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DEVELOPMENT, CALIBRATION AND VALIDATION OF AN INDEX OF BIOTIC INTEGRITY FOR THE WABASH RIVER

Thomas P. Simon: U.S. Fish and Wildlife Service, 620 South Walker Street, Bloomington, Indiana 47403 USA

ABSTRACT. Fish assemblage data were collected using daytime electrofishing during 1993–2001 from 275 river reaches found throughout the Interior River Lowland and Eastern Corn Belt Plain ecoregions to construct, test, and apply an index of biotic integrity (IBI). The index was developed from a rapid assessment procedure that was used to assess the environmental quality of large and great river ecosystems in the state. The reference condition was based on 275 sites that were representative of the Wabash River, but were not pristine or least-impacted. These sites were not randomly chosen, but met specific least-impacted criteria to develop the IBI. We used another 36 sites exposed to point-source discharges to test the index. Prior to sampling, sites were classified as "least-impacted" or as affected by point source pollution from industrial discharges. Of the 24 potential IBI metrics considered, 12 metrics were chosen based on statistical relevance for large and great rivers. For the test subset, the least-impacted sites had significantly higher mean scores and lower temporal variation than the point-source site classification, showing they possessed the best ecosystem quality. Point-source sites had the lowest means and most variable scores, signifying degraded ecosystem quality. Least-impacted sites had the highest IBI scores and the lowest variability, while representative sites typical of agricultural land uses had slightly but not significantly worse scores. Regional estimates of stream conditions showed that 42% of the stream reaches in the Interior River Lowland ecoregion had fish assemblages in poor or fair ecological condition, while large-river reaches in the Eastern Corn Belt Plain ecoregion had 36% fair and 23% good.

Keywords: Biological integrity, reference condition, IBI, Interior River Lowland, Eastern Corn Belt Plain

The index of biological integrity (IBI) is a multimetric index that integrates structure, composition, trophic ecology, and reproductive attributes of fish assemblages at multiple levels of ecological organization (Karr 1981; Karr et al. 1986; Simon & Lyons 1995; Simon 1999). Indices of biological integrity can be viewed as a family of indices for rating the health of an aquatic ecosystem (Simon 2001). These indices provide a valuable framework for assessing the status and evaluating the restoration of aquatic communities (Fausch et al. 1990; Karr & Chu 1999; Simon et al. 2003). Standard procedures are used to compare existing biological conditions in order to assess the current status of the biota.

Indices of biotic integrity have been widely based on fish assemblages in "wadeable" streams, but applications to large and great warm water rivers are few (Simon & Lyons 1995; Hughes & Oberdorff 1999; Emery et al. 2003). Simon & Stahl (1998) calibrated an IBI for the Wabash River. This calibration was a preliminary index that was based on a limited number of sites and only a portion of the river from Lafayette (Tippecanoe County) to Wabash Island (Posey County). Gammon (2000) calibrated an index for the middle Wabash River, but this calibration was not based on an entire fish assemblage assessment; rather it focused on large, long-lived fish species. The State of Illinois does not have a large-river calibration for their water monitoring program.

In this paper, an IBI is presented that is designed to assess the quality of fish assemblages in the Wabash River. The index was developed using a large statewide database of standardized fish assemblage samples from numerous reaches of varying human impact. An objective procedure was followed to select and score the metrics that comprise the IBI, choosing metrics that represent a variety of the structural, compositional, and functional attributes of large and great rivers (Karr & Chu 1999). The index was then validated with independent data from 36 other river reaches that had anthropogenic disturbances, using as validity criteria the accurate and precise ranking of these other reaches in accordance with their degree of environmental degradation based on water quality, habitat, and use measures. Finally, this IBI was applied to the entire dataset to assess the relative effects of human impacts on river health.

METHODS

Survey design.—Between 1993 and 2001, teams of U.S. Environmental Protection Agency (USEPA), U.S. Fish and Wildlife Service, Indiana Department of Environmental Management, and Indiana Department of Natural Resources professionals sampled 275 large and great river (as defined by Simon & Emery 2000) sites as part of routine monitoring on the Wabash River. The Wabash River includes sites in wadeable stream (<2590 km^2), large- and great river categories. Data used for this project were part of the USEPA's ecoregion project in Indiana (Simon & Stahl 1998), probabilistic assessment for water quality impairment, and monitoring of sport fishes in the Wabash River (Fig. 1). Sampling protocols followed boat electrofishing methods developed by USEPA (1988). In response to criticisms of the Simon & Stahl (1998) paper, large-river criteria development in the Wabash River (EA Engineering, Science, and Technology, Inc. 1999) were reassessed by external peer review, and comments were responded to by Simon & Stahl (2001). The arguments presented in EA Engineering, Science, and Technology, Inc. (1999) were not found to be credible by the external review panel. Protocols, data, and analysis of results were found to be consistent and reproduceble. The conclusion of the external peer review panel was fully supported by both the State of Indiana and the U.S. Environmental Protection Agency.

The Wabash River traverses two ecoregions in Indiana, including the Interior River Lowland and the Eastern Corn Belt Plain (Omernik & Gallant 1988). The Interior River Lowland (IRL) extends from central Indiana along the Wabash River floodplain to the Ohio River and includes the Mississippi River floodplain. The IRL has varied land use including forestry, diverse cropland agriculture, orchards, livestock production, and oil and gas production. The IRL consists of dissected glacial till plains, which are covered by thick mantle loess, rolling narrow ridgetops, and hilly to steep ridge and valley slopes. Woods et al. (1995) subdivided the ecoregion into two subregions that include the area along the Wabash River floodplain to the White River mouth. The Eastern Corn Belt Plain (ECBP) extends from Lafayette to the river's headwaters in Ohio. The ECBP consists of gently rolling glacial till plain, which is broken by moraines, kames, and outwash plains.

Large rivers are defined as drainage units with watersheds greater than 2590 km^2 (1000 mi^2) but less than 5957 km^2 (2300 mi^2) (Simon & Emery 2001), which are effectively sampled using a boat-mounted electrofishing unit. Great rivers include drainage areas greater than 5957 km^2. Following the definition of Lyons et al. (1996) and Mundahl & Simon (1999), the thermal classification for all portions of the Wabash River is warmwater, which means that summer temperatures are too warm to allow the survival of salmonid fishes. Site selection was chosen to maximize different locations along the Wabash River so that various river reaches incorporating different sizes along the regional gradient were sampled. These sites are representative of the condition of the Wabash River; however, sites were picked to deliberately encompass the full range of natural habitat and flow conditions that exist among the Wabash River. The inclusion of the entire suite of sites enables the entire range of conditions to be used to develop both negative and positive metrics. Also, inclusion of sites were selected so that all geographic portions of the drainage were included. By including drainage areas ranging from 1139.6 to 85,231.7 km^2, we provide data from sites that are smaller than typical large-river sites. Site information does not suggest that this is a violation of the River Continuum Concept, since these sites do not reflect an accretion of data sufficient to warrant a drainage area metric calibration. By testing ecoregion and drainage area hypotheses, this enables the creation of a single IBI that does not warrant unnecessary separation of expectations based on ecoregion or size. Although the literature shows that small headwater (<54 km^2) and wadeable streams (>54–2590 km^2) demonstrate a strong species area relationship with drainage area, the size of the main stem Wabash River data used in this study is clearly larger than these size categories; thus it is not surprising that a drainage area calibration correction was not warranted.

