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September 11, 2008

Ms. Sara Beard
Kentucky Division of Water
Surface Water Permits Branch
14 Reilly Road
Frankfort, KY 40601

Dear Ms. Beard:

Re: North American Stainless
KPDES Permit 0095877
Section 316(a) Thermal Discharge Demonstration

Thank you for your e-mail of August 20, 2008 that provided comments and questions from EPA Region 4 regarding the Section 316(a) demonstration North American Stainless (NAS) prepared for the Outfall 001 discharge to the Ohio River.[1]

We appreciate the opportunity to explain and clarify the basis of the Section 316(a) demonstration. Presented below is an explanation of our use of the EPA guidance upon which the 316(a) demonstration is based, followed by responses to the EPA Region 4 comments and questions. I have asked Ron King of EA Engineering Science & Technology and Gary Amendola of Amendola Engineering, Inc. to provide responses for NAS.

NAS Section 316(a) Thermal Demonstration

NAS currently discharges approximately 2.9 million gallons per day of treated process waters and non-contact cooling water recirculating system blowdowns directly to the Ohio River through Outfall 001. Recent maximum discharge temperatures have been in the range of 110°F. The discharge pipeline extends approximately 75 feet into the Ohio River and is equipped with a multi-port effluent diffuser anchored to the bottom of the river. NAS is undergoing a plant

[1] *Evaluation of Thermal Discharge from Outfall 001 Under Section 316(a) of the Clean Water Act and 40 CFR Part 125, Subpart H.* Prepared for North American Stainless, Ghent, KY. Prepared by Amendola Engineering, Inc., Lakewood, OH and EA Engineering, Science and Technology, Hunt Valley MD. February 20, 2008 (Draft).

expansion that will result in a maximum expected discharge flow of approximately 4.5 mgd. This flow is only 0.06% of the $Q_{7,10}$ flow of the Ohio River in the McAlpine Pool (11,000 cfs).

Based on guidance from the Kentucky Division of Water, the 316(a) demonstration was conducted following EPA guidance for the "Low Potential Impact Type III" discharge category (see Figure 3 of the NAS report referenced as footnote 1).[2,3] The NAS Outfall 001 discharge clearly qualifies for this type of demonstration. The 1977 EPA technical guidance manual regarding Low Impact Type III demonstrations states:

> "3a) **Low Potential Impact Type III Predictive Studies** is (are) appropriate for facilities located on receiving waters for which available data indicate low potential for adverse impacts of cooling water discharges. The Type III Demonstration is appropriate for a site that can be shown to have low potential for significant impacts to aquatic biota, based on available literature, site characterization studies performed for other purposes, or previous thermal discharge studies on similar sites. This study design is flexible and determined by the applicant and the permitting authority, with consideration given to each biotic category, while avoiding collection of unneeded data."

The demonstration submitted by NAS is fully consistent with the EPA guidance presented above. Site-specific temperature modeling was conducted to estimate the impacted zone (i.e., area of the Ohio River above maximum ambient water quality standard); and, available literature information from area biological studies was evaluated by qualified biologists to assess possible impacts on biota in the Ohio River.

The draft report for the 316(a) demonstration shows that even with an unrealistically high Outfall 001 discharge temperature of 125°F and a background Ohio River temperature of 88°F (1°F below the maximum ambient water quality standard of 89°F), the area of the Ohio River impacted would be approximately 16 feet downstream from the discharge. Under more realistic and typical discharge and Ohio River conditions the impacted area would be only a few feet downstream from the discharge. Figure 1 from the NAS report (attached) shows the limited zone of influence in relation to the size of the Ohio River. The conclusion of the biological assessment is that significant adverse impacts on Ohio River biota cannot reasonably be anticipated.

Response to EPA Region 4 Comments/Questions

EPA Comment No. 1: List of organisms that make up the Balanced, Indigenous Population (BIP) - The permittee did not define the BIP to be used in the 316(a) demonstration study. Identification of the BIP is necessary to show compliance with the Section 316(a) requirement that the thermal component of the discharge assures the protection and propagation of a

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[2] *Interagency 316(a) Technical Guidance Manual and Guide to Thermal Effects Sections of Nuclear Facilities Environmental Impacts Statements*. U.S. Environmental Protection Agency, Office of Water Enforcement, Permits, Division, Industrial Permits Branch, Washington, DC. May 1, 1977 (Draft)

[3] *Basic Guide to the Design of 316 Demonstrations*. Region IV, U.S. Environmental Protection Agency. Atlanta, GA. August 7, 1974.

balanced, indigenous population of shellfish, fish and wildlife in and on that body of water into which the discharge is to be made.

Response: The 1977 guidance indicates that a Type III, Low Potential Impact Demonstration should follow the information needs for the Type I and II demonstrations except that the Representative Important Species (RIS) sections should be deleted (see page 64). Also, as indicated in our response to Comment No. 4, use of the Ohio River Fish Index (ORFIn) represents an improved assessment tool over that called for in the 1977 EPA guidance document.

EPA Comment No. 2: A diagram depicting the spawning areas and information on temperature tolerance ranges for organisms in the BIP - The permittee did not identify and submit data and/or information on the thermal tolerance ranges and known spawning areas in the study area(s) for each life stage (e.g., larval, juvenile and adult) of each species selected as a Representative Important Species (RIS) or for each species selected to be in the BIP.

Response: It is our understanding that this information is not required for a Type III, Low Potential Impact discharge because, as indicated above in our response to EPA Comment No. 1, it is not necessary to select RIS. Also, because the discharge temperatures decline so rapidly to the 89°F water quality standard, there appears to be no need to provide detailed temperature tolerance data because the zone of influence is so small that even if fish were attracted to the discharge, they would avoid elevated temperatures through well documented avoidance responses.

As stated in the demonstration, any potential thermal effects on the fish community will not be significant based on the low potential for exposure to the elevated water temperatures at the point of discharge from the NAS Outfall 001 effluent diffuser. That low potential for thermal effects reflects the bottom location of the discharge; the extremely small discharge zone (i.e., less than 16 feet under unrealistically conservative conditions); and, the well documented avoidance responses of adult and juvenile fish to water temperatures that exceed their upper tolerance limits. The warm and cool water species known to occur in the Ohio River will not be at risk even though their avoidance temperatures may occasionally be exceeded in a small portion of the NAS plume during the warmest months. Fishes will avoid such temperatures, either by staying outside of that portion of the plume that reaches their avoidance temperatures, or by swimming underneath the buoyant plume. This requires access to thermal refugia and/or a zone of passage, which in this case is highly likely because of the very small discharge (6.96 cfs; 4.5 mgd) compared to the mean summer (June to September) Ohio River flows (45,300 to 91,100 cfs) at the Markland Dam. The NAS discharge represents less than 0.02 percent of the mean summer flow, which means most of the river functions as a thermal refugia and/or a zone of passage from water temperatures discharged from NAS Outfall 001.

EPA Comment No. 3: The decision criteria used to determine "appreciable harm." – The Permittee did not determine, before commencing the study, the decision criteria that will be used to determine if "appreciable harm" has occurred.

Response: As detailed in the report, criteria were applied in making the no impact determination, e.g., a very small plume that fish will avoid when water temperatures are elevated

and the available ORFIn scores show fish communities upstream and downstream of the NAS discharge are meeting expectations.

EPA Comment No. 4: Detailed information on ORSANCO's Ohio River Fish Index - The permittee did not submit detailed information describing the methodology used to develop the bioassessment index, which was used in the decision process.

Response: The Ohio River Fish Index (ORFIn) was developed specifically to assess the condition of fish assemblages along the Ohio River. ORFin applies 13 metrics that were selected because they respond predictably to measures of human disturbance or indicate desirable features of the Ohio River. See attached published paper by Emery et. al., 2001 for details on the development and application of the index.[4]

Application of ORFIn to the NAS 316(a) demonstration represents an improvement over the traditional RIS approach because, rather than focusing on the temperature requirements of selected species (i.e., the BIP approach), the ORFIn incorporates community level measures that reflect the overall health of the receiving water and alternatively addresses the balanced, indigenous community (BIC).

We believe the Section 316(a) evaluation approach referenced by EPA Region 4 (quantitative evaluations against known biotic populations) goes beyond the EPA guidance for low impact thermal discharges. The area impacted by the NAS Outfall 001 discharge is so small in relation to the area of the Ohio River that comprehensive *in-situ* biological studies that would be required to assemble the necessary data to make such evaluations are not warranted. Such studies would be exceedingly difficult to conduct given the small size of the discharge mixing zone.

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[4] Emery, E.B., Simon, T., McCormick, F., Angermeier, P., Deshon, J., Yoder, C., Sanders, R., Pearson, W., Hickman, G., Reash, R., Thomas, J., *Development of a Multimetric Index for Assessing the Biological Condition of the Ohio River*, Transactions of the American Fisheries Society, 132: 791-808, 2003.

In conclusion, NAS submits the Section 316(a) thermal discharge demonstration meets the requirements of a Low Potential Impact Type III demonstration as set out in EPA guidance and demonstrates that a Water Quality Based thermal limit is not necessary to assure protection and propagation of aquatic life in the Ohio River.

