# Single-Pass versus Two-Pass Boat Electrofishing for Characterizing River Fish Assemblages: Species Richness Estimates and Sampling Distance 

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#### Abstract

Determining adequate sampling effort for characterizing fish assemblage structure in nonwadeable rivers remains a critical issue in river biomonitoring. Two-pass boat electrofishing data collected from 500-1,000-m-long river reaches as part of the U.S. Geological Survey's National Water-Quality Assessment (NAWQA) Program were analyzed to assess the efficacy of singlepass boat electrofishing. True fish species richness was estimated by use of a two-pass removal model and nonparametric jackknife estimation for 157 sampled reaches across the United States. Compared with estimates made with a relatively unbiased nonparametric estimator, estimates of true species richness based on the removal model may be biased, particularly when true species richness is greater than 10 . Based on jackknife estimation, the mean percent of estimated true species richness collected in the first electrofishing pass ( $\hat{p}_{j, s_{1}}$ ) for all 157 reaches was $65.5 \%$. The effectiveness of single-pass boat electrofishing may be greatest when the expected species richness is relatively low ( $>10$ species). The second pass produced additional species (1-13) in $89.2 \%$ of sampled reaches. Of these additional species, centrarchids were collected in $50.3 \%$ of reaches and cyprinids were collected in $45.9 \%$ of reaches. Examination of relations between channel width ratio (reach length divided by wetted channel width) and $\hat{p}_{j, s}$ values provided no clear recommendation for sampling distances based on channel width ratios. Increasing sampling effort through an extension of the sampled reach distance can increase the percent species richness obtained from single-pass boat electrofishing. When single-pass boat electrofishing is used to characterize fish assemblage structure, determination of the sampling distance should take into account such factors as species richness and patchiness, the presence of species with relatively low probabilities of detection, and human alterations to the channel.


Compared with the biological assessment of wadeable streams, assessment of nonwadeable riverine fish assemblages has lagged (Reash 1999). Criticism of the development of assessment techniques for nonwadeable rivers has largely focused on issues concerning stability and consistency of sampling (Simon and Sanders 1999). Among the complex sampling issues to be considered is how to determine the appropriate level of sampling effort. Angermeier and Smogor (1995) noted that determination of the appropriate level of sampling effort needed to characterize fish species richness is difficult. Too little sampling may negatively influence the reliability of conclusions, whereas too much sampling may be unnecessarily expensive. A thorough sampling effort occurs when measurement of the attribute of interest (such as species richness) approaches an asymptotic level and additional sampling yields comparatively little new information (Lyons 1992; Paller 1995).

Determination of the appropriate level of sam-

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pling effort in nonwadeable rivers is confounded by the consideration of study purpose and objectives and the size of the area to be sampled. Though the sampling method chosen for nonwadeable rivers is often boat electrofishing, the sampling volume can range from any navigable river (regardless of channel width or drainage area) to surface waters described as "large" rivers (drainage area $=2,590-5,180 \mathrm{~km}^{2}$ ) or "great" rivers ( $>5,180$ $\mathrm{km}^{2}$; Simon 1992).
Although standardized sampling of fish assemblage structure on a large geographic scale has many benefits (Bonar and Hubert 2002), protocols for nonwadeable sampling effort vary. In some cases, sampling is conducted for a fixed distance, whereas in other cases sampling is conducted for a distance proportional to channel width. Gammon (1976) and Yoder and Smith (1999) indicated that a single boat-electrofishing pass for a reach length of 500 m was sufficient to give consistent data for assessment of fish assemblage structure. Lyons et al. (2001) suggested that asymptotic species richness, or about $95 \%$ of "true" species richness, could be achieved with a single electrofishing pass along a $1,600-\mathrm{m}(1 \mathrm{mi})$ reach for warmwater rivers

Table 1.-List of the nine U.S. Geological Survey National Water-Quality Assessment (NAWQA) Program study units and sampling reach characteristics that were included in the present study. Channel width ratio equals reach length divided by wetted channel width.