A five step process in IBI development was followed, including validation, and application that was modified by Lyons et al. (2001) after the recommendations in Hughes et al. (1998) and Karr & Chu (1999). First, an appropriate sampling methodology was identified and tested. Second, this methodology was used to collect fish assemblage data in a standardized manner from river reaches across the two ecoregions. Some reaches had minimal human impact (least-impacted), while others had varying amounts of different types of human impact from point and non-point source pollution (impacted). Third, we used our fish assemblage data to evaluate potential metrics and develop an IBI. We used data from our least-impacted sites to characterize relatively high-quality fish communities and to investigate the influence of natural factors on community attributes. We contrasted data from least-impacted sites with data from degraded impacted sites to quantify the metric range and sensitivity to human impacts. We then selected final metrics, developed metric scoring criteria, and completed the IBI. Fourth, this IBI was tested with a new set of independent field data that had not been used in the development phase. Finally, IBI scores were compared and ratings among river reaches that had been grouped by type of human impact in order to assess the relative effect of each impact on biotic integrity.

Study area.—Sampling on the Wabash River included 275 large and great river sites collected between 1993 and 2001 for the development of the reference condition, and an independent set of 36 point-source sites collected during 2002 and 2005 was used to validate the index (Fig. 1). The Wabash River extends from the headwaters in Ohio to the mouth of the river at Wabash Island. The Wabash River is the longest free-flowing river east of the Mississippi River and is the largest northern tributary of the Ohio River. The river begins in northwestern Ohio in the Eastern Corn Belt Plain and flows west to southwest; the river bends and flows south through the Interior River Lowland. The Wabash River at the Indiana state line is between 678.6 km^2 (262 mi^2) to 85,236.9 km^2 (32,910 mi^2) at the junction with the Ohio River. Land uses in these areas are principally dominated by agriculture, with some urban, and forested areas.

Figure 1.—Distribution of sites sampled as part of the development, validation, and application of an Index of Biotic Integrity for the Wabash River in Indiana.

Data collection.—Daytime fish assemblage sampling was done along a 500 m river reach at each site, based on time criteria using boat-mounted, pulsed-DC electrofishing equipment. Preliminary sampling to establish standard operating procedures were conducted between 1988 and 1990 (Davis & Simon 1989; Simon 1991; Simon & Saunders 1999). Data from this preliminary sampling were not used in IBI development, validation, or application. Large-river (>2509.3 km^2 and <5957 km^2 drainage area) and great river (>5957 km;^{2} drainage area) sites were sampled using a Smith-Root DC mounted electrofishing unit in a jon boat (Simon & Sanders 1999). The boat electrofishing method of U.S. Environmental Protection Agency was used by all agencies, with the only exception being that the state Department of Natural Resources added two seine hauls at each sampling site to better quantify small non-game minnow and darter diversity. A validation of this approach was conducted by repeat sampling of five sites that were sampled using this procedure by DNR. We used an ANOVA to compare differences between metric results and total IBI score for each site. No significant difference was observed between DNR electrofishing + seine samples compared to electrofishing only results. The addition of seining to the standard method by DNR personnel was to ensure that total catch included small nongame species in order to compensate for inherent personnel bias towards large game species. Easily recognized species, including sport fish were identified and released. Voucher specimens of smaller individuals of each species and unidentified specimens were retained for museum verification. Collections were archived at the Indiana Biological Survey, Division of Fishes, Aquatic Research Center, Bloomington, Indiana.

A 500 m reach length is the point distance that has been shown to be representative of a large-river habitat cycle (Simon & Sanders 1999). The adequacy of our stream length criteria was tested by sampling three continuous 500 m segments, for a total of 1500 m. This distance ranged from 2–40× the wetted stream width. The cumulative number of species captured from each consecutive segment was evaluated and analyzed with non-linear regression equations to estimate asymptotic species richness and the sampling distance that would attain 95% of this richness. The 95%-richness distance is a very conservative sampling length. The minimum sampling distance selected was 500 m because no significant difference was observed with species richness or percent metrics with the addition of distance. Since these river reaches do not typically possess riffle-run-pool habitat, reach structure increases species diversity by the presence of woody debris and scour pools.

For sampling, time duration ranged from 60–90 min, depending on stream complexity. The objective was to collect a representative sample of the fish assemblage using methods designed to collect all except very rare species and provide an unbiased measure of the proportional abundances of species.

During sampling, a single person positioned on the bow, used a dip net with 6 mm mesh (stretch) and attempted to capture all fish seen. This mesh size was effective in retaining small species and individuals such as minnows, darters, and topminnows. Captured fish were identified to species, counted, weighed in aggregate by species, and inspected for deformities, eroded fins, lesions, and tumor (DELT) anomalies (Sanders et al. 1999). Consistent with other IBI's, specimens less than 25 mm TL were considered young-of-year (Fausch et al. 1984; Karr et al. 1986), with the exception of some species that only attain these sizes, i.e., mosquitofish (*Gambusia affinis*). These young-of-year individuals were excluded from the analysis.

Data analyses.—Regional literature references were used to classify adult fish into taxonomic and ecological categories for computation of metrics (Appendix; Gerking 1945; Simon 1999b; Goldstein & Simon 1999). An analysis of variance (ANOVA) was used to test for sub-ecoregional differences in richness metrics, adjusted for catchment area. Finding no such differences, data from all ecoregions were aggregated. All of our impacted sites ($n = 36$) were classified into one of four categories according to the predominant type of human impact. Classification was done prior to sampling and was based on physical-chemical attributes related to hydrology and water and habitat quality. "Agricultural" sites were located in watershed with at least 50% of their surface area in intensive agriculture or less than 20% in urban land uses. "Point source" sites had been affected by major point source discharges of industrial or municipal waste (IDEM 2002). Since the 1990s, most major discharges into Indiana streams have been eliminated or have been heavily treated to reduce water quality impacts, and violations of water quality standards are much less common (IDEM 2002). Thus, the point source category largely represents a historical impact.

The least-impacted sites had relatively few impacts and represented the best remaining river segments in the ecoregions. These sites are not pristine, but generally had intact riparian corridors, minimal non-point source pollution, and limited point source pollution. We considered some agriculture impacts to represent background conditions at almost every site in Indiana.