We believe that it will be helpful to all concerned if we have the opportunity to meet with Region 4 representatives to further explain the above responses. Please advise as to who we should contact at Region 4 to request an appointment.

Sincerely yours,

Steven Shaver, CSIH, MEHS
Corporate Environmental Manager

Enclosures

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KDOW

William J. Jones, Jr., Esq.
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Gary A. Amendola, P.E.
Amendola Engineering, Inc.

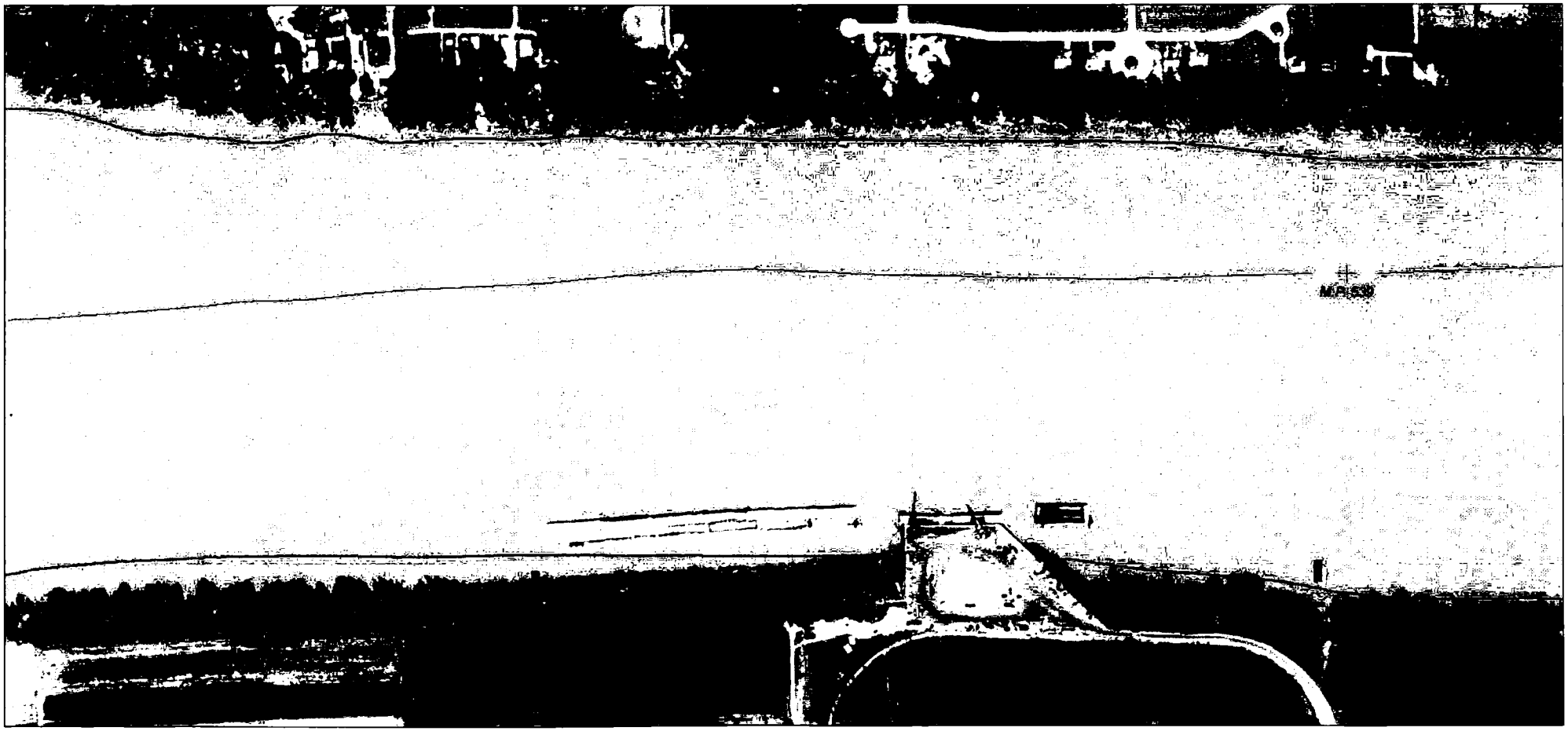
Figure 1

NORTH AMERICAN STAINLESS
GHENT, KY

AERIAL PHOTO OF OHIO RIVER WITH DIFFUSER STRUCTURE AND MIXING ZONE

0 50 100 150 200 ft

DIFFUSER STRUCTURE
MIXING ZONE (35 ft)
NORMAL POOL SHORELINE
OHIO RIVER SAILING LINE



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Development of a Multimetric Index for Assessing the Biological Condition of the Ohio River

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[1] The Unit is jointly sponsored by the U.S. Geological Survey, Virginia Polytechnic Institute and State University, and the Virginia Department of Game and Inland Fisheries.

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Abstract.—The use of fish communities to assess environmental quality is common for streams, but a standard methodology for large rivers is as yet largely undeveloped. We developed an index to assess the condition of fish assemblages along 1,580 km of the Ohio River. Representative samples of fish assemblages were collected from 709 Ohio River reaches, including 318 "least-impacted" sites, from 1991 to 2001 by means of standardized nighttime boat-electrofishing techniques. We evaluated 55 candidate metrics based on attributes of fish assemblage structure and function to derive a multimetric index of river health. We examined the spatial (by river kilometer) and temporal variability of these metrics and assessed their responsiveness to anthropogenic disturbances, namely, effluents, turbidity, and highly embedded substrates. The resulting Ohio River Fish Index (ORFIn) comprises 13 metrics selected because they responded predictably to measures of human disturbance or reflected desirable features of the Ohio River. We retained two metrics (the number of intolerant species and the number of sucker species [family Catostomidae]) from Karr's original index of biotic integrity. Six metrics were modified from indices developed for the upper Ohio River (the number of native species; number of great-river species; number of centrarchid species; the number of deformities, eroded fins and barbels, lesions, and tumors; percent individuals as simple lithophils; and percent individuals as tolerant species). We also incorporated three trophic metrics (the percent of individuals as detritivores, invertivores, and piscivores), one metric based on catch per unit effort, and one metric based on the percent of individuals as nonindigenous fish species. The ORFIn declined significantly where anthropogenic effects on substrate and water quality were prevalent and was significantly lower in the first 500 m below point source discharges than at least-impacted sites nearby. Although additional research on the temporal stability of the metrics and index will likely enhance the reliability of the ORFIn, its incorporation into Ohio River assessments still represents an improvement over current physicochemical protocols.

Protecting the biological integrity of aquatic ecosystems is a fundamental goal of water resource policy in the United States and is mandated by the U.S. Water Pollution Control Act Amendment of 1972 and its reauthorizations. Achieving this goal requires, among other things, scientifically sound protocols for assessing biotic condition, including monitoring designs, sampling methods, and analytical tools. However, biological monitoring and assessment remain weakly implemented for many aquatic systems (Karr 1991; Karr and Chu 1999), and few states have developed quantitative criteria for assessing the biotic status of water bodies (Southerland and Stribling 1995). Instead, physicochemical measures of condition focused on the success of pollution abatement programs are emphasized over biological ones (Adler 1995; Sparks 1995). Environmental assessments of large rivers exemplify this deemphasis of biotic condition (Karr 1985a).

Large-floodplain rivers (hereafter called great rivers) are distinctive in terms of their ecological operation and how humans have modified them. River components, including catchments, are physically and biologically connected along longitudinal, lateral, and vertical dimensions (Vannotte et al. 1980; Ward and Stanford 1995). Great rivers are subject to a variety of stressors, including impoundments that alter the flow regimes of water and sediments (Ward and Stanford 1989; Bayley 1995), pollution and land use practices that alter water quality and temperature, and intensive agriculture and wetlands reclamation that interrupt the connectivity of the floodplain and its associated wetlands (Bayley 1995) and thereby disrupt energy flow (Power et al. 1995). In great rivers, the disruption of the natural hydrologic and sediment regimes is evident in channelization (Braaten and Guy 1999), impoundment by dams (Dynesius and Nilsson 1994; Pearson and Krumholz 1984; Ligon et al. 1995), inundation and embayment of backwaters and tributaries (Stalnaker et al. 1989), isolation and loss of wetlands, water withdrawal for irrigation and industrial uses, and excessive loading of fine sediment via land use in their catchments (Berkman and Rabeni 1987; Carlson and Muth 1989; Ebel et al. 1989; Poff et al. 1997). Flow regulation has cascading effects on all aspects of the ecological structure and function of rivers, including altered sediment transport and temperature regimes, reduced production, fewer native species, and more nonnative species (Ward and Stanford 1995; Stanford et al. 1996; Poff et al. 1997). As such, assessments of biological integrity for large rivers should indicate substantial impairment from the cumulative stressors of great-river basins.