| NAWQA study unit name | Study unit <br> abbreviation | Number of <br> reaches | Mean reach <br> length $(\mathrm{m})$ | Mean wetted <br> channel <br> width $(\mathrm{m})$ | Mean <br> channel <br> width ratio |
| :--- | :--- | :---: | :---: | :---: | :---: |
| Albemarle-Pamlico drainage basin | ALBE | 6 | 575.0 | 25.9 | 22.9 |
| Mississippi embayment | MISE | 56 | 495.5 | 33.9 | 21.6 |
| Red River of the North | REDN | 11 | 432.4 | 41.5 | 12.3 |
| Rio Grande Valley | RIOG | 7 | 329.6 | 61.5 | 5.4 |
| Sacramento River basin | SACR | 5 | 500.0 | 79.1 | 8.6 |
| San Joaquin-Tulare River basins | SANJ | 7 | 466.7 | 35.7 | 13.9 |
| Trinity River basin | TRIN | 16 | 951.1 | 60.8 | 15.7 |
| Upper Colorado River basin | UCOL | 8 | 583.3 | 79.5 | 7.0 |
| Upper Mississippi River basin | UMIS | 41 | 819.5 | 115.5 | 10.2 |
| Combined |  | 157 | 565.8 | 56.3 | 15.3 |

in Wisconsin. Hughes et al. (2002) reported that a single electrofishing pass along a distance equal to 85 times the channel width for Oregon rivers that were 7-210 m wide (reach lengths of 595$17,850 \mathrm{~m}$ ) could produce $95 \%$ of total species richness in $75 \%$ of the samples collected. Thus, considerable debate remains regarding the sampling distance required to characterize fish assemblage structure in nonwadeable rivers.

Determination of the sampling distance that is adequate for producing $95 \%$ species richness requires an accurate estimation of "true" species richness. The removal model has been used to estimate true species richness via backpack electrofishing (Meador et al. 2003) and to assess the sampling effectiveness of a single backpack-electrofishing pass (Heimbuch et al. 1997; Mitro and Zale 2000; Meador et al. 2003). However, Burnham and Overton (1979), noting that the assumptions of the removal model are often violated, proposed the nonparametric jackknife estimator as an alternative to the removal model. Palmer (1990) used the jackknife estimator to determine species richness and reported that of eight estimation techniques examined, the jackknife estimator provided the least biased and most precise estimation of species richness.

Characterization of stream fish assemblage structure is one component of the U.S. Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) Program. Two-pass boat electrofishing is the primary NAWQA collection method used for nonwadeable rivers (Meador et al. 1993). I analyzed NAWQA data to assess the effectiveness of single-pass boat electrofishing for estimating fish species richness across nonwadeable rivers of varying sizes and geographic locations. Specific objectives included (1) comparing two-
pass removal model and jackknife estimates of true species richness for each sampling reach, (2) assessing relative percentages of species richness collected in first-pass sampling, (3) assessing relations between the percentage of species richness collected in first-pass sampling and true species richness estimates for the reach, and (4) evaluating relations between the percentage of species richness collected in first-pass sampling and sampling reach length or channel width ratio (reach length divided by channel width).

## Methods

The NAWQA Program focuses on major river basins (study units) across the United States (Gilliom et al. 1995). Fish collected in nine study units (Table 1) were sampled during summer low-flow periods from 1993 to 1997 by use of a standard sampling protocol (Meador et al. 1993). Study sites typically were located near USGS gaging stations, and at least one sampling reach was identified at each site. The strategy for defining a sampling reach represented a combination of sampling distances that were of fixed lengths and those that were proportional to channel width. Reach lengths were determined based on consideration of meander wavelength and fixed minimum and maximum length criteria. Reach length was determined based on a distance of 20 times the mean wetted channel width, roughly equivalent to one meander wavelength (Fitzpatrick et al. 1998). A minimum reach length of 500 m and a maximum reach length of $1,000 \mathrm{~m}$ were also established. For each sampling reach, reach length (m) was recorded. Wetted channel width (m) was measured perpendicular to the streamflow. A total of 157 reaches was sampled by use of two-pass boat electrofishing in the nine study units.