Two datasets were used in developing the IBI. One set ($n = 275$) included representative, best-remaining, least-impacted sites and was used in the development group to identify appropriate metrics, devise metric scoring criteria, and construct the final IBI. Test data included 36 independent sites that were downstream of point source discharges that were used to validate the IBI and determine how well it reflected known patterns of human impacts.

Twenty-four candidate metrics were considered for inclusion in the Wabash River IBI (Table 1). These contained all of the relevant metrics used in previous warmwater stream IBIs, plus several additional metrics (Simon & Lyons 1995; Hughes & Oberdorff 1999). Prior to the analyses the metrics were transformed to better approximate normality (a $\log_e$ transformation for the number of individuals or biomass and an arcsine-square-root transformation for proportional metrics). Results of analyses were considered significant if $\alpha < 0.05$.

First, the variation in metric values was examined in relation to two natural factors, drainage area and geographic location, that might influence fish assemblages. Appropriate metrics would have either little variation relative to these two factors or a strong, monotonic, biologically meaningful relation that could be easily taken into account in IBI calculations (Hughes et al. 1998; Lyons et al. 2001). This analysis was limited to the 275 least-impacted samples from the development group to minimize the potential confounding effects of human impacts. Drainage area upstream of the sampling site ($\log_e$ transformed) was used as a measure of stream size. Data from large and great river reaches and preliminary analyses of a subset of our large-river reaches based on ecoregions (Eastern Corn Belt Plain ("north"); n = 83 and Interior River Lowland ("south"); n = 192) and sub-ecoregions in the Interior River Lowland (Woods et al. 1995) classified as "north" (Glaciated Wabash Lowlands sub-ecoregion; n = 110) and "south" (Wabash Bottomland sub-ecoregion; n = 82) did not show any substantial structural or compositional differences, so it was not necessary to derive separate reference condition expectations for either the two ecoregions nor the two sub-ecoregions in the final analyses. Regression analysis was used to evaluate patterns between each metric and drainage area, while an Analysis of Variance (ANOVA) for each metric was used to compare the "north" and "south" potential differences for ecoregion or sub-ecoregions. No statistically significant relationship was observed for drainage area, ecoregion, or sub-ecoregion.

Next, metric performance relative to a gradient of human impact was evaluated using the development samples. When examining the most- and least-degraded stream reaches, the assumption was that multiple-impact sites would have the most modified fish assemblages and least-impacted would have the least, with the intermediate impact classes somewhere in between. Metrics that fit this pattern, that is, that showed least-impacted sites as having the best values (highest or lowest depending on the specific metric) and multiple-impact sites having the worst values, were considered appropriate for our IBI. For each potential metric, an analysis of variance (ANOVA) was used with a Duncan multiple-range, multiple-comparison test (DMC) to assess differences among impact classes. If the metric value at the least-impacted sites were related to stream size, drainage area ($\log_e$ transformed) was included as a covariate in this analysis.

The final metrics chosen for inclusion in the IBI were based on their variation relative to natural factors, their relation to human impact, and whether they represented a unique aspect of the structure, composition, or functional organization of the fish assemblage (Hughes et al. 1998). Each final metric had an appropriate response pattern to both natural factors and human impacts. For those metrics that involved the same species and that were strongly correlated with each other (Pearson's $r >$ 0.6), a single representative metric was chosen for use in the index. The final metrics selected included at least one metric for each of the five attributes of fish assemblages that an IBI should include: species richness and composition, indicator species, trophic function, reproductive function, and individual abundance and condition (Simon & Lyons 1995).

Scoring criteria followed the classic 1, 3, and 5 scoring criteria established by Karr (1981) and Karr et al. (1986), which is consistent with previous adaptations of the IBI for other Indiana ecoregions (Simon 1991, 1994; Simon & Dufour 1998a, b) and large rivers (Simon 1992; Simon & Stahl 1998; Emery et al. 2003). A minimum possible score (0 points) was assigned when the metric value was below the level achieved by the development data set. For example, when a site did not possess a particular indicator species or guild, then the specific metric was assigned 0 points. The overall IBI

Table 1.—Candidate metrics considered for inclusion in a calibration of the index of biotic integrity (IBI) for the Wabash River. Species designations are provided in the appendix. The abbreviation wt stands for weight (biomass); n is the total number of fish captured. Metrics in bold are included in the final IBI.

Metric	Definition
CPUE	Catch of individuals per standard sampling distance (500-m).
CPUE2	Catch of individuals per standard sampling sistance, excluding individuals of tolerant species.
Total species	Total number of species collected.
Native species	Total species excluding exotic and non-indigenous species.
Number sucker species	Total number of species in the sucker family (Catostomidae).
Sunfish species	Number of species in the sunfish family (Centrarchidae), excluding black basses (genus *Micropterus*).
Centarchid species	Number of species in the sunfish family (Centrarchidae) including black basses (genus *Micropterus*).
Minnow species	Number of species in the minnow family (Cyprinidae).
Darter species	Number of species in the perch family (Percidae) in the genera *Ammocrypta, Etheostoma, Crystallaria*, and *Percina*.
Sensitive species	Number of species sensitive to anthropogenic disturbance of physical and chemical integrity.
% DELT (n)	Percentage of total fish captured that upon gross inspection possessed deformities, eroded fins, lesions, or tumors.
% Top carnivores (n)	Percentage of total fish captured that were top carnivores.
% Insectivores (n)	Percentage of total fish captured that were insectivores.
% Detritivores (n)	Percentage of total fish captured that were detritivores.
% Omnivores (n)	Percentage of total fish captured that were omnivores; i.e., consumed at least 25% animal and 25% plant material.
% Great River (n)	Percentage of total fish captured that were obligate great-river species.
% Large-river species (n)	Percentage of total fish captured that were obligate large-river species.
% Lithophil (n)	Percentage of total fish captured that were simple lithophilic spawners (i.e., first spawned on clean rocky surface without preparing a nest or guarding their eggs).
% Round-bodied suckers (n)	Percentage of total fish captured in the genera *Cycleptus* (blue sucker), *Hypentelium* (hog sucker), *Minytrema* (spotted sucker), *Erimyzon* (chubsuckers), and *Moxostoma* (redhorses).
% Tolerant (n)	Percentage of total fish captured that were considered tolerant of environmental degradation.
% Top carnivore (wt)	Percentage of total biomass accounted for by top carnivores.
% Insectivores (wt)	Percentage of total biomass captured that were insectivores.
% Detritivores (wt)	Percentage of total biomass captured that were detritivores.
% Omnivores (wt)	Percentage of total biomas captured that were omnivores; i.e., consumed at least 25% animal and 25% plant material.

score was the sum of 12 metric scores and ranged between 0 and 60.