Great rivers are also distinctive in the difficulties associated with assessing their biotic condition. Foremost among these are their size and the spatial scales over which habitat patches and biota are distributed. Scale has important implications for

defining reference conditions and sampling biotic assemblages. Unlike smaller water bodies, which are typically replicated across a given region, large rivers are typically unique, at least within the jurisdiction of a typical (e.g., state or province) management agency. This lack of comparable replicates severely limits the development of region-specific reference conditions, which commonly provide a basis for biotic assessments (Hughes 1995), and forces a disproportionate reliance on historical accounts and expert judgment to define assessment benchmarks. This difficulty is exacerbated by the virtual absence of only slightly modified reaches from most large rivers; thus, even pseudoreplicate reference reaches are largely unavailable for comparison. Consequently, unless historical accounts are very explicit, which is rare, attributing observed patterns of variation (physicochemical or biological) to natural as opposed to anthropogenic sources might be arbitrary. Nevertheless, biological benchmarks can be defined on the basis of a general understanding of the ecology of riverine species and historical faunal conditions and by comparing the assemblage structure and function at anthropogenically impacted sites with those from relatively unimpacted sites. As such, they can substantially improve environmental assessments of large rivers.

The biotic assemblages of large water bodies are difficult to sample thoroughly. Fish sampling protocols for small streams commonly apply uniform sampling effort to the entire volume of multiple habitat units (e.g., riffles and pools), which collectively provides a "sample" (McCormick et al. 2001). In contrast, there are no sampling technologies that can thoroughly sample a single habitat unit of a large river, let alone be uniformly applicable to multiple unit types. All available sampling gears have strong biases with respect to taxa, habitat morphology, or water conditions (e.g., clarity and conductivity). Even if thorough sampling were technologically feasible, the cost (monetary and biotic) of sampling a major portion of the fishes in a large river would generally be prohibitive. Thus, biotic assessments of large rivers are necessarily based on relatively small samples with strong, but often predictable, biases.

Analytical tools that efficiently convey biological information to both biologists and nonbiologists are crucial to the implementation of biological monitoring programs. Over the past two decades, multimetric indices (Karr et al. 1986; Karr 1991) have been developed in many areas to serve this function. These tools typically integrate information on many attributes of a biotic community (one attribute per metric) into a numerical index scaled to reflect the ecological health of the community.

A major strength of this approach is its broad ecological foundation, with individual metrics representing selected aspects of the taxonomic and functional composition of the biotic community. This enables detection of a broader array of human impacts than is possible using only physicochemical measures of water quality, including the impacts on flow regime, habitat structure, and biotic interactions (Yoder and Smith 1999). However, the sensitivity and general applicability of multimetric indices are contingent on appropriate customization during their development. In particular, the component metrics and their scoring criteria should reflect system-specific attributes of natural biotic communities and the system-specific responses of those communities to human impacts. For example, dozens of metrics have been substituted for Karr's (1981) original metrics in applications to different ecosystems (Simon and Lyons 1995). This flexibility enhances the ability of multimetric indices to accurately measure environmental degradation. Most adaptations of multimetric indices to new ecosystems, including those for large rivers (Simon and Emery 1995; Emery et al. 1999; Gammon and Simon 2000), have relied largely on expert knowledge and intuition. However, recently developed protocols call for increasing reliance on empirical relations to select metrics and derive scoring criteria (Barbour et al. 1995; Hughes et al. 1998; Karr and Chu 1999; Angermeier et al. 2000).

Species that are native to great rivers have life history traits that enable them to survive and reproduce in a highly fluctuating environment (Dettmers et al. 2001). Sampling considerations (Simon and Sanders 1999), metric development and testing (Simon 1992; Simon and Emery 1995; Simon and Stahl 1998; Emery et al. 1999), and the variability of index of biotic integrity (IBI) metrics (Gammon and Simon 2000) complicate the assessment of great-river fish assemblages. Reash (1999) cited the distinctive abiotic features and unique biological characteristics of large rivers as factors that complicate metric development for great-river bioassessment. The unique nature of great rivers and the lack of other systems of comparable size hinder development of a reference condition based on a reference site approach (Hughes et al. 1986; Hughes 1995). Recent studies have addressed the development of biological in-

dicators for assessing the condition and ecological health of great rivers (Hickman and McDonough 1996; McDonough and Hickman 1999; Simon and Sanders 1999; Lyons et al. 2001). The purpose of this research was to develop an assessment tool that would detect impairment from known sources of impact and assess the biological condition of the aquatic resources of the main-stem Ohio River. We attempted to include metrics that represented measures of habitat protection, antidegradation, and ecosystem restoration in the Ohio River. We describe three major steps in the development process: (1) defining reference conditions, (2) selecting metrics and analyzing the relationships between these metrics and human impacts on water and substrate quality, and (3) setting metric scoring criteria. We also identify research topics that would enhance index performance.

Methods

Study area.—The Ohio River begins at the confluence of the Monongahela and Allegheny rivers (river kilometer [rkm] 0) and flows southwesterly for 1,578 km through six states into the Mississippi River (Figure 1). The Ohio River crosses four ecoregions (the Western Allegheny Plateau, Interior Plateau, Interior River Lowland, and Mississippi Alluvial Plain [Omernik 1987]). Nearly 10% of the U.S. population, more than 25 million people, resides in the Ohio River basin. The Ohio River has over 600 permitted discharges to its waters under the National Pollutant Discharge and Elimination System, including ones from industry, power generating facilities, and municipalities. Between 1885 and 1927, the Ohio River was impounded by 50 low-head navigation dams (Pearson and Pearson 1989). Currently, 20 high-lift dams provide a 2.75-m minimum depth for commercial navigation, which transports approximately 250 million tons of cargo annually.

Trautman (1981) relates accounts from early settlers along the Ohio River describing abundant shifting sandbars, sandbanks, rock and gravel bars, and bedrock and rock ledges as well as clean bottoms and clear water except during floods. Degradation of the Ohio River occurred initially as a result of logging, agriculture, mining, and sewage effluent (Taylor 1989; Lowman 2000). Water quality in the Ohio River declined between 1810 and 1960 as a result of deforestation, increased agricultural activities, and increases in mining, industrialization, and urban sprawl that led to increases in mean turbidity, total dissolved solids, chlorides, nitrates, and sulfates. Acid mine drainage resulted in degradation of the upper 161 km of the river before 1950 (Pearson and Krumholz 1984). Pearson and Krumholz (1984) and Lowman (2000) documented the decline of pollution-sensitive species and the dominance of pollution-tolerant species.

Site selection.—From 1991 to 2001, the Ohio River Valley Water Sanitation Commission sampled 709 sites along the entire 1,578-km length of the Ohio River. Each 500-m zone incorporated the predominant habitat types within a pool, ranging from shallow, sandy shorelines with no cover to rocky shorelines with a variety of cover types and variable depths. Samples were collected during summer and fall (from early July until late October) when the river was at stable low to moderate flow.

Habitat and water quality data.—Physical habitat data were collected from each 500-m zone. Depth and substrate composition were measured at six longitudinal transects (spaced at 100-m intervals along the shoreline) that were divided into ten 3-m lengths. Visual estimates of the in-channel area containing woody debris (e.g., brush, logs, and stumps), habitat unit (right or left descending bank, inside or outside bend or straight channel), riparian land use and the occurrence and proximity of riparian human disturbances (e.g., roads, buildings, industry, and agriculture), and bank stability were recorded. Water quality data (pH, temperature, dissolved oxygen, conductivity, and Secchi depth) were measured at a single point in each sample area.

Electrofishing.—Fish were collected by nighttime DC boat electrofishing. Sanders (1991) and Simon and Sanders (1999) found that electrofishing success (measured by species richness and abundance) was greater at night than during the day. Electrofishing was conducted on a single shoreline over a linear distance of 500 m using a serpentine travel route within the zone to incorporate all available habitat types (Gammon 1998; Simon and Sanders 1999). Simon and Sanders (1999) found that 500 m was long enough to capture sufficient numbers of species to characterize biological integrity but not biological diversity. Fish were collected in 709 site visits using a Smith-Root Type 6A (350-V, 8-A) electrofishing unit deployed on a 5.5-m johnboat. Amperage was maintained by varying the pulse width according to individual site conditions. We varied the pulse width to obtain an 8-A output for at least 1,500 s. Because boat electrofishing was most effective when employed within 30 m of the shoreline (i.e.,