Boat electrofishing was conducted with pulsed DC. Pulse frequencies ranged from 30 to 60 pulses per second (Meador et al. 1993). Field technicians that used the electrofishing equipment received training in the sampling methods (Meador et al. 1993) and in electrofishing principles (e.g., power transfer theory) to help standardize sampling effort and increase the efficiency of electrofishing operations (Reynolds 1996). All boat electrofishing was conducted in the daytime and in a downstream direction. An electrofishing pass equivalent to the length of the sampling reach was made near the shoreline. Upon completion of the first pass, field technicians counted the fish that could be identified in the field and transported the fish downstream of the sampling reach. A second pass was then conducted along the opposite shoreline. Fish that could not be identified in the field were retained for identification and enumeration in the laboratory (Walsh and Meador 1998).

Data analysis.-True species richness was estimated by use of the removal model (Zippin 1956; Seber 1982; Nichols and Conroy 1996). The assumptions of the simplest removal model (when only two samples are used) are (1) the population is closed, (2) the probability of detection is constant among all individuals in the population, and (3) the probability of detection is constant between samples. It should be noted that whereas the simplest removal model can be used with two capture events, it does not allow for heterogeneity of detection probabilities.

For each reach, total species richness from the first pass was determined, and the second pass was evaluated to determine whether additional species were collected. Following the removal model formula of Seber and Le Cren (1967), true species richness was estimated based on the formula:

$$
\hat{S}_{r}=\left(s_{1}\right)^{2} /\left(s_{1}-q_{2}\right)
$$

where $s_{1}$ is the total species richness collected in the first pass, $q_{2}$ is the number of additional species collected as a result of the second pass (i.e., collected species that are unique to the second pass), and $\hat{S}_{r}$ is the estimated true species richness based on the removal model. Percent species richness of the first pass (percent of estimated true species richness) was determined with the formula

$$
\hat{p}_{r, s_{1}}=\left(s_{1} / \hat{S}_{r}\right) \times 100
$$

In 2 of the 157 reaches ( $1 \%$ ), the number of unique fish species collected in the second pass was equal to or greater than that collected in the first pass;
thus, true species richness could not be estimated via the removal model for those reaches.

True species richness was also estimated by use of the first-order jackknife estimation method (Burnham and Overton 1979) based on the formula

$$
\hat{S}_{j}=S_{\mathrm{obs}}+q_{1}(m-1) / m
$$

where $S_{\text {obs }}$ represents the total number of species collected ( $S_{\text {obs }}=s_{1}+q_{2}$ ), $q_{1}$ equals the number of collected species that are unique to the first pass, $m$ equals the number of passes, and $\hat{S}_{j}$ is the estimated true species richness based on the jackknife estimation method. Each estimate was based on means calculated from 1,000 randomizations of the original reach data performed in EstimateS software (Colwell 1997). Percent species richness of the first pass was determined with the formula

$$
\hat{p}_{j, s_{1}}=\left(s_{1} / \hat{S}_{J}\right) \times 100
$$

Analysis of variance was conducted to assess differences between removal model and jackknife estimates of percent species richness for the first pass within and among study units. Percent species richness values were examined for normality by use of normal probability plots and were arcsine-square-root transformed to improve normality. Pearson's correlation was used to examine relations between percent species richness of the first pass and estimates of true species richness.

More than 40 reaches were sampled in the Mississippi Embayment (MISE) and the Upper Mississippi River Basin (UMIS); thus, additional analyses were conducted based on these data. With one exception, all sampling reaches for the MISE were 500 m long, whereas reach lengths varied from 210 to $1,219 \mathrm{~m}$ for the UMIS. Pearson's correlation was used to examine relations between percent species richness values and channel width ratio (reach length divided by wetted channel width) for MISE reaches, and to examine relations between percent species richness values and reach length or channel width ratio for UMIS reaches. The significance level was 0.05 for all statistical analyses.

