software, Gleneden Beach, Oregon, USA; variously paginated.

Mississippi Department of Environmental Quality. 2007. State of Mississippi Water Quality Criteria for Intrastate, Interstate, and Coastal Waters; 36. Available at: http://www.deq.state.ms.us/MDEQ.nsf/pdf/WMB_adopted_wqsstandoc_aug07/$File/WQS_std_adpt_aug07.pdf?OpenElement (accessed 04/19/2010).

Moore WG. 1942. Field studies on the oxygen requirements of certain fresh-water fishes. *Ecology* **23**: 319–329.

Moulton SR, II, Kennen JG, Goldstein RM, Hambrook JA. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program. United States Geological Survey Open-File Report 02-150; 75.

National Agriculture Statistical Service. 2009. 2007 Census of Agriculture. Available at: http://www.nass.usda.gov (accessed 06/01/2010).

Omernik JM. 1987. Ecoregions of the conterminous United States, map (scale1:7,500,000). *Annals of the Association of American Geographers* **77**: 118–125.

Ortiz JD, Puig MA. 2007. Point source effects on density, biomass, and diversity of benthic macroinvertebrates in a Mediterranean stream. *River Research and Applications* **23**: 155–170.

Ostrand LG, Wilde GR. 2001. Temperature, dissolved oxygen, and salinity tolerances of five prairie stream fishes and their role in explaining fish assemblage patterns. *Transactions of the American Fisheries Society* **130**: 742–749.

Petraitis PS, Latham RE, Niesenbaum RA. 1989. The maintenance of species diversity by disturbance, *The Quarterly Review of Biology* **64**: 393–418.

Robertson DM, Saad DA, Wieben AM. 2001. An alternative regionalization scheme for defining nutrient criteria for rivers and streams. US Geological Survey Water-Resources Investigations Report 01-4073; 57.

Robison HW, Buchanan TM. 1988. *Fishes of Arkansas* (5th edn). The University of Arkansas Press: Fayetteville, Arkansas; 536.

Ross ST. 2001. *The Inland Fishes of Mississippi*. University Press of Mississippi: Hattiesburg, Mississippi; 624.

Schofield PJ. 2007. Hypoxia tolerance of two centrarchid sunfishes and an introduced cichlid from karstic Everglades wetlands of southern Florida, USA. *Journal of Fish Biology* **71**: 87–99.

Smale MA, Rabeni CF. 1995. Hypoxic and hyperthermia tolerances of headwater stream fishes. *Transactions of the American Fisheries Society* **124**: 698–710.

Systat Software. 2008. SigmaPlot 11, User's Guide, Parts 1 and 2. San Jose, California; variously paginated.

Terrel JW, Cade BS, Carpenter J, Thompson JM. 1996. Modeling stream fish habitat limitations from wedge-shaped patterns of variation in standing stock. *Transactions of the American Fisheries Society* **125**: 104–117.

Texas Commission on Environmental Quality. 2007. Seventeen Total Maximum Daily Loads for Bacteria, Dissolved Oxygen, and pH in Adams Bayou, Cow Bayou, and Their Tributaries. Available at: http://www.tceq.state.tx.us/assets/public/implementation/water/tmdl/37orange-county/37-orangetmdl-adopted.pdf (accessed 04/19/10).

Todd MJ, Vellidis G, Lowrance RR, Pringle CM. 2009. High sediment oxygen demand within an instream swamp in southern Georgia: Implications for low dissolved oxygen levels in coastal blackwater streams. *Journal of the American Water Resources Association* **45**(6): 1493–1507.

Toms JD, Lesperance ML. 2003. Piecewise regression: a tool for identifying ecological thresholds. *Ecology* **84**: 2034–2041.

Tramer EJ. 1977. Catastrophic mortality of stream fishes trapped in shrinking pools. *American Midland Naturalist* **97**: 469–478.

US Geological Survey. 2005a. Land Cover data for Louisiana, National Biological Information Infrastructure. Available at: http://gapanalysis.nbii.gov/xml/th_landcover_la_16.html (accessed 03/25/10).

US Geological Survey. 2005b. Land Cover data for Texas, National Biological Information Infrastructure. Available at: http://gapanalysis.nbii.gov/xml/th_landcover_tx_24.html (accessed 03/25/10).

Val AL, Silva MNP, Almeida-Val VMF. 1998. Hypoxia adaptation in fish of the Amazon: A never-ending task. *African Zool* **33**: 107–114.

Wagner RJ, Mattraw HC, Ritz GF, Ritz GF, Smith BA. 2000. Guidelines and standard procedures for continuous water-quality monitors: site selection, field operation, calibration, record computation, and reporting. US Geological Survey Water-Resources Investigations Report 00-4252; 53.

Ward JS, Stanford JA, Fontaine TD, Bath SM. 1983. The intermediate-disturbance hypothesis: An explanation for biotic diversity pattern in lotic ecosystems. In *Dynamics of Lotic Ecosystems*. Ann Arbor Science Publishers: Ann Arbor, Michigan; 347–356.

Weaver K. 2004. *Everglades marsh dissolved oxygen site specific alternative criterion technical support document*. Florida Department of Environmental Protection: Tallahassee, FL; 61.

Wetzel RG. 2001. *Limnology* (3rd edn). Academic Press: San Diego, CA.

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