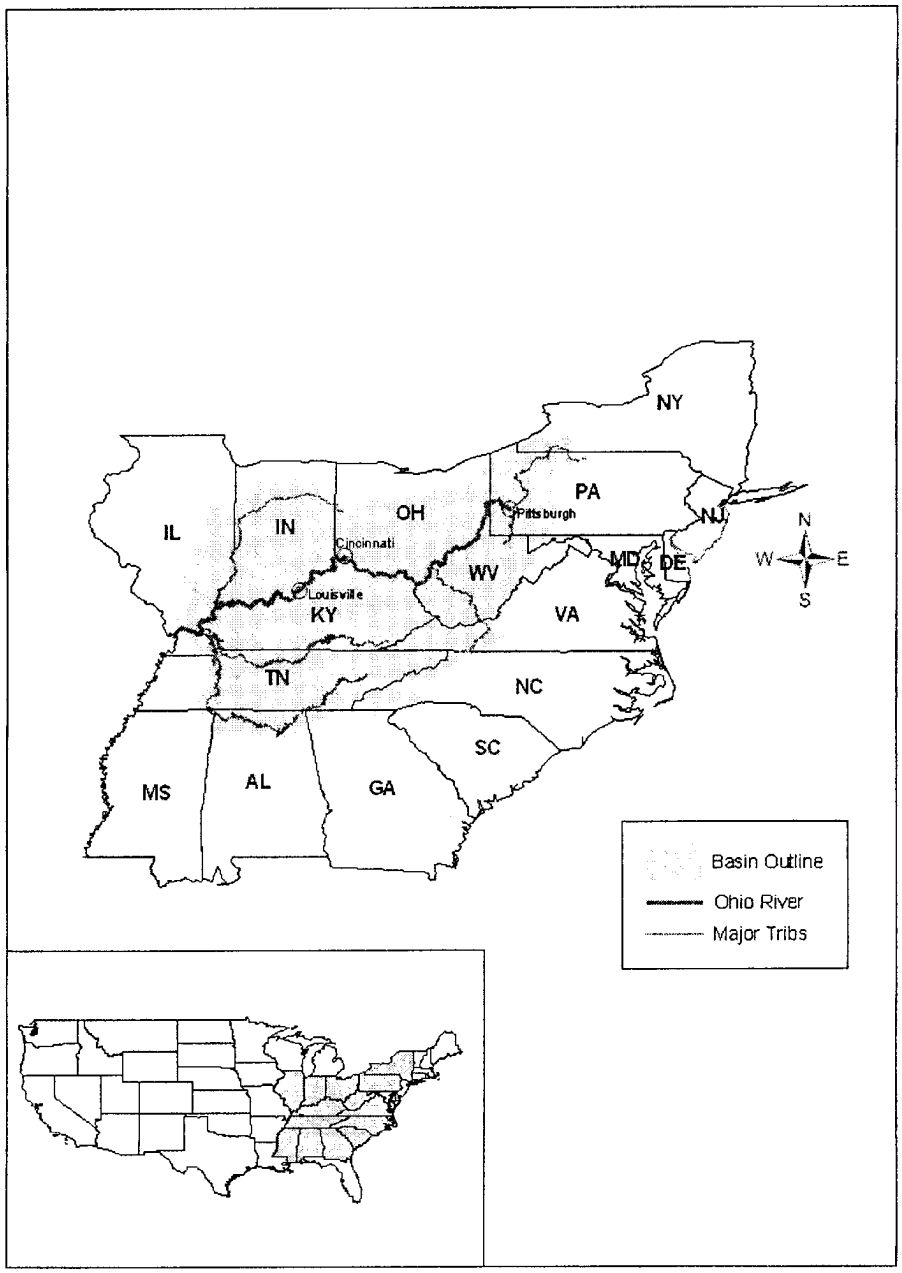

FIGURE 1.—Map of the main-stem Ohio River (dark line) and its tributaries.

at depths less than 4 m), sampling was conducted only under stable, low-flow conditions at a stage level within 1 m of "normal flat pool" and when Secchi depths were at least 0.3 m. Every attempt was made to capture all observed fish using 6.35-mm-mesh nets; captured fish were placed in an onboard holding tank for later processing. The mesh size of the nets was selected to avoid capturing young-of-year individuals; if captured, individuals less than 20 mm (standard length) were not identified. At the conclusion of site sampling, fish were identified to species, counted, and inspected for deformities, eroded fins and barbels, lesions, and tumors (DELT anomalies; Sanders et al. 1999). All fish were released except for small species (e.g., minnows [Cyprinidae], darters *Eth-*

TABLE 1.—Metrics rejected in the evaluation process, by reason for rejection. Lists 1 and 2 comprise groups of species created for test purposes; see text for descriptions of other species groups. The acronym OEPA is for the Ohio Environmental Protection Agency.

Failed range test	Failed redundancy test	Failed responsiveness test
Number of darter species	Number of species	Catch per unit effort (species; list 1)
Number of minnow species	Number of bass and crappie species	Catch per unit effort (species; list 2)
Proportion of great-river species (biomass)	Number of sunfish species excluding basses	Proportion of great-river species
Number of hybrids	Proportion of hybrids	Proportion of large-river species
Proportion of sensitive species	Number of round-bodied suckers	Proportion of round-bodied suckers (biomass)
Proportion of fish with DELT anomalies[a]	Proportion of round-bodied suckers (number)	Proportion of deep-bodied suckers (numbers)
	Proportion of round-bodied suckers (species)	Proportion deep-bodied suckers (biomass)
	Number of deep-bodied sucker species	Proportion of sucker biomass
	Proportion of green sunfish	Number of sensitive species
	Proportion of intolerant species	Proportion of tolerant species (list 2)
	Proportion of nonnative individuals	Proportion of tolerant species (list 1; biomass)
	Proportion of omnivores (biomass; OEPA)	Proportion of tolerant species (list 2; biomass)
	Proportion of omnivores (biomass; new list)	Proportion of insectivores (OEPA)
	Proportion of omnivores (new list)	Proportion of tolerant species (OEPA)
	Proportion of omnivores (OEPA)	Proportion of top piscivores (list 1)
	Number of catfish and sucker species	Proportion of carnivores (OEPA)
	Number of piscivores (list 1)	
	Number of piscivores (list 2)	
	Number of piscivore species (list 1)	
	Number of piscivore species (list 2)	

[a] Deformities, eroded fins and barbels, lesions, and tumors.

eostoma and *Percina* spp., and madtoms *Noturus* spp.), which were retained for laboratory identification using regional fish references (Trautman 1981; Etnier and Starnes 1993; Jenkins and Burkhead 1994; Simon 1999a).

Reference data set.—With its long history of flow alteration and water quality impairment, the Ohio River lacks reference sites representative of pristine conditions. In adopting criteria reflective of the least-impacted conditions, we recognized that most of the changes to the Ohio River are permanent alterations of the system (i.e., hydrologic and channel modifications associated with dams; Ward and Stanford 1989). Metric scoring was conducted on a data set of 318 least-impacted sites. We selected these sites according to the following criteria: (1) they were at least 1 km upstream or downstream from the restricted areas in the vicinity of navigational dams; (2) they were at least 1.61 km downstream from any point source discharge; and (3) they were at least 500 m from any tributary mouth. We eliminated sites with other sources of disturbance in the electrofishing zone (e.g., barge fleeting operations, boating activity, docks or mooring sites, and artificial structures such as pipes or other metal debris in the water). Of the 709 sites sampled, 391 failed to meet the criteria for least-impacted condition and were retained as test sites for metric calibration to evaluate metric response.

Metric selection.—All species collected were classified into various taxonomic, tolerance, feeding, and reproductive guilds (Appendix 1) using regional references (Trautman 1981; Etnier and Starnes 1993; Jenkins and Burkhead 1994; Simon 1999a) and consultation with professional ichthyologists and fisheries biologists. We developed a set of 55 candidate metrics incorporating the original metrics described by Karr (1981), modifications suggested by Miller et al. (1988), the Ohio Environmental Protection Agency (1989), Hughes and Oberdorff (1999), and Emery et al. (1999), and new metrics developed specifically for this study (including various combinations of species that were designated as lists 1–3). The metrics chosen for the Ohio River Fish Index (ORFIn) focus on six areas of fish assemblage structure and function: species richness, pollution tolerance, breeding habits, feeding habits, fish health, and abundance. The metrics were chosen to reflect biological and habitat integrity, trophic complexity, and future restoration and recovery.

The evaluation process followed Hughes et al. (1998) and McCormick et al. (2001) in that we examined each candidate metric for its scoring range, variability, responsiveness, and redundancy. Metrics were rejected (Table 1) if they failed a range test (i.e., if their raw values were between 0 and 2 species or were otherwise too small to provide a range of response to disturbance). We

used Spearman correlations and scatter plots to test the responsiveness of the remaining candidate metrics to physical habitat structure and water quality. We retained metrics with significant correlations ($r > 0.15$; $P < 0.001$) for which scatter plots reflected the predicted responses to physical habitat and water quality variables (Hughes et al. 1998). We tested for redundancy among metrics and rejected one metric of any pair with a high Pearson's correlation ($r > 0.75$). In such cases, we consulted regional fish references, professional ichthyologists, and fisheries biologists and retained the metric more representative of the Ohio River fish assemblage than of other systems. We retained some metrics, such as the number of great-river species (a smaller subset of large-river taxa), the number of DELT anomalies, and percent individuals as nonindigenous species, because we believed that they reflect historical conditions or they constitute important measures of recovery or represent direct measures of individual health or biological pollution. We tested the response of each metric to a multivariate (principal components analysis) axis of disturbance that represented a gradient of abiotic conditions derived from 11 habitat and 5 water quality variables. Repeat sampling was conducted at 8 locations in Markland Pool (rkm 702–855) and 6 locations in Greenup Pool (rkm 450–549) and in a riverwide outfall study at 11 effluent locations (Emery et al. 2002) to assess signal-to-noise ratios.