The IBI was validated with data from the test group by performing an ANOVA and a DMC on the 36 test samples, with impact categories as the main effect and IBI score as the response variable. Index of biotic integrity scores were converted to a proportion from 0 to 1 and then arcsine-square-root transformed prior to analysis. The IBI was considered valid if there were significant differences among impact categories, with the least-impacted samples having the highest scores and the point source samples the lowest.

RESULTS

Fish assemblages were sampled at 275 Indiana sites in the Wabash River between

1993–2001 (Fig. 1). An independent test sample set of 36 sites exposed to human-impacted conditions were collected between 2002–2005 to evaluate the final IBI. Of the 275 least-impacted sites, 31 were classified as non-impacted, 231 as agriculture exposed, and 13 as point source pollution impacted. Eighty-three sites were in the northern ECBP, while 110 sites were in the northern portion of the IRL ecoregion, and the remaining 82 sites were located in the southern portion of the IRL ecoregion. Watershed areas ranged from 1139.6 (440 mi^2) to 85,231.7 km^2 (32,908 mi^2).

A total of 119 fish species was collected (Appendix) including 57,519 individuals and 19,825 kg of biomass. The study reaches had a wide variety of fish assemblages. Individual samples yielded from 2–47 species, from 23–5437 individuals (minus schooling species), and from 2.14–34.71 kg of biomass. The most frequently encountered species were carp (92% of samples), channel catfish (84%), gizzard shad (82%), and freshwater drum (77%). The most numerous species were spotfin shiner (12,878 individuals), emerald shiner (9959 individuals), gizzard shad (5296 individuals), and river shiner (4366 individuals), and the greatest biomass was for carp (1057.3 kg), freshwater drum (201.7 kg), and channel catfish (160.5 kg).

Index development.—Of the 24 potential metrics considered (see Table 1 for designations and definitions), none varied significantly in relation to either river size or geographic (ecoregion or sub-ecoregion) location for our 31 least-impacted development group samples. This is most likely due to large and great rivers being an assimilator of upstream conditions. Large and great rivers most likely are already beyond the inflexion or accretion curve that is so dramatic in headwater and wadeable streams and thus would not demonstrate the pronounced drainage area relationships seen in small systems. These results are consistent with other large and great river calibrations (Simon & Emery 1998; Niemela et al. 1999; Emery et al. 2003). In addition, the River Continuum Concept (RCC) suggests increasing species richness with downstream drainage area increase; however, it is important to note that the Wabash River main stem is the trunk of the RCC since the increase in species richness occurs in the tributaries. Only a single metric, % great river species, had a positive but weak correlation with stream size ($P = 0.036$). None of the metrics had values that differed between drainage area, or northern and southern ecoregions or between or northern and southern sub-ecoregions.

Twenty metrics met the criteria for inclusion in the IBI based on an analysis of all 275 development group samples. Four of these metrics were excluded because of redundancy. The metrics total species and native species provided almost identical results ($r = 0.924$) and differed by more than one species at only one site. The number of native species metric was retained, and the total number of species metric was dropped. The metrics % omnivore ($r = 0.893$), % tolerant ($r = 0.888$), and % detritivore (r = 0.872), had similar patterns across the impact classes regardless of whether calculated based on the number of individuals or the biomass collected. Since biomass was used as a separate indicator, the % omnivore and % tolerant species metrics was retained.

One metric, % DELT was retained that did not meet the criteria for inclusion. This metric has been shown to be particularly sensitive to industrial and toxic discharges in numerous other studies (Sanders et al. 1999). In this data set, the DELT percentages were consistently low and did not differ among impact categories; but since sites with major untreated point source discharges were difficult to find during the time of our sampling, this was not considered a problem. However, such pollution types were common in this ecoregion as recent as the 1970s, so the DELT metric was retained to provide sensitivity to potential impacts that were not encompassed within the dataset.

Scoring criteria for the final twelve metrics are provided in Fig. 2 and Table 2. Different criteria were not needed for northern and southern portions of the ecoregion; nor were different metric calibrations needed for stream sizes including % large-river species (< 5,957 km^2) and % great-river species (> 5,957 km^2). The overall IBI score was the sum of the individual scores for the 12 metrics and could range from 0 (worst) to 60 (best).

Index validation.—Overall IBI scores for 36 test group samples ranged from 16 (very poor) to 31 (fair) (Fig. 3), while the entire 275 combined set of development and test samples ranged from 12 (very poor) to 45 (fair-good) (Fig. 4). The least-impacted category was significantly greater than the agriculture and non-

Table 2.—Final metrics and scoring criteria for the Wabash River, Indiana.

Metric	Location	Scoring criteria and rating (points)		
		Poor (1)	Fair (3)	Good (5)
Native species (Total)	All	<10	10–20	>20
Centrarchid species	All	≤2	3–4	≥5
Round-bodied sucker	All	<2	2–4	≥5
Sensitive species	All	≤3	4–7	≥8
% Tolerant	All	<71.6%	43.3–71.6%	>43.3%
% Omnivores	All	<68.3%	36.7–68.3%	>36.7%
% Insectivores	All	<25.0%	25.0–50.0%	>50.0%
% Carnivores	All	<10% or >40%	10–20% & 30–40%	>20–30%
% Large-river species	All	<28.3%	28.3–56.6%	>56.6%
CPUE	All	<600	600–1200	>1200
% Lithophils	All	<15%	15–30%	>30%
% DELT	All	>1.3%	0.1–1.3%	<0.1%

point source categories, which did not differ from each other. Least-impacted samples (n = 31) had a mean of 34 (fair) and a range of 22–45 compared to agriculture samples (n = 231), which had a mean of 24 (poor) and a range of 13–44, and a mean of 22 (very poor) and a range of 16–29 for point source samples. Ninety percent of the least-impacted samples were rated between poor and fair, and 83% of the impact samples were rated as poor or very poor.

Variation within years.—Substantial annual variation in IBI scores among samples occurred at some sites but not at others. Generally, variation was lowest at the least-impacted sites and highest at the point-source impact sites. Within-year variation in IBI scores for sites ranged from 0 to 8 points with a mean of 3.2 points, and among- year variation ranged from 0 to 12 points with a mean of 4.1 points. All of these sites remained within the same integrity class. One site had ratings that ranged from very poor to fair between years. A single point-source pollution site varied 12 points and fluctuated in rating from very poor to poor between years.

DISCUSSION

Metric selection.—A wide range of metrics representative of the structure, composition, and functional organization of the Wabash River in the Interior River Lowland and Eastern Corn Belt Plain was considered. Most of the selected metrics have been found useful in other stream IBI applications, though they were modified to reflect understanding of river assemblages in this area. For example, previous stream versions of IBI have not used a "0" score when a metric attribute is not present. This simple adjustment in the scoring procedure reduced inherent natural variation in the degraded sites. Simon et al. (1998) used this procedure in vernal ponds when evaluating a multi-species assemblage and coastal wetlands in Lake Michigan (Simon & Stewart 2006).