## Results

Mean reach lengths ranged from 329.6 to 951.1 m (Table 1). Reach lengths less than the minimum sampling distance of 500 m occurred in $15.9 \%$ of the reaches and in seven of the nine study units. The channel width ratio averaged 15.3 across all study units.

Across all reaches, the mean estimated true species richness was 20.1 based on the removal model

Table 2.-Statistical results of true species richness estimates based on the removal model ( $\hat{S}_{r}$ ) and jackknife estimation $\left(\hat{S}_{j}\right) . P$-values are based on analysis of variance between estimates of percent species richness collected in the first electrofishing pass for the removal model ( $\hat{p}_{r, s_{1}}$ ) and estimates from jackknife method ( $\hat{p}_{j, s_{1}}$ ). Abbreviations for study units are defined in Table 1.

| Study unit | Mean <br> $\hat{S}_{r}$ | Mean <br> $\hat{S}_{j}$ | Mean <br> $\hat{P}_{r, s_{1}}$ | Mean <br> $\hat{P}_{j, s_{1}}$ | $P$ |
| :--- | ---: | ---: | ---: | ---: | :---: |
| ALBE | 24.2 | 26.7 | 75.2 | 66.5 | 0.352 |
| MISE | 21.9 | 24.4 | 74.3 | 65.4 | 0.001 |
| REDN | 19.8 | 21.1 | 66.6 | 62.6 | 0.181 |
| RIOG | 7.6 | 8.9 | 86.4 | 78.7 | 0.297 |
| SACR | 15.0 | 18.2 | 67.0 | 62.8 | 0.673 |
| SANJ | 15.3 | 16.6 | 76.7 | 63.4 | 0.219 |
| TRIN | 17.1 | 18.4 | 69.4 | 64.2 | 0.214 |
| UCOL | 8.8 | 10.6 | 87.3 | 77.3 | 0.076 |
| UMIS | 24.4 | 25.6 | 71.5 | 62.9 | 0.014 |
| Combined | 20.1 | 21.8 | 74.1 | 65.5 | 0.001 |

and 21.8 based on jackknife estimation (Table 2). Variation in true species richness estimated by use of the two approaches can be visualized as the deviation from a $1: 1$ relationship between $\hat{S}_{r}$ and $\hat{S}_{j}$ values (Figure 1).

Analysis of variance indicated significant differences in $\hat{p}_{r, s_{1}}(P=0.023)$ and in $\hat{p}_{j, s_{1}}(P=0.002)$ among study units. Across all reaches, the mean $\hat{p}_{r, s_{1}}(74.1 \%)$ was significantly greater than the mean $\hat{p}_{j, s_{1}}(65.5 \%)(P=0.001$; Table 2). Values for $\hat{p}_{r, s_{1}}$ were also significantly greater than $\hat{p}_{j, s_{1}}$ values for the MISE and UMIS study units (Table 2). No other significant differences were detected between $\hat{p}_{r, s_{1}}$ and $\hat{p}_{j, s_{1}}$ values among study units.

Across all reaches, $\hat{p}_{r, s_{1}}$ values exhibited a significant negative correlation with $\hat{S}_{r}$ values $(r=$ $-0.67, P=0.0001$ ), and $\hat{p}_{j, s_{1}}$ values also had a significant negative correlation with $\hat{S}_{r}$ values ( $r$ $=-0.63, P=0.0001)$. A scatter plot of these


Figure 1.-Relationship between estimated true species richness values determined from a two-pass removal model and the jackknife method. The line represents a 1:1 relationship between values derived from the two methods.
relations suggested that values for both $\hat{p}_{r, s_{1}}$ and $\hat{p}_{j, s_{1}}$ were approximately $80 \%$ or greater for reaches where estimated true species richness was less than 10 species (Figure 2). Values for both $\hat{p}_{r, s_{1}}$ and $\hat{p}_{j, s_{1}}$ were less than $60 \%$ for reaches where estimated true species richness was greater than 30 species.