Scoring procedures.—We performed linear regressions of the species richness metrics on river kilometer, which we used as a surrogate for watershed area (Figure 2). Historical records and surveys showed that 10 species have been extirpated from the Ohio River and many others have declined due to human impacts (Pearson and Krumholz 1984). To account for these historical changes in fish assemblage structure, we used the maximum value for observed species richness (interpreted as the y-intercept) for the maximum observed line (MOL) for scoring species richness metrics instead of the 95th percentile (Fausch et al. 1984). The MOL was drawn through the data and parallel to the regression line. The area below the MOL was evenly trisected into regions providing scores of 1, 3, or 5.

Large numbers of individuals of some schooling species can distort the responsiveness of percentage metrics. Because gizzard shad and emerald shiners can occur unpredictably and in large numbers (Simon and Emery 1995; Simon and Sanders 1999), we excluded them from the calculations of

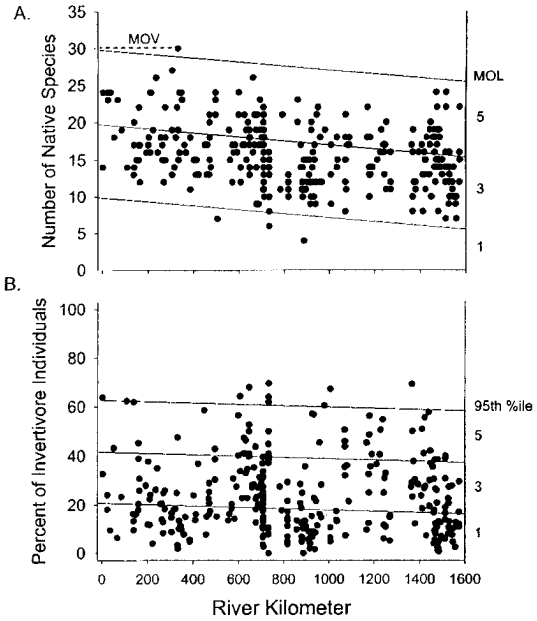

FIGURE 2.—Examples of scoring criteria for the (**A**) richness and (**B**) percentage metrics. The line labeled MOV points to the maximum observed value, which was used as the y-intercept; that labeled MOL represents the maximum observed line drawn parallel to the regression line with river kilometer as the dependent variable. The 95th percentile line in (B) is also parallel to the regression line.

percentile metrics; however, both species are included in species richness metrics. Each percentile metric was scored following the methods described by Fausch et al. (1984). That is, the data for each metric were plotted against river kilometer and a line was drawn at the 95th percentile; the area beneath the line was then trisected into regions representing scores of 1, 3, and 5. In cases where fewer than 50 individuals were collected (after removing gizzard shad, emerald shiners, tolerant fishes, nonindigenous species, and hybrids), all proportional metrics were scored as 1 (Yoder and Rankin 1995). In the event that no individuals in a particular metric category were collected, the metric was scored as 0.

Results

We rejected 6 metrics because they failed our range test, 20 metrics because they were redundant with other metrics, and 16 metrics because they were not responsive to anthropogenic disturbance (Table 1). None of the final metrics selected for consideration failed the signal-to-noise test. We selected 13 metrics, each of which was signifi-

TABLE 2.—Spearman correlations of fish assemblage metrics and Ohio River Fish Index (ORFIn) scores with habitat and water quality variables. Habitat data were available for 166 "least-impacted" sites, but water quality data were available for only 66 sites. All correlations are significant at the 0.0001 level.

Metric and index	Variable				
	Mean depth	% boulder	% cobble	% gravel	% coarse substrate
Native species	0.41	0.43	0.44	0.33	0.43
Intolerant species	0.39	0.49	0.51	0.43	0.57
Sucker species	0.15		19	0.24	0.23
Centrarchid species	0.47	0.29	0.47	0.27	0.41
Great-river species		0.12			
% Piscivores	0.21			−0.27	
% Invertivores	0.23		0.22	−0.27	0.19
% Detritivores				−0.18	−0.22
% Tolerant species	0.19			0.15	0.2
% Lithophils	0.18				0.2
% Nonindigenous species			−0.19		
Number of DELT anomalies[b]		0.14	0.19	0.24	
CPUE[c]				0.19	
ORFIn	0.34	0.17	0.39	0.31	0.43

[a] First principal components axis of abiotic conditions (see text).
[b] Deformities, eroded fins and barbels, lesions, and tumors.
[c] Catch per unit effort.

cantly correlated ($P < 0.0001$, $r > 0.2$) with one or more of the habitat or chemical variables, and from these we calculated the ORFIn (Table 2). In a separate study, Emery et al. (2002) found that native-species richness, intolerant-species richness, sucker species richness, centrarchid species richness, great-river-species richness and the proportions of top piscivores, invertivores, and simple lithophils were lower at outfall sites than at reference sites. The proportion of detritivores, catch per unit effort (CPUE), and the number of DELT anomalies were higher at outfall sites than at reference sites (Emery et al. 2002).

The first principal component axis of abiotic conditions explained 42% of the variability and was strongly and positively correlated with fine substrates ($r = 0.95$) and negatively correlated with depth ($r = -0.59$), coarse substrates ($r = -0.86$ to -0.56), water clarity ($r = -0.4$), and conductivity ($r = -0.3$). Correlations of fish assemblage metrics with the first principal component axis reflected their response to critical habitat features. The number of native, centrarchid, and intolerant species increased in areas with high-quality habitat characterized by greater depth, coarse substrates, and high water clarity (Table 2). Among the proportional metrics, the proportions of simple lithophils, nonindigenous species, invertivores, and piscivores declined and the proportions of detritivores and tolerant species increased with measures of habitat disturbance associated with increased fine sediments and embeddedness (Table 2).

Metric Descriptions

Native-species richness was modified from Karr's (1981) species richness metric. It focuses on native-species diversity (Simon and Lyons 1995; Hughes and Oberdorff 1999) by excluding nonindigenous species and hybrids that indicate a loss of biological integrity. The number of native species decreases with river kilometer as species found primarily in the upper 500 km of the Ohio River disappear downstream. Changes in river geomorphology from a high-gradient, constrained-floodplain system to a low-gradient floodplain system are accompanied by the replacement of round-bodied suckers and other species associated with higher-gradient river systems by a more depauperate fauna (Emery et al. 1999). The number of native species was greater at deeper sites with coarse substrates (cobble, boulder, and gravel) than at shallower sites with more sand and fines and was greater at sites with good water clarity and cooler temperatures and more available cover (Table 2). Native species declined with degraded water quality (Emery et al. 2002) and at sites with abundant sand and fines and highly embedded substrates (Table 2). We expected the number of native species to decline with increased environmental disturbance (Karr 1981; Karr et al. 1986).

The number of intolerant species is intended to

TABLE 2.—Extended.

Variable									
% sand and fines	% highly embedded substrate	% total woody cover	% submerged vegetation	% overhanging vegetation	Secchi depth	Dissolved oxygen	Temperature	Conductivity	PC 1[a]
−0.42	−0.43	0.23	0.28	0.23	0.17		−0.24	0.26	−0.36
−0.56	−0.57		0.24		0.27	0.28	0.18	0.3	−0.53
−0.24	−0.23	0.16	0.16				−0.31	−0.26	
−0.41	−0.41	0.31	0.22	0.23	0.15		−0.27	0.31	−0.34
		0.18					−0.25		
−0.19	−0.42	0.22					−0.25	0.17	
0.22	0.2			0.17	−0.15		0.19		0.29
−0.21	−0.2	0.25		0.22				0.18	
−0.16							−0.34		
−0.24	0.22	0.26			−0.27		−0.16		
−0.26	−0.25				−0.19	−0.21			
	−0.3								
−0.42	−0.43		0.2		0.23	0.21	−0.25	0.22	−0.56

distinguish areas of the highest quality. Species that are especially sensitive to anthropogenic stressors are the first to be eliminated and the last to return to the reach. Only species that are highly sensitive to habitat disturbance, toxins, and thermal and nutrient stressors are included in this metric. Species that are sensitive to only one type of stressor are not included (Appendix 1). Karr et al. (1986) warned that designating too many species as intolerant would prevent this metric from discriminating among the highest-quality areas and recommended that a maximum of 10% of the fauna be included in this classification. Our list contains 22 species, although 3 of these species have not been collected in the river using electrofishing techniques. The total number of intolerant species decreased with river kilometer. The number of intolerant species decreased significantly with degraded water quality (Emery et al. 2002) and at sites with increased sand, fines, and highly embedded substrates (Table 2). This metric reflected the highest levels of biological integrity and was expected to increase with improved water and habitat quality.