The choice of metrics reflected a balance between different types of metrics (i.e., structure and function) and different measures of assemblage characteristics (i.e., composition, tolerance, trophic guild, reproductive guild, abundance, and condition). As recommended by Simon & Lyons (1995) and Karr & Chu (1999), metrics that related to species richness and composition (number of native, minnow, sucker, and sunfish species), indicator species (sensitive species, % tolerant species, % pioneer species), trophic function (% insectivores, % detritivores, % carnivores), reproductive function (% lithophils), abundance (CPUE), and fish condition (% DELT). Some previous IBIs have used biomass to assess biological integrity when there are large differences in adult size among species or when species richness is low (Hughes & Gammon 1987; Goldstein et al. 1994; Minns et al. 1994; Niemela et al. 1999; Lyons et al. 2000; Emery et al. 2003). Percent metrics were based entirely on individuals since this biomass data are used for another indicator other than the IBI, i.e., index of well being (Gammon 1976).

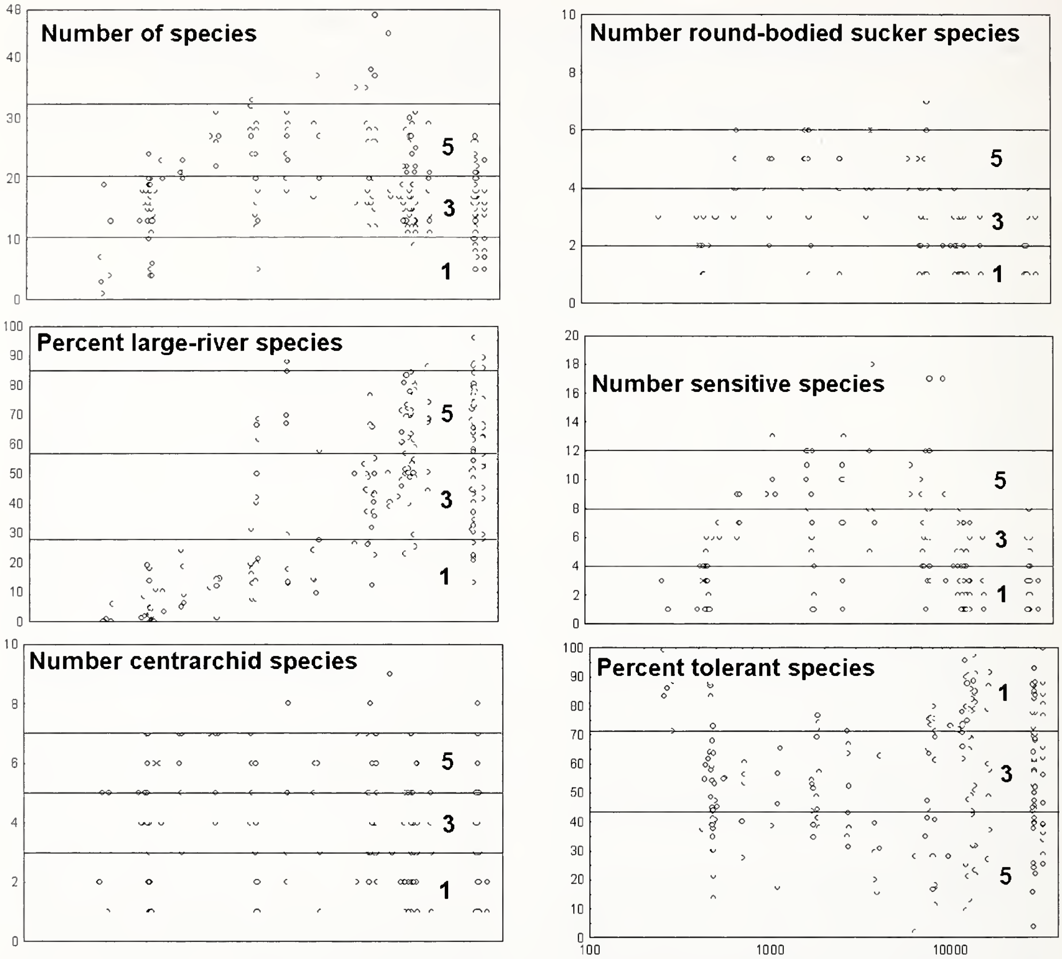

Figure 2.—Index of Biotic Integrity (IBI) metric scoring criteria for the Wabash River.

The IBI is an important component of an assessment toolbox that can be used by fisheries and environmental professionals.

Only a single metric included in the final metrics of the calibration related weakly to river size, as measured by drainage area. This is consistent with the results of other large-river IBIs (Simon 1992; Simon 1994; Simon & Dufour 1998a, b; Simon & Emery 1995; Emery et al. 2003), and Ohio (Ohio EPA 1989) calibrations, which did not show any positive correlation with species richness metrics.

Validation and variation.—An analysis of the test dataset validated the effectiveness of the Wabash River IBI (Fig. 3). As is necessary for an effective index, the sites were judged based on *a priori*, independent (i.e., non-fish) criteria: our least impacted sites had the highest IBI scores, and sites that we judged worst—the point-source sites—had the lowest scores. Based on the entire developmental dataset, the same patterns were observed with agricultural sites attained an intermediate level of impact with associated intermediate scores (Fig. 4). Because the test data were not used in any phase of the index development, these results are strong evidence that the IBI accurately measures the condition of large and great rivers (Karr & Chu 1999; Simon 1999a). These results support the utility of an IBI based on a subset of the river fish community for rapid biological assessment.