The second pass produced additional species in $89.2 \%$ of the reaches sampled. Of these reaches, the number of additional species collected in the second pass ranged from 1 to 13 . Five common families-Catostomidae, Centrarchidae, Cyprinidae, Ictaluridae, and Percidae-were selected for comparison (Table 3). Combined, these five families contributed 109 of the 151 ( $72 \%$ ) total collected taxa that were unique to the second pass. Other families included Acipenseridae, Amiidae, Anguillidae, Aphredoderidae, Atherinidae, Bothidae, Clupeidae, Cottidae, Cyprinodontidae, Elo-


Figure 2.-Scatter plot of the relation between percent species richness from the first pass of a boat electrofisher and true fish species richness estimated by a two-pass removal model and the jackknife method. Lines represent linear trends.

Table 3.-Percent of U.S. Geological Survey National Water-Quality Assessment Program reaches and study units where taxa unique to the second electrofishing pass were collected and the number of second-pass unique species for five selected families collected in all 157 reaches combined.

|  | Reaches <br> $(\%)$ | Study <br> units $(\%)$ | Unique <br> species <br> $(N)$ |
| :--- | :---: | :---: | :---: |
| Family | 50.3 | 88.9 | 20 |
| Centrarchidae | 45.9 | 100 | 43 |
| Cyprinidae | 34.4 | 88.9 | 20 |
| Catostomidae | 31.2 | 66.7 | 11 |
| Ictaluridae | 23.6 | 77.8 | 15 |
| Percidae |  |  |  |

pidae, Esocidae, Gadidae, Gasterosteidae, Hiodontidae, Lepisosteidae, Mugilidae, Percichthyidae, Percopsidae, Petromyzontidae, Poeciliidae, Sciaenidae, and Umbridae. Centrarchidae and Cyprinidae were the most frequently collected families unique to the second pass across all reaches sampled. Collection of these two families was unique to the second pass in at least eight of the nine study units sampled (Table 3).

For the MISE study unit, the mean channel width was 33.9 m (Table 1) and ranged from 8.1 to 116.3 m . The channel width ratio averaged 21.6 and ranged from 4.3 to 47.7. The channel width ratio was not significantly correlated with $\hat{p}_{r, s_{1}}(r$ $=0.10, P=0.281)$ or $\hat{p}_{j, s_{1}}(r=0.16, P=0.242)$.

For the UMIS study unit, the mean reach length was 819.5 m (Table 1). Mean channel width was 115.5 m ; channel width ranged from 7.0 to 223.8 m . The channel width ratio averaged 10.2 and ranged from 4.3 to 30.0 . The channel width ratio was not significantly correlated with $\hat{p}_{r, s_{1}}(r=0.03$, $P=0.322)$ or $\hat{p}_{j, s_{1}}(r=0.07, P=0.374)$. However, reach length showed a significant positive correlation with both $\hat{p}_{r, s_{1}}(r=0.37, P=0.045)$ and $\hat{p}_{j, s_{1}}(r=0.33, P=0.049)$.