The number of sucker (Catostomidae) species was one of the original IBI metrics proposed by Karr et al. (1986) for small streams and rivers. Suckers are a major component of the Ohio River fish fauna (Emery et al. 1999). Round-bodied suckers, such as *Moxostoma*, *Hypentelium*, *Cycleptus*, *Catostomus*, and *Minytrema* spp., are generally sensitive to habitat and water quality degradation (Karr 1981; Trautman 1981; Karr et al. 1986), and their long life span provides a metric influenced by long-term environmental changes (Emery et al. 1999). Decreases in the round-bodied sucker distribution in the lower reaches of the Ohio River suggest that redhorse suckers are not a major component of the structure of the great-river fish assemblage (Emery et al. 1999). In contrast, Emery et al. (1999) reported that the relative abundance and diversity of deep-bodied sucker species, such as *Carpiodes* spp. and *Ictiobus* spp., increased in the lower Ohio River. The number of sucker species was significantly correlated with coarse substrates and the presence of submerged vegetation, woody cover, and conductivity, and negatively correlated with elevated temperature, an abundance of sand and fines, and generally degraded abiotic conditions (Table 2). We expected sucker species to decline with increased disturbance (Karr 1981).

The number of centrarchid species was modified from Karr's (1981) metric (the number of sunfish species) to include the black basses (*Micropterus* spp.), which are the dominant centrarchids in Ohio River pool habitats. The number of centrarchid species did not change significantly with river kilometer. It was greater at deeper sites over coarse substrates and at sites with abundant woody or vegetative cover and lower at shallower sites with more sand, fines, or highly embedded substrates (Table 2). Centrarchid species richness declined with increased turbidity and water temperature. This metric should decline with the degradation of pool habitat.

The number of great-river species represents the fish species that are expected to predominate in

great rivers (Pflieger 1971; Simon 1992; Simon and Emery 1995) and to decline with the loss of associated floodplain habitat (Appendix 1). Great-river species have declined in the Ohio River because of hydrologic modification and poor water quality (Pearson and Krumholz 1984; Pearson and Pearson 1989; Poff et al. 1997). The number of great-river species was not strongly correlated with any abiotic variables (Table 2) but was retained because it expresses historical conditions in the river. We expected that the number of great-river species would increase with improvements in water quality and restoration of floodplain habitats.

Percent top piscivores was modified from Karr's (1981) percent top carnivore metric. Top piscivores represent the top of the aquatic food web and should be those that no other fishes feed on. We selected only species that feed exclusively on vertebrates or crayfish as adults (Appendix 1). Species that switch among prey items during ontogeny (e.g., smallmouth bass) are included, but adult species that eat both macroinvertebrates and fish (e.g., green sunfish) were excluded. The percentage of top piscivores in the Ohio River increased slightly with river kilometer. It also increased with increased depth and woody cover but declined with increased water temperature (Table 2). We expected the percentage of top piscivores to decrease with habitat degradation in the absence of any intensive stocking program.

Percent invertivores was modified from Karr's (1981) proportion of cyprinid insectivores metric to measure the proportion of specialized sight feeders in the assemblage (Goldstein and Simon 1999; Appendix 1). A scarcity of insectivorous fish species may reflect a disturbance that has reduced the production of benthic insects. The proportion of invertivores ranged from 0% to 100% and decreased with river kilometer. It was higher at deeper sites with coarse substrates (cobble) and lower at sites with more sand and fines and higher temperature (Table 2). We expected the percentage of invertivores to decline with increased disturbance.

Percent detritivores replaced the percent omnivores metric of Karr et al. (1986) because the original metric did not discriminate between species that switched between food types or were behaviorally plastic in feeding ecology as a result of disturbance (Goldstein and Simon 1999). The percentage of detritivores increased with increasing proportions of sand and fine substrates and higher water temperature (Table 2). The percentage of detritivores should have increased as habitat quality declined and the abundance of ultrafine-particulate organic matter increased.

Percent tolerant individuals is meant to represent the worst conditions in the Ohio River prior to the implementation of the Clean Water Act of 1972. Historical lock chamber data (Lowman 2000; Emery et al. 2002) revealed fish assemblage patterns associated with widespread water quality degradation that are still seen in the most impaired areas of the river. Tolerant species are becoming increasingly scarce as the impacts of degradation become more localized, allowing riverwide recolonization by more-sensitive species (Emery et al. 1999). The percentage of tolerant individuals increased with degraded water quality (increased turbidity and low dissolved oxygen; Table 2). We expected the percentage of tolerant individuals to increase with increased disturbance.

Percent simple lithophils represents the reproductive guilds that are sensitive to substrate disturbance and degradation (Ohio Environmental Protection Agency 1989; Simon 1999b). Simple lithophils decreased with river kilometer, presumably for lack of habitat given that coarse substrates become less common in the lower segments of the river. Emery et al. (1999) related the decrease to the absence of redhorse species in the lower river. As expected, the percentage of simple lithophils declined with increased sand and fine substrates (Table 2). They also declined with increased temperature. We expected the percentage of simple lithophils to decrease with the loss of clean substrates for spawning.

Percent nonindigenous individuals measures the degree to which nonindigenous species and hybrids have reduced biological integrity in the Ohio River. Many nonindigenous species increase at degraded sites because the behavioral and ecological mechanisms of species segregation are disrupted (Courtenay and Stauffer 1984; Fuller et al. 2000). The percentage of nonindigenous species was significantly correlated with increased turbidity (Table 2). We retained this metric to document the increasing impacts of nonindigenous and hybrid species in the Ohio River.

The number of DELT anomalies measures the effects of contaminants, diet, and overcrowding (Sanders et al. 1999). We chose the number rather than the percentage of such anomalies (which the Ohio Environmental Protection Agency employs) because of the greater number of individuals captured at great-river sites and the scarcity of DELT anomalies observed. Karr (1981) considered a high proportion of disease to be a reflection of the low-

TABLE 3.—Scoring criteria based on the maximum observed line adjusted for river kilometer (rkm) or the actual value of the unscored metric. For each metric, the letter "X" represents the actual recorded value for that metric.

Metric	Score 1	Score 3	Score 5
Number of species	$X \leq (-0.0046 \cdot (\text{rkm}) + 48.28) \cdot 0.33$	$(-0.0046 \cdot (\text{rkm}) + 48.28) \cdot 0.33 < X < (-0.0046 \cdot (\text{rkm}) + 48.28) \cdot 0.66$	$X \geq (-0.0046 \cdot (\text{rkm}) + 48.28) \cdot 0.66$
Number of sucker species	$X \leq (-0.0035 \cdot (\text{rkm}) + 14.48) \cdot 0.33$	$(-0.0035 \cdot (\text{rkm}) + 14.48) \cdot 0.33 < X < (-0.0035 \cdot (\text{rkm}) + 14.48) \cdot 0.66$	$X \geq (-0.0035 \cdot (\text{rkm}) + 14.48) \cdot 0.66$
Number of centrarchid species	$X < 3$	$3 \leq X < 6$	$X \geq 6$
Number of great-river species	$X < 2$	$2 \leq X \leq 3$	$X > 3$
Number of intolerant species	$X \leq (-0.004 \cdot (\text{rkm}) + 12.87) \cdot 0.33$	$(-0.004 \cdot (\text{rkm}) + 12.87) \cdot 0.33 < X < (-0.004 \cdot (\text{rkm}) + 12.87) \cdot 0.66$	$X \geq (-0.004 \cdot (\text{rkm}) + 12.87) \cdot 0.66$
% Tolerant individuals	$X > 6.66$	$3.33 < X \leq 6.66$	$X \leq 3.33$
% Simple lithophilic individuals	$X \leq (-0.0237 \cdot (\text{rkm}) + 105.09) \cdot 0.33$	$(-0.0237 \cdot (\text{rkm}) + 105.09) \cdot 0.33 < X < (-0.0237 \cdot (\text{rkm}) + 105.09) \cdot 0.66$	$X \geq (-0.0237 \cdot (\text{rkm}) + 105.09) \cdot 0.66$
% Nonnative individuals	$X > 8.58$	$4.3 < X \leq 8.58$	$X \leq 4.3$
% Detritivorous individuals	$X \geq (-0.006 \cdot (\text{rkm}) + 51.49) \cdot 0.66$	$(-0.006 \cdot (\text{rkm}) + 51.49) \cdot 0.33 < X < (-0.006 \cdot (\text{rkm}) + 51.49) \cdot 0.66$	$X \leq (-0.006 \cdot (\text{rkm}) + 51.49) \cdot 0.33$
% Invertivorous individuals	$X \leq (-0.0335 \cdot (\text{rkm}) + 138.4) \cdot 0.33$	$(-0.0335 \cdot (\text{rkm}) + 138.4) \cdot 0.33 < X < (-0.0335 \cdot (\text{rkm}) + 138.4) \cdot 0.66$	$X \geq (-0.0335 \cdot (\text{rkm}) + 138.4) \cdot 0.66$
% Piscivorous individuals	$X \leq (-0.0047 \cdot (\text{rkm}) + 96.56) \cdot 0.33$	$(-0.0047 \cdot (\text{rkm}) + 96.56) \cdot 0.33 < X < (-0.0047 \cdot (\text{rkm}) + 96.56) \cdot 0.66$	$X \geq (-0.0047 \cdot (\text{rkm}) + 96.56) \cdot 0.66$
Number of DELT anomalies	$X \geq 4$	$2 \leq X < 4$	$X < 2$
CPUE	$X \leq (-0.018 \cdot (\text{rkm}) + 740.29) \cdot 0.33$	$(-0.018 \cdot (\text{rkm}) + 740.29) \cdot 0.33 < X < (-0.018 \cdot (\text{rkm}) + 740.29) \cdot 0.66$	$X \geq (-0.018 \cdot (\text{rkm}) + 740.29) \cdot 0.66$

est extreme in biological integrity. These anomalies are absent or occur infrequently in areas with high water quality, but their occurrence increases at impacted sites (Mills et al. 1993; Baumann et al. 1987; Ohio Environmental Protection Agency 1989; Sanders et al. 1999). We expected low levels of DELT anomalies because of improvements in water quality since the 1970s (Emery et al. 1999). Despite the rarity of DELT anomalies, we retained this metric to capture any future degradation or impacts specifically associated with point- and non-point-source pollution. The number of DELT anomalies increased with increased turbidity and at sites with low dissolved oxygen (Table 2).