Although our new IBI appears to provide an accurate measure of stream ecosystem condition, this measure is not particularly precise, especially between years. This may be due to

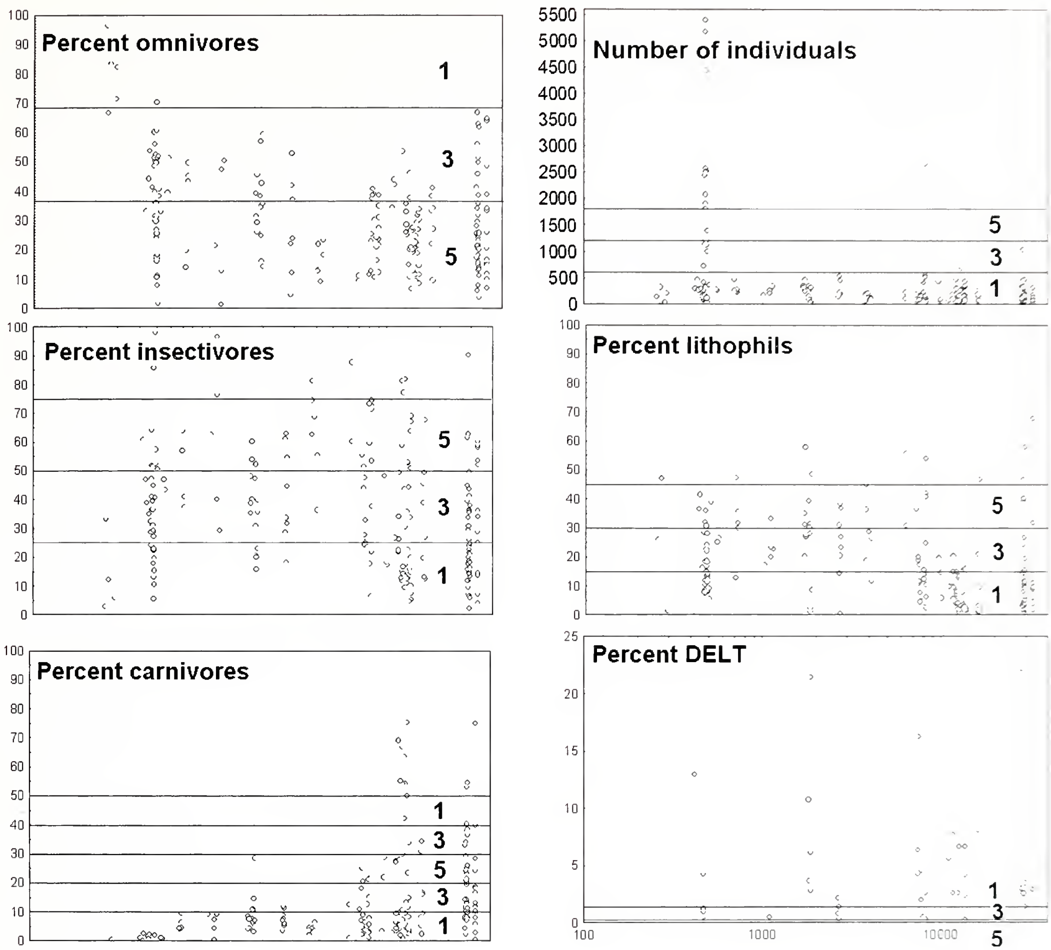

Figure 2.—Continued.

extremes in hydrologic conditions between years; however, at the highest quality river reaches little variability was observed. The temporal variation within high-quality reaches was relatively low, at 0–5 points, or about 0–8.3% of actual IBI scores, but much higher within degraded reaches, at 4–12 points or 6.7–20% of actual scores. Several other studies from midwestern United States streams have also found greater variation over time in IBI scores at degraded sites, although variation has typically been in the range of 25–60% of actual scores (summarized in Fore et al. 1994; Yoder & Rankin 1995; Gammon & Simon 2000). These findings suggest that strong temporal variation in fish assemblage characteristics is a real phenomena at degraded sites and not an artifact of the particular IBI used. Variation in IBI scores may be a signal of degradation (Karr & Chu 1999). Additional studies are needed to document the status and trends in biotic integrity at sites with human impacts than will be needed at least-impacted sites. Additional sampling is recommended from different periods to assess the condition of a site of unknown quality. Gammon & Simon (2000) found that four metrics (i.e., total number of species, number of centrarchid species, number of sensitive species, and % lithophils) responded at sites across the Eastern Corn Belt Plain ecoregion and a portion of the Interior River Lowland ecoregion; however, this was not anticipated to be an observed relationship for reference condition calibration since no single site is expected to represent the highest integrity for all

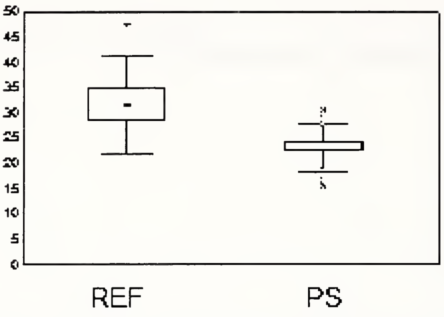

Figure 3.—Mean IBI scores and 95% confidence intervals for reference IBI categories and 36 test samples for point source discharge. Abbreviations are as follows: REF = reference condition, and PS = point source.

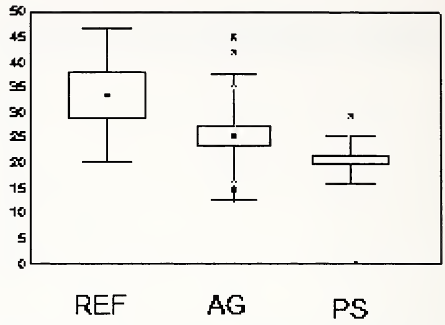

Figure 4.—Distribution of IBI scores among ratings for impact categories for all 275 developmental samples. Abbreviations are as follows: REF = reference condition, AG = agriculture, and PS = point source.

metrics. Thus, individual metrics may show a trend in scores across ecoregions without metric expectations showing similar trends since the upper line is derived by either the maximum observed line for percentage metrics or 95 percentile for species structural and compositional metrics.

Application.—Since no statistically significant difference was observed in metric response for drainage area, ecoregion, or sub-ecoregion expectations for the reference condition, a single IBI was calibrated for the Wabash River. All studies of large and great rivers have not shown a relationship with drainage area (Goldstein et al. 1994; Minns et al. 1994; Niemela et al. 1999; Lyons et al. 2000; Emery et al. 2003; Simon & Stewart 2006), or across ecoregion or sub-ecoregion (Goldstein et al. 1994; Minns et al. 1994; Niemela et al. 1999; Lyons et al. 2000; Emery et al. 2003). Thus, a relationship between drainage area, ecoregion, or sub-ecoregion and metric expectations was not expected in this study. Simon & Stahl (1998) and Simon (1992) did not observe a relationship between fish assemblages and ecoregions or sub-ecoregions for the Eastern Corn Belt Plain, Interior River Lowland, and Interior River Plateau. Although a drainage area relationship is usually seen with increasing species accretion in headwater and wadeable streams, the RCC predicts that large and great rivers should not show increasing expectations. Once species diversity accretion is attained in large rivers, the replacement of small headwater species with large-river species does not increase substantially since the drainage area is already at the maximum for the watershed.