## Discussion

The removal model can be used when only two capture events (electrofishing passes) are available (Heimbuch et al. 1997) and has been used to estimate total species richness for two-pass backpack electrofishing (Meador et al. 2003). When data from two-pass electrofishing are used, the assumption that detection probabilities are constant both among individuals and between passes cannot be tested. However, White et al. (1982) reported that if all animals have an average detection probability ( $\hat{p}_{r, s_{1}}$ in the present study) of at least $80 \%$, then two capture events will suffice because failure
of the constant detection probability assumption will not matter. Even if all of the assumptions are valid, the simplest removal model can fail if $q_{2}$ is greater than or equal to $s_{1}$. Although the two-pass removal model failure rate was relatively low $(1 \%), \hat{p}_{r, s,}$ values for seven of the nine study units were less than $80 \%$.
In cases when two capture events are used, if parameters are estimated with the removal model in the presence of heterogeneous detection probabilities, $\hat{S}_{r}$ tends to be underestimated and $\hat{p}_{r, s_{1}}$ tends to be overestimated (Seber 1982). This could result in a deflated estimate of true species richness and an inflated estimate of the percentage of species richness collected in the first pass. Deviation from a 1:1 relationship between $\hat{S}_{r}$ and $\hat{S}_{j}$ values suggested relatively close agreement between the two approaches for species richness less than 10 , an underestimation of $\hat{S}_{r}$ values compared to $\hat{S}_{j}$ values for species richness from approximately 10 to 30 , and an overestimation of $\hat{S}_{r}$ values compared to $\hat{S}_{j}$ values for species richness greater than 30 . In the MISE and UMIS study units, values for $\hat{p}_{r, s_{1}}$ were significantly greater than $\hat{p}_{j, s_{1}}$ values. Compared to the use of a relatively unbiased nonparametric estimator, estimates of true species richness based on the removal model may be biased, particularly when true species richness is greater than 10. Peterson et al. (2004) noted that overestimates of capture efficiency based on removal models may lead to insufficient sampling effort, increasing the chances of falsely concluding that a species is absent. Thus, biologists should consider approaches like the nonparametric jackknife estimator when estimating true species richness based on boat electrofishing data.
The results of this study indicate that across a large geographic area, a single boat electrofishing pass for reaches ranging from 500 to $1,000 \mathrm{~m}$ may not be adequate for characterizing fish species richness and thus fish assemblage structure. Mean $\hat{p}_{j, s_{1}}$ values were less than the $95 \%$ of estimated total species richness recommended by Lyons et al. (2001) as a target indicator of adequate sampling effort in streams. Hughes et al. (2002) designated the effort needed to collect $95 \%$ of species richness obtained at $75 \%$ of sampled reaches as a target of adequate sampling effort. In the present study, $\hat{p}_{j, s_{1}}$ values of $95 \%$ or greater were noted at only 2 of the 157 reaches ( $1 \%$ ). Thus, greater sampling effort than a single boat electrofishing pass for a $500-1,000-\mathrm{m}$ reach would be required to collect $95 \%$ of species richness for a variety of rivers across a large geographic area.

Examination of relations between channel width ratio and $\hat{p}_{j, s_{1}}$ values provided no clear recommendation for determining sampling distances based on channel width ratios. In the present study, two of the nine study units (Albemarle-Pamlico Drainage Basin [ALBE] and MISE) had mean channel width ratios of greater than 21 , yet the corresponding mean $\hat{p}_{j, s_{1}}$ values were less than $67 \%$. No significant correlation between channel width ratio and $\hat{p}_{j, s_{1}}$ values was detected for the MISE or UMIS study units. In the ALBE, MISE, and UMIS study units, $\hat{S}_{j}$ values were relatively high and exceeded 24. Hughes et al. (2002) suggested that channel width ratios of 85 were necessary to produce $95 \%$ of estimated true species richness in $75 \%$ of sampled reaches; jackknife estimation indicated that estimated true species richness ranged from 2 to 20 species. For rivers in Idaho, sampling of a reach length equal to 20 channel widths yielded an average of $80 \%$ of estimated true species richness collected by single-pass boat electrofishing (Maret and Ott 2003), whereas sampling of a reach length between 30 and 40 channel widths yielded $95 \%$ of estimated true species richness. However, Maret and Ott (2003) collected between 1 and 14 fish species per reach. Cao et al. (2001) noted that the number of channel widths recommended to obtain $95 \%$ of estimated true species richness varied greatly among rivers of similar size, and suggested that sampling effort based on sampling distances proportional to channel widths was related to a number of factors, including estimated true species richness and species abundance distribution (patchiness).