Our CPUE metric, namely, that for species list 3, was modified from Karr's (1981) number of individuals metric. The number of fish is a measure of community productivity. However, because it is difficult to obtain a quantitative measure of fish abundance in open systems such as the Ohio River, we employ CPUE for a standard sampling technique. We believe that an increase in abundance reflects greater biological integrity, although nutrient inputs often exaggerate the productivity of the reach by causing an increase in abundance. Specific taxa often respond in a predictable manner to this type of stimulation. These increases have been accounted for in our CPUE metric by removing the species designated as tolerant, nonindigenous, and hybrids (Appendix 1).

Index Scoring and Responsiveness

We generated the scoring calculations for each of the 13 metrics (Table 3). Metrics that were significantly correlated with river kilometer were adjusted by the regression equations for those metrics. The sum of the scores of the 13 metrics resulted in ORFIn scores that ranged from 7 to 59 (mean ± SD, 30.4 ± 11.8). The potential range is 0–65. The ORFIn scores from nonoutfall sites were significantly higher than those from sites within the first 500 m of point source of chemical, thermal, and wastewater effluents (analysis of variance [ANOVA]: $F = 8.127$; $P < 0.05$; Figure 3). The mean ORFIn scores showed a pattern of recovery over a distance of 300 m downstream (methods described in Emery and Thomas 2002). The ORFIn scores were lowest at shallow sites with sand and fine substrates (ANOVA; $P < 0.05$) and highest at deeper sites with coarse substrates, clear water, and cooler temperatures (Table 2; Figure 4).

Discussion

Because they exhibit diverse morphological, ecological, behavioral, and evolutionary adaptations to their natural habitat, fish species are particularly effective indicators of the condition of aquatic systems (Karr et al. 1986; Fausch et al. 1990; Simon and Lyons 1995). Human disturbance of streams and landscapes alters key attributes of

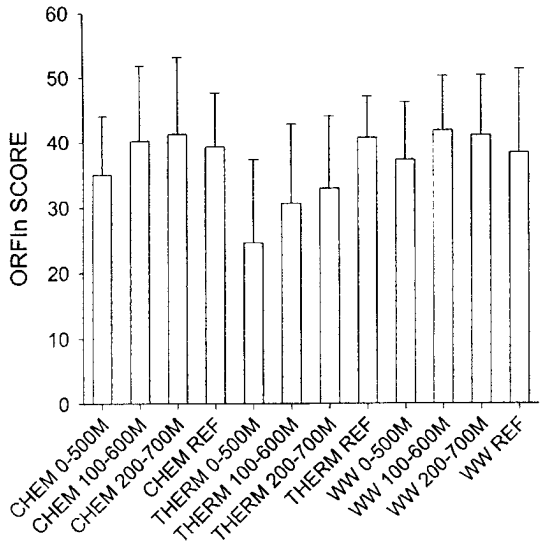

FIGURE 3.—Mean Ohio River Fish Index (ORFIn) scores (+SD) for three overlapping 500-m electrofishing zones affected by chemical (CHEM), thermal (THERM), or wastewater (WW) point source discharges and control sites (REF) not affected by point source discharges.

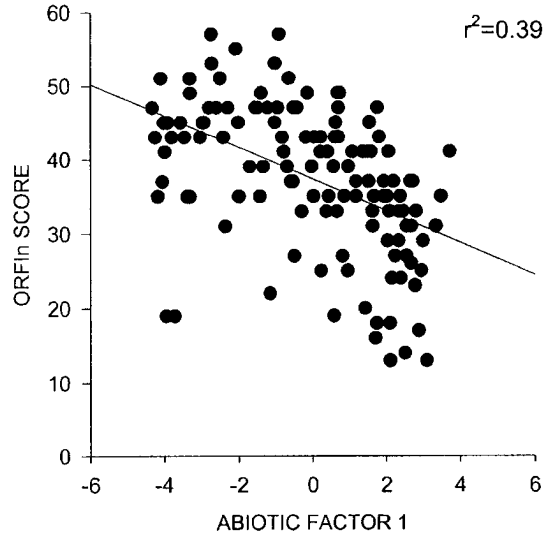

FIGURE 4.—Regression of ORFIn scores on a multivariate axis of abiotic variables ($P < 0.001$). Sites on the left (negative) side of the x-axis have better water quality and physical habitat conditions (i.e., they are deeper and have coarser substrates, lower turbidity, and higher dissolved oxygen) than sites on the right (positive) side of the axis.

aquatic ecosystems, namely, water quality, habitat structure, hydrological regime, energy flow, and biological interactions (Karr and Dudley 1981). We were able to identify fish assemblage variables that were strongly correlated with degraded substrate quality and water quality variables that reflected anthropogenic disturbance. In our analyses, the strongest correlations between ORFIn metrics and environmental variables were with those measures that described the heterogeneity of depth, substrate quality, dissolved oxygen, and temperature. Nine metrics that we expected to be sensitive to disturbance decreased with degraded substrate quality. Three metrics that we expected to be relatively insensitive to disturbance increased with increased pH and turbidity. Seven metrics decreased as disturbance (measured by a multivariate axis of substrate and water quality) increased. The resulting IBI for the Ohio River was significantly correlated with an aggregate (multivariate) measure of habitat quality that represented different types and intensities of anthropogenic disturbance.

This approach may be applied to other large rivers, particularly those that have comparable evolutionary histories (i.e., large Midwestern rivers) and similar fish assemblages. The identification of least-impacted sites, particularly the incorporation of a criterion for a minimum distance from point source discharges and hydrologic modifications, should be transferable to any large river system. The assemblage classifications may differ because of local adaptations of fish assemblages to prevailing natural conditions. However, researchers developing multimetric indices of biotic integrity may elect to adopt metrics that reflect past conditions (e.g., the percentage of tolerant individuals), metrics that are likely to respond to future water quality improvement (e.g., the number of intolerant species) or degradation (e.g., the percentage of tolerant individuals and the number of DELT anomalies), or metrics that are likely to reflect ecosystem restoration (e.g., the number of great-river species).

Additional efforts to assess the nutrient loadings or trophic status of the Ohio River and to relate changes in land use to conditions in the Ohio River and trends in water quality to changes in the fish assemblage could provide a more defensible way to define least-impacted conditions. We could not test the response of ORFIn metrics to nutrient loading because we lacked the data to assess the relationship between nutrient chemistry and fish assemblages. However, we did find that ORFIn scores increased with increasing distance from point sources associated with municipal wastewater treatment plants. While these results are consistent with those of Karr et al. (1985b), we cannot

directly attribute the decline in ORFIn scores to a particular constituent of the effluent. Comparison of the ORFIn results with those of the modified Index of Well Being (Ohio Environmental Protection Agency 1989) may be used to indirectly assess the responses of fish assemblages to nutrient loading.

Many great-river systems have been hydrologically modified, leading to physicochemical and biotic alterations (Ward and Stanford 1989; Ligon et al. 1995; Poff et al. 1997). Water quality degradation as a result of point- and non-point-source pollution further impacts the ecological integrity of large rivers such as the Ohio (Sparks et al. 1990; Bayley 1995). Clearly, the lack of reference sites representing minimally disturbed conditions affected the metric selection and calibration process. The impoundment of the Ohio River has interrupted the abiotic processes (erosion, sedimentation, and floodplain inundation) and biotic processes (colonization and succession from refugia) that enable it to maintain and restore itself (Gore and Shields 1995; Ligon et al. 1995; Sparks 1995; Poff et al. 1997). Such alterations tend to reduce the abundance and diversity of fishes (Schlosser 1991; Ligon et al. 1995). Loss of biological diversity as a result of the introduction of nonindigenous species (Courtenay and Stauffer 1984), loss of endangered and threatened species (Carlson and Muth 1989), habitat fragmentation (Dynesius and Nilsson 1994; Ward and Stanford 1995; Pringle 1997; Pringle et al. 2000), and declining genetic diversity (Nehlsen et al. 1991) have imperiled the aquatic assemblages of great rivers. However, despite the pervasive and persistent disturbance of the Ohio River by these factors, we were able to identify least-impacted sites that had little evidence of poor water quality or degraded habitat and to verify their status with the ORFIn. The relationship of the ORFIn to habitat variables suggests the need to include calibration of the ORFIn scores with specific habitat classes. Such modifications should improve the ability of the ORFIn to detect water quality impairment.