The least-impacted sites had higher IBI scores and better ecosystem quality than sites that are more strongly impacted by human activities. Most least-impacted samples were rated as fair, and seldom were sites rated as good. On the contrary, many impacted sites were rated as poor. The sites rated as poor were representative of widescale land use changes that affected entire river reaches, but would not have been apparent from the local riparian and instream condition. Regional estimates of stream conditions showed that 42% of the stream reaches in the Interior River Lowland ecoregion had fish assemblages in poor or fair ecological condition, while large-river reaches in the Eastern Corn Belt Plain ecoregion had 36% fair and 23% good. Much of Indiana is in agricultural land use and serves as a background condition, thus sediment and nutrient runoff from upstream agriculture may well have reduced ecosystem quality below least-impacted site conditions on other large rivers. Despite the inclusion of lower quality sites in the developmental data base for the IBI, the classification of these sites indicated that both the metrics and the final IBI classification accurately portrayed the actual stream condition.

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Appendix.—Classification of fishes captured during this study. For feeding, P = parasite, F = filter, C = "carnivore" indicates the top carnivore, I = insectivore, H = herbivore, and O = omnivore. For habitat, "large" indicates streams greater than 2,590 but less than 5,957 square kilometer drainage area. For spawning, SL = simple lithophil. "Other" indicates that the species was not included within one of the categories used in calculating particular metrics. Species are listed in taxonomic order by family and alphabetically within family by scientific name. Classifications were taken from Simon (1999b), Goldstein & Simon (1999), and unpublished data.

Common name	Scientific name	Origin	Tolerance	Feeding	Habitat	Spawning
Lamprey	Petromyzontidae					
Chestnut lamprey	*Ichthyomyzon castenaus*	Native	Other	P	Large	Other
Silver lamprey	*Ichthyomyzon unicuspis*	Native	Other	P	Large	Other
American brook lamprey	*Lampetra appendix*	Native	Intolerant	F	Other	Other
Least brook lamprey	*Lampetra aepyptera*	Native	Intolerant	F	Other	Other
Gar	Lepisosteidae					
Spotted gar	*Lepisosteus oculatus*	Native	Other	C	Other	Other
Longnose gar	*Lepisosteus osseus*	Native	Other	C	Other	Other
Shortnose gar	*Lepisosteus platostomus*	Native	Other	C	Large	Other
Sturgeon	Acipenseridae					
Lake sturgeon	*Acipenser fulvescens*	Native	Other	I	Large	SL
Shovelnose sturgeon	*Scaphirhynchus platorhynchus*	Native	Other	I	Large	SL
Paddlefish	Polyodontidae					
Paddlefish	*Polyodon spathula*	Native	Intolerant	P	Large	SL
Bowfin	Amiidae					
Bowfin	*Amia calva*	Native	Other	C	Other	Other
Herring	Clupeidae					
Skipjack herring	*Alosa chrysochloris*	Native	Other	C	Large	Other
Gizzard shad	*Dorosoma cepedianum*	Native	Other	O	Other	Other
Threadfin shad	*Dorosoma petenense*	Native	Other	O	Large	Other
Mooneye	Hiodontidae					
Goldeye	*Hiodon alsoides*	Native	Intolerant	I	Large	Other
Mooneye	*Hiodon tergisus*	Native	Intolerant	I	Large	Other
Minnow	Cyprinidae					
Stoneroller minnow	*Campostoma anomalum*	Native	Other	H	Other	Other
Goldfish	*Carassius auratus*	Exotic	Tolerant	O	Other	Other
Spotfin shiner	*Cyprinella spiloptera*	Native	Other	I	Other	Other
Steelcolor shiner	*Cyprinella whipplei*	Native	Other	I	Other	Other
Common carp	*Cyprinus carpio*	Exotic	Tolerant	O	Other	Other
Grass carp	*Ctenopharyngodon idella*	Exotic	Tolerant	O	Other	Other
Silverjaw shiner	*Ericymba buccata*	Native	Other	I	Other	Other
Streamline chub	*Erimystax dissimilis*	Native	Intolerant	I	Large	SL
Gravel chub	*Erimystax x-punctata*	Native	Intolerant	I	Large	SL
Bigeye chub	*Hybopsis amblops*	Native	Intolerant	I	Other	SL
Mississippi silvery minnow	*Hybognathus nuchalis*	Native	Other	O	Large	SL

Appendix.—Continued.

Common name	Scientific name	Origin	Tolerance	Feeding	Habitat	Spawning
Silver carp	*Hypopthalmichthys molitrix*	Exotic	Tolerant	H	Large	Other
Bighead carp	*Hypophthalmichthys nobilis*	Exotic	Tolerant	I	Other	Other
Striped shiner	*Luxilus chrysocephalus*	Native	Other		Other	Other
					Other	
Ribbon shiner	*Lythrurus fumeus*	Native	Other	I	Other	Other
Redfin shiner	*Lythrurus umbratilis*	Native	Other	I	Other	Other
Shoal chub	*Macrhybopsis hyostoma*	Native	Other	I	Large	Other
Silver chub	*Macrhybopsis storeriana*	Native	Other	I	Large	Other
Hornyhead chub	*Nocomis biguttatus*	Native	Intolerant	I	Other	Other
River chub	*Nocomis micropogon*	Native	Intolerant	I	Large	Other
Golden shiner	*Notemigonus crysoleucas*	Native	Tolerant	I	Other	Other
Emerald shiner	*Notropis atherinoides*	Native	Other	I	Large	Other
River shiner	*Notropis blennius*	Native	Other	I	Large	SL
Bigeye shiner	*Notropis boops*	Native	Intolerant	I	Other	SL
Ghost shiner	*Notropis buchanani*	Native	Other	I	Other	Other
Spottail shiner	*Notropis hudsonius*	Native	Other	I	Large	Other
Silver shiner	*Notropis photogenis*	Native	Intolerant	I	Other	SL
Rosyface shiner	*Notropis rubellus*	Native	Intolerant	I	Other	SL
Silverband shiner	*Notropis shumardi*	Native	Intolerant	I	Large	SL
Sand shiner	*Notropis stramineus*	Native	Other	I	Other	Other
Mimic shiner	*Notropis volucellus*	Native	Intolerant	I	Large	Other
Channel shiner	*Notropis wickliffi*	Native	Other	I	Large	Other
Suckermouth minnow	*Phenacobius mirabilis*	Native	Other	I	Other	SL
Bluntnose minnow	*Pimephales notatus*	Native	Tolerant	O	Other	Other
Fathead minnow	*Pimephales promelas*	Native	Tolerant	O	Other	Other
Bullhead minnow	*Pimephales vigilax*	Native	Other	I	Large	Other
Western blacknose dace	*Rhinichthys obtusus*	Native	Tolerant	—	Other	SL
Creek chub	*Semotilus atromaculatus*	Native	Tolerant	—	Other	Other
Sucker	Catostomidae					
River carpsucker	*Carpiodes carpio*	Native	Other	O	Other	Other
Quillback	*Carpiodes cyprinus*	Native	Other	O	Other	Other
Highfin carpsucker	*Carpiodes velifer*	Native	Intolerant	O	Other	Other
White sucker	*Catostomus commersonii*	Native	Tolerant	O	Other	SL
Blue sucker	*Cycleptus elongatus*	Native	Intolerant	I	Large	SL
Lake chubsucker	*Erimyzon sucetta*	Native	Other	I	Other	Other
Northern hogsucker	*Hypentelium nigricans*	Native	Intolerant	I	Other	SL
Smallmouth buffalo	*Ictiobus bubalus*	Native	Other	O	Large	Other
Bigmouth buffalo	*Ictiobus cyprinellus*	Native	Other	O	Large	Other
Black buffalo	*Ictiobus niger*	Native	Other	O	Large	Other
Spotted sucker	*Minytrema melanops*	Native	Other	I	Other	SL
Silver redhorse	*Moxostoma anisurum*	Native	Intolerant	I	Other	SL
River redhorse	*Moxostoma carinatum*	Native	Intolerant	I	Other	SL
Black redhorse	*Moxostoma duquesnei*	Native	Intolerant	I	Other	SL
Golden redhorse	*Moxostoma erythrurum*	Native	Intolerant	I	Other	SL
Shorthead redhorse	*Moxostoma macrolepidotum*	Native	Intolerant	I	Other	SL