The results of the present study also suggest that the effectiveness of single-pass boat electrofishing is related to estimated true species richness. Mean $\hat{p}_{j, s_{1}}$ values from first-pass sampling varied significantly across study units and were greater than $77 \%$ for the Rio Grande Valley and Upper Colorado River basin study units, where mean $\hat{S}_{j}$ values were less than 11 . Values for $\hat{p}_{j, s_{1}}$ were about $80 \%$ or greater for reaches with estimated true species richness less than 10 , whereas $\hat{p}_{j, s_{1}}$ values were less than $60 \%$ for reaches with estimated true species richness greater than 30. Cao et al. (2001) also reported that the percent of estimated true species richness decreased significantly with increasing species richness. Therefore, single-pass boat electrofishing along $500-1,000-\mathrm{m}$ reaches may yield the highest percent total species richness estimates for geographic regions or reaches where true species richness is relatively low ( $>10$ species). However, Cao et al. (2001) noted that although sam-
pling effectiveness may be greatest for reaches where diversity is relatively low, this relation was confounded by species abundances that were patchy in distribution. Greater sampling effort may be needed for reaches where diversity is low but patchiness of species distribution is high, compared to reaches with greater diversity and lower patchiness (Cao et al. 2001). Thus, sampling effort recommendations may be influenced by a combination of expected species richness and species distribution within a reach.

A single boat electrofishing pass for a distance of $500-1,000 \mathrm{~m}$ often under-represented centrarchid and cyprinid species richness. Of the collected species that were unique to the second pass, members of Centrarchidae and Cyprinidae were collected most frequently across at least eight of the nine study units. In a study that used backpack electrofishing data to estimate fish detection probabilities for wadeable streams in Maryland, Heimbuch et al. (1997) reported relatively low detection probabilities for largemouth bass Micropterus salmoides and bluegill Lepomis macrochirus, species with a tendency to associate with cover that may be difficult to sample adequately with a single electrofishing pass. Similar results were reported for single-pass backpack electrofishing reaches (Meador et al. 2003). Electrofishing efficiency is influenced by many environmental and biological factors and their interactions (Reynolds 1996). Environmental factors include conductivity, water clarity (as it affects operator visibility), depth, cover (such as submerged trees, brush, and rooted aquatic macrophytes), and discharge. Biological factors include variation in fish species size, morphology, physiology, and behavior. Pierce et al. (1985) reported that electrofishing efficiency along shoreline areas of the upper Mississippi River decreased due to increased water velocity and decreased water clarity. Although species that typically inhabit riverine shoreline areas (e.g., centrarchids and cyprinids) are considered to be generally more vulnerable than benthic species (e.g., ictalurids) because of factors like water depth (Reynolds 1996), this did not appear to be the case in the present study. However, sampling in this study was limited to shoreline areas and did not include the relatively deep mid-channel areas. It should be recognized that fish species that utilize primarily main-channel habitats (e.g., ictalurids) may be under-represented in electrofishing efforts that are conducted primarily along the shoreline, regardless of the number of passes. Also, representation of catostomid species may be influenced
by the relatively high interannual variability of catostomids observed in boat electrofishing of rivers (Meador and McIntyre 2003).

Sampling effort increases that are accomplished by extending the sampled reach distance to more than $1,000 \mathrm{~m}$ may increase the percent species richness estimated by single-pass boat electrofishing. For the UMIS study unit, $\hat{p}_{j, s_{1}}$ increased with increasing sampling distance. Lyons et al. (2001) suggested that asymptotic species richness could be achieved at a distance of $1,600 \mathrm{~m}$. However, in the present study, the minimum sampling distance of 500 m was not achieved for $15.9 \%$ of the reaches in seven of the nine study units. Reasons for this varied, but were in all cases related to hydrologic discontinuities, such as low-head dams, at less than $500-\mathrm{m}$ intervals in the sampling reach. Such structures not only create physical barriers to boat access, but also facilitate the formation of differing fish assemblages above and below the barrier (Porto et al. 1999). Also, some species, such as common carp Cyprinus carpio, may tend to be associated with structures like low-head dams and may not occur in the absence of such structures (Kanehl et al. 1997). Depending on the objectives of the study, it may be desirable to avoid sampling near such structures so as to minimize the introduction of additional sources of variation in fish assemblage structure attributed solely to hydrologic discontinuities. Thus, alternatives to sampling distance increases may need to be considered for some reaches. Means of increasing sampling effort could include conducting additional electrofishing passes or the use of multiple gears.