This research describes an approach for determining least-impacted conditions in the Ohio River and provides a set of fish assemblage metrics that may be applied to the development of IBIs for other great-river systems. By selecting sites that were not immediately influenced by the hydrologic modifications of dams or by point source discharges, we minimized the impacts of human disturbance on our selected sampling reaches. We developed fish assemblage metrics that represent the diversity of native-fish assemblages, preimpoundment conditions, and the impacts associated with the introduction of nonindigenous species as well as important elements of food web structure.

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Appendix: Guild Assignments for Fish Assemblages

TABLE A.1.—Guild assignments for fish assemblages used in metric development for the Ohio River Fish Index. The abbreviation GRS stands for great-river species. Trophic categories are detritivore (D), invertivore (I), and piscivore (P). Reproductive guild designates whether species are simple lithophils (SL) or not. The list includes species collected by electrofishing on the Ohio River since 1991 along with species deemed important based on the possibility of their occurrence in future collections. Species assignments were made by consulting regional fish references as well as professional ichthyologists and fisheries biologists.

Species	Family	GRS	Tolerance	Trophic category	Reproductive guild	Alien
Ohio lamprey *Ichthyomyzon bdellium*	Petromyzontidae		Intolerant			
Chestnut lamprey *I. castaneus*						
Silver lamprey *I. unicuspis*		X				
Lake sturgeon *Acipenser fulvescens*	Acipenseridae	X		I	SL	
Shovelnose sturgeon *Scaphirhynchus platorynchus*		X		I	SL	
Paddlefish *Polyodon spathula*	Polyodontidae	X	Intolerant		SL	
Spotted gar *Lepisosteus oculatus*	Lepisosteidae			P		
Longnose gar *L. osseus*				P		
Shortnose gar *L. platostomus*		X		P		
Alligator gar *L. spatula*		X		P		
Bowfin *Amia calva*	Amiidae			P		
Goldeye *Hiodon alosoides*	Hiodontidae	X	Intolerant			
Mooneye *H. tergisus*		X	Intolerant			
American eel *Anguilla rostrata*	Anguillidae	X				
Skipjack herring *Alosa chrysochloris*	Clupeidae	X		P		
Alewife *A. pseudoharengus*					X	
Gizzard shad *Dorosoma cepedianum*				D		
Central stoneroller *Campostoma anomalum*	Cyprinidae					
Goldfish *Carassius auratus*			Tolerant	D		X
Grass carp *Ctenopharyngodon idella*			Tolerant			X
Red shiner *Cyprinella lutrensis*			Tolerant			X
Spotfin shiner *C. spiloptera*						
Steelcolor shiner *C. whipplei*				I		
Common carp *Cyprinus carpio*			Tolerant	D		X
Cypress minnow *Hybognathus hayi*						
Mississippi silvery minnow *H. nuchalis*		X				
Bighead carp *Hypophthalmichthys nobilis*			Tolerant			X
Striped shiner *Luxilus chrysocephalus*				I		
Speckled chub *Macrhybopsis aestivalis*		X		I		
Silver chub *M. storeriana*		X		I	SL	
Hornyhead chub *Nocomis biguttatus*				I		
River chub *N. micropogon*						
Golden shiner *Notemigonus crysoleucas*			Tolerant			
Bigeye chub *Notropis amblops*			Intolerant	I	SL	
Emerald shiner *N. atherinoides*				I		
River shiner *N. blennius*		X		I	SL	
Silverjaw minnow *N. buccatus*				I		
Ghost shiner *N. buchanani*		X		I		
Spottail shiner *N. hudsonius*				I		

TABLE A.1.—Continued.

Species	Family	GRS	Tolerance	Trophic category	Reproductive guild	Alien
Silver shiner *N. photogenis*						
Rosyface shiner *N. rubellus*			Intolerant	I		
Silverband shiner *N. shumardi*						
Sand shiner *N. stramineus*			Intolerant			
Mimic shiner *N. volucellus*			Intolerant	I		
Channel shiner *N. wickliffi*		X				
Suckermouth minnow *Phenacobius mirabilis*				I		
Bluntnose minnow *Pimephales notatus*			Tolerant	D		
Fathead minnow *P. promelas*			Tolerant	D		
Bullhead minnow *P. vigilax*						
Blacknose dace *Rhinichthys atratulus*					SL	
River carpsucker *Carpiodes carpio*	Catostomidae			D		
Quillback *C. cyprinus*				D		
Highfin carpsucker *C. velifer*				D		
White sucker *Catostomus commersoni*			Tolerant	I/D	SL	
Blue sucker *Cycleptus elongatus*		X	Intolerant	I	SL	
Northern hog sucker *Hypentelium nigricans*			Intolerant	I	SL	
Smallmouth buffalo *Ictiobus bubalus*				D		
Bigmouth buffalo *I. cyprinellus*				D		
Black buffalo *I. niger*				D		
Spotted sucker *Minytrema melanops*				I	SL	
Silver redhorse *Moxostoma anisurum*				I	SL	
River redhorse *M. carinatum*			Intolerant	I	SL	
Black redhorse *M. duquesnei*			Intolerant	I	SL	
Golden redhorse *M. erythrurum*				I	SL	
Shorthead redhorse *M. macrolepidotum*			Intolerant	I	SL	
Grass Pickerel *Esox americanus vermiculatus*	Esocidae			P		
Northern pike *E. lucius*				P		
Muskellunge *E. masquinongy*				P		
White catfish *Ameiurus catus*	Ictaluridae					X
Black bullhead *A. melas*			Tolerant			
Yellow bullhead *A. natalis*			Tolerant			
Brown bullhead *A. nebulosus*			Tolerant			
Blue catfish *Ictalurus furcatus*		X				
Channel catfish *I. punctatus*						
Mountain madtom *Noturus eleutherus*				I		
Slender madtom *N. exilis*				I		
Stonecat *N. flavus*			Intolerant	I		
Tadpole madtom *N. gyrinus*				I		
Brindled madtom *N. miurus*				I		
Freckled madtom *N. nocturus*				I		
Northern madtom *N. stigmosus*				I		
Flathead catfish *Pylodictis olivaris*				P		
Trout perch *Percopsis omiscomaycus*	Percopsidae			I		
Pirate perch *Aphredoderus sayanus*	Aphredoderidae			I		
Banded killifish *Fundulus diaphanus*	Fundulidae			I		X
Blackstripe topminnow *F. notatus*				I		
Western mosquitofish *Gambusia affinis*	Poeciliidae			I		
Brook silverside *Labidesthes sicculus*	Atherinidae			I		
Inland silverside *Menidia beryllina*						X
White perch *Morone americana*	Percichthyidae			P		
White bass *M. chrysops*				P		
Yellow bass *M. mississippiensis*			Intolerant	P		
Striped bass *M. saxatilis*				P		X
Rock bass *Ambloplites rupestris*	Centrarchidae			P		
Green sunfish *Lepomis cyanellus*			Tolerant	I		
Pumpkinseed *L. gibbosus*				I		
Warmouth *L. gulosus*				I		
Orangespotted sunfish *L. humilis*				I		
Bluegill *L. macrochirus*				I		
Longear sunfish *L. megalotis*				I		
Redear sunfish *L. microlophus*				I		X
Smallmouth bass *Micropterus dolomieu*			Intolerant	P		
Spotted bass *M. punctulatus*				P		

TABLE A.1.—Continued.

Species	Family	GRS	Tolerance	Trophic category	Reproductive guild	Alien
Largemouth bass *M. salmoides*				P		
White crappie *Pomoxis annularis*				P		
Black crappie *P. nigromaculatus*				I		
Crystal darter *Ammocrypta asprella*	Percidae	X		I		
Eastern sand darter *A. pellucida*				I	SL	
Mud darter *Etheostoma asprigene*				I		
Greenside darter *E. blennioides*			Intolerant	I		
Rainbow darter *E. caeruleum*				I	SL	
Bluebreast darter *E. camurum*			Intolerant	I		
Bluntnose darter *E. chlorosoma*				I		
Fantail darter *E. flabellare*				I		
Johnny darter *E. nigrum*				I		
Orangethroat darter *E. spectabile*				I	SL	
Variegate darter *E. variatum*			Intolerant	I		
Banded darter *E. zonale*			Intolerant	I		
Yellow perch *Perca flavescens*						
Logperch *Percina caprodes*			Intolerant	I	SL	
Channel darter *P. copelandi*		X	Intolerant	I	SL	
Blackside darter *P. maculata*				I	SL	
Slenderhead darter *P. phoxocephala*			Intolerant	I	SL	
Duskey darter *P. sciera*			Intolerant	I	SL	
River darter *P. shumardi*		X		I	SL	
Sauger *Stizostedion canadense*				P	SL	
Walleye *S. vitreum*				P	SL	
Freshwater drum *Aplodinotus grunniens*	Sciaenidae					
Striped mullet *Mugil cephalus*	Mugilidae					X