Appendix.—Continued.

Common name	Scientific name	Origin	Tolerance	Feeding	Habitat	Spawning
Bullhead catfish	Ictaluridae					
Yellow bullhead	*Ameiurus natalis*	Native	Other	I	Other	Other
Black bullhead	*Ameiurus melas*	Native	Tolerant	I	Other	Other
Brown bullhead	*Ameiurus nebulosus*	Native	Other	I	Other	Other
Channel catfish	*Ictalurus punctatus*	Native	Other	C	Large	Other
Blue catfish	*ictalurus furcatus*	Native	Other	C	Large	Other
Mountain madtom	*Noturus eleutherus*	Native	Intolerant	I	Other	Other
Stonecat	*Noturus flavus*	Native	Intolerant	I	Other	Other
Brindled madtom	*Noturus miurus*	Native	Intolerant	I	Other	Other
Freckled madtom	*Noturus nocturnus*	Native	Intolerant	I	Other	Other
Flathead catfish	*Pylodictis olivaris*	Native	Other	C	Large	Other
Pike	Esocidae					
Grass pickerel	*Esox americanus*	Native	Other	C	Other	Other
Mudminnow	Umbridae					
Central mudminnow	*Umbra limi*	Native	Tolerant	O	Other	Other
Topminnow	Fundulidae					
Blackstripe topminnow	*Fundulus notatus*	Native	Other	I	Other	Other
Livebearer	Peciliidae					
Mosquitofish	*Gambusia affinis*	Native	Other	I	Other	Other
Silverside	Atherinidae					
Brook silverside	*Labidesthes sicculus*	Native	Other	I	Other	Other
Pirate perch	Aphredoderidae					
Pirate perch	*Apheredoderus sayanus*	Native	Other	I	Other	Other
Sculpin	Cottidae					
Mottled sculpin	*Cottus bairdi*	Native	Other	I	Other	Other
Banded sculpin	*Cottus carolinae*	Native	Other	I	Other	Other
Temperate bass	Moronidae					
White bass	*Morone chrysops*	Native	Other	C	Large	Other
Yellow bass	*Morone mississippensis*	Native	Other	C	Large	Other
Striped bass	*Morone saxatilis*	Native	Other	C	Large	Other
Sunfish	Centrarchidae					
Rock bass	*Ambloplites rupestris*	Native	Other	C	Other	Other
Green sunfish	*Lepomis cyanellus*	Native	Tolerant	I	Other	Other
Warmouth	*Lepomis gulosus*	Native	Other	C	Other	Other
Orangespotted sunfish	*Lepomis humilis*	Native	Other	I	Other	Other
Bluegill	*Lepomis macrochirus*	Native	Other	I	Other	Other
Redear sunfish	*Lepomis microlophus*	Native	Other	I	Other	Other
Longear sunfish	*Lepomis megalotis*	Native	Intolerant	I	Other	Other
Bantam sunfish	*Lepomis symmetricus*	Native	Other	I	Other	Other
Smallmouth bass	*Micropterus dolomieu*	Native	Intolerant	C	Other	Other
Spotted bass	*Micropterus punctulatus*	Native	Other	C	Other	Other
Largemouth bass	*Micropterus salmoides*	Native	Other	C	Other	Other
White crappie	*Pomoxis annularis*	Native	Other	—	Other	Other
Black crappie	*Pomoxis nigromaculatus*	Native	Other	—	Other	Other

Appendix.—Continued.

Common name	Scientific name	Origin	Tolerance	Feeding	Habitat	Spawning
Perch	Percidae					
Western sand darter	*Ammocrypta clara*	Native	Intolerant	I	Large	SL
Eastern sand darter	*Ammocrypta pellucida*	Native	Intolerant	I	Large	SL
Mud darter	*Etheostoma asprigene*	Native	Other	I	Other	Other
Greenside darter	*Etheostoma blennioides*	Native	Intolerant	I	Other	Other
Rainbow darter	*Etheostoma caeruleum*	Native	Intolerant	I	Other	SL
Bluebreast darter	*Etheostoma camurum*	Native	Intolerant	I	Other	SL
Bluntnose darter	*Etheostoma chlorosoma*	Native	Other	I	Other	Other
Fantail darter	*Etheostoma flabellare*	Native	Other	I	Other	Other
Slugh darter	*Etheostoma gracile*	Native	Other	I	Other	Other
Harlequin darter	*Etheostoma histrio*	Native	Intolerant	I	Large	SL
Johnny darter	*Etheostoma nigrum*	Native	Other	I	Other	Other
Orangethroat darter	*Etheostoma specatbile*	Native	Other	I	Other	SL
Tippecanoe darter	*Etheostoma tippecanoe*	Native	Intolerant	I	Other	SL
Logperch	*Percina caprodes*	Native	Other	I	Other	SL
Channel darter	*Percina copelandi*	Native	Intolerant	I	Other	SL
Gilt darter	*Percina evides*	Native	Intolerant	I	Other	SL
Blackside darter	*Percina maculata*	Native	Other	I	Other	SL
Slenderhead darter	*Percina phoxocephala*	Native	Intolerant	I	Other	SL
Dusky darter	*Percina sciera*	Native	Other	I	Other	SL
River darter	*Percina shumardi*	Native	Intolerant	I	Large	SL
Sauger	*Sander canadense*	Native	Other	C	Large	SL
Walleye	*Sander vitreus*	Native	Other	C	Large	SL
Drum	Scianidae					
Freshwater drum	*Aplodinotus grunniens*	Native	Other	—	Large	Other