Decisions regarding standardized sampling effort in nonwadeable rivers must be based on study objectives, and those decisions will affect estimated true species richness and sampling efficiency. The increased physical dimensions of nonwadeable rivers combined with habitat zonation affect sampling efficiency relative to that of small streams. Because of the reduced surface area subject to shading in rivers compared to smaller streams, many fish use depth as thermal refugia (Stalnaker et al. 1989). Stalnaker et al. (1989) indicated that river fish species show a tendency toward segregation among habitat zones that include the main channel, main-channel border, and offchannel areas (e.g., backwaters and tributaries). Sampling to characterize river fish assemblages as part of biological assessment tends to be conducted primarily along shorelines in the main-channel border habitat zone (Meador et al. 1993; Yoder and Smith 1999; Hughes et al. 2002). Mann and

Penczak (1984) electrofished a large river in Poland and collected $82.6 \%$ of the fish from the river margin compared to $6.6 \%$ from the main channel.

Determination of sampling distances by using the relation between channel width and meander wavelength has been proposed on the assumption that replicate habitat types occur within a meander wavelength. This assumption may or may not hold true for large rivers. Nearly all unregulated alluvial channels exhibit some tendency to develop curves or meanders that are proportional to the size of the channel. The distance between inflection points is a measure of the wavelength of a meander. Meander wavelengths of natural alluvial channels range from 10 to 14 channel widths (Leopold et al. 1964). Because the spacing of successive riffles is related to meander wavelength and averages 57 times the channel width, one could expect replicate pool/riffle sequences to occur in streams within a meander wavelength. Fish habitat types can vary depending upon the riverbank sampled, because geomorphic processes like scour can occur at a meander bend ("outside") while simultaneous deposition can occur at the crossover ("inside") (Leopold et al. 1964). A sampling distance for nonwadeable rivers greater than 14 channel widths would seem logical, because the relation between meander wavelength and channel width holds true for small streams and large rivers (Leopold et al. 1964). However, the relation between meander wavelength and pool/riffle patterns is complex in large rivers and is controlled by many factors, including bank and channel-bed substrate composition, gradient, discharge and water depth, and human alteration of the channel. Therefore, replicate habitat types, such as pool/riffle sequences, may not occur in all nonwadeable rivers. Hughes et al. (2002) indicated that the sampling of higher-gradient rivers in the western United States might require longer sampling distances compared to relatively low-gradient systems. Human alteration is often commonplace among rivers and can result in changes in flow regime, habitat, and fish assemblage structure (Penczak 1995; Slavik and Bartos 2001; Pegg and Pierce 2002). Hughes et al. (2002) noted that the number of channel widths needed to collect $95 \%$ of fish species richness increased as nearshore anthropogenic disturbance increased. River sampling efforts based on sampling distances proportional to channel widths may need to be varied in relation to a combination of natural and anthropogenic factors.

Similarly, the standardization of sampling effort based on fixed sampling distances would appear
to have limitations across a broad range of sampling conditions. However, fixed distances provide guidance in placing bounds on sampling effort. In order to maximize the number of reaches sampled and minimize the number of visits per reach, total effort (e.g., travel time, sampling effort, and sample processing) should be limited to a single day. Determination of a maximum fixed distance should thus be considered within the context of the total effort. However, results of the present study indicate that a maximum distance greater than 1,000 m may be needed to collect $95 \%$ of true species richness at a majority of reaches when a single electrofishing pass is used.

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