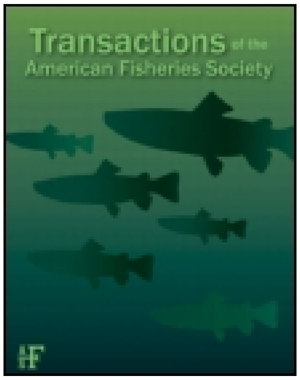

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Comparison of Two Sampling Designs for Fish Assemblage Assessment in a Large River

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ARTICLE

Comparison of Two Sampling Designs for Fish Assemblage Assessment in a Large River

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Abstract
We compared the efficiency of stratified random and fixed-station sampling designs to characterize fish assemblages in anticipation of dam removal on the Penobscot River, the largest river in Maine. We used boat electrofishing methods in both sampling designs. Multiple 500-m transects were selected randomly and electrofished in each of nine strata within the stratified random sampling design. Within the fixed-station design, up to 11 transects (1,000 m) were electrofished, all of which had been sampled previously. In total, 88 km of shoreline were electrofished during summer and fall in 2010 and 2011, and 45,874 individuals of 34 fish species were captured. Species-accumulation and dissimilarity curve analyses indicated that all sampling effort, other than fall 2011 under the fixed-station design, provided repeatable estimates of total species richness and proportional abundances. Overall, our sampling designs were similar in precision and efficiency for sampling fish assemblages. The fixed-station design was negatively biased for estimating the abundance of species such as Common Shiner *Luxilus cornutus* and Fallfish *Semotilus corporalis* and was positively biased for estimating biomass for species such as White Sucker *Catostomus commersonii* and Atlantic Salmon *Salmo salar*. However, we found no significant differences between the designs for proportional catch and biomass per unit effort, except in fall 2011. The difference observed in fall 2011 was due to limitations on the number and location of fixed sites that could be sampled, rather than an inherent bias within the design. Given the results from sampling in the Penobscot River, application of the stratified random design is preferable to the fixed-station design due to less potential for bias caused by varying sampling effort, such as what occurred in the fall 2011 fixed-station sample or due to purposeful site selection.

Characterizing fish assemblage structure is an important component of fisheries research and management. Some assessments are conducted within relatively large ecosystems over multiple seasons or years, and sampling effort required for researchers to provide repeatable estimates is often unknown. Assessments are particularly difficult in large rivers where longitudinal variation and impacts of dams on fish assemblage structure can be profound. Low levels of sampling effort yield imprecise data, which could yield ambiguous results and poorly informed recommendations for management. Researchers must balance precision with many other considerations, including potential bias within the sampling design and budget limitations

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(Hughes and Peck 2008). Therefore, it is important to evaluate precision, efficiency, and bias when designing or choosing a sampling design for fish assemblage assessment.

Considerable research has been performed on large rivers to compare different fish sampling methods (Edwards and Mandrak 2006; Lapointe et al. 2006) and to evaluate sampling design (Flotemersch et al. 2011); however, most evaluations of sampling design have focused on sampling effort within a reach (Flotemersch and Blocksom 2005; Meador 2005; Flotemersch et al. 2011) and did not consider spatial distribution or whether site selection was probabilistic or nonprobabilistic. In general, a number of different sampling designs can be used for sampling large rivers, but in practice two major types of designs appear most common. Fixed-site designs are perhaps most common, in part because of simpler logistics, and in part because of statistical guidance that such designs provide through greater power for trend detection (e.g., Urquhart et al. 1998; Quist et al. 2006). Often, fixed-station designs are also implemented whereby sites are selected for logistical convenience or to include sites where catches are believed to be particularly high. As such, fixed-site designs are often implemented in a nonprobabilistic way and have the potential to be biased due to nonrandom site selection. On the other hand, probabilistic designs are unbiased by design (e.g., Cochran 1977) but may require more effort to capture a comparable number of individual fish and have been reported to require more sample sites to provide power for trend detection for fish populations (e.g., Quist et al. 2006). Comparison of fixed-station nonprobabilistic sampling and random sampling designs for large rivers has rarely been undertaken. Recently, McClelland and Sass (2012) compared results from fixed-station and random sampling designs as part of evaluating a long-term monitoring program on the Illinois River. They determined that fixed-station and random sampling designs yielded similar fish assemblage data (McClelland and Sass 2012), but each design provided benefits to sampling fish assemblages on a large river that were dependent on monitoring goals. The fixed-station design yielded higher relative abundances with slightly lower total effort expended; the authors acknowledged that the sites had been chosen based on areas that were favorable to fish and were susceptible to bias (McClelland and Sass 2012). Alternatively, the random design yielded unbiased results and could achieve greater spatial coverage within a large river (McClelland and Sass 2012).

Optimal sampling design depends on study objectives, fish assemblage characteristics being evaluated, and availability of prior data. In this study, sampling was conducted in order to characterize the fish species present and their relative abundance in the lower reaches of the Penobscot River, Maine, prior to removal of two main-stem dams. Previous studies have shown that measures of species presence or species richness increase nonlinearly with increased sampling effort and catch (e.g., Lyons 1992; Hughes et al. 2002, 2012), whereas measures of relative abundance show no trend with sample size. Thus, the choice of a sampling design and sampling effort may differ for each of these measures. In our study, we also faced the situation where prior data were available from a nonprobabilistic fixed-station design. We had concerns that following the previous design could introduce bias into our results, and as such, chose to implement both designs simultaneously, allowing us to objectively evaluate the strengths of each design for each fish assemblage metric.

Our evaluation of sampling was part of monitoring efforts to document effects of dam removal within the Penobscot River Restoration Project (PRRP). Through removal of Veazie Dam and Great Works Dam, along with the installation of a fish lift at Milford Dam, the PRRP is anticipated to increase passage of diadromous and resident fishes and improve connectivity among currently fragmented habitat of the main stem (PRRT 2011). The study was designed to specifically inform decisions regarding sampling effort and design for future monitoring of fish assemblages in the Penobscot River, but it was also intended to be generally informative for designing sampling programs in other large rivers. Our specific objectives were to (1) determine whether our sampling effort produces repeatable estimates of species richness and proportional abundance, (2) compare the efficiency between sampling designs, and (3) compare estimates of species richness and proportional abundance between sampling designs.

STUDY AREA

The Penobscot River watershed is the largest in Maine, and the second largest in New England, draining 2.2 million hectares through more than 8,800 river kilometers (rkm) of river and streams (Opperman et al. 2011). This study focuses on the lower 70 rkm of river (Figure 1), which ranges from 170 to 600 m wide with an average annual discharge of about 440 m^3/s during recent years (USGS 2011). This reach contains approximately 257 km of shoreline, and includes freshwater tidal, impounded, and free flowing areas. Excluding impoundments, most areas are relatively heterogeneous in shoreline habitat and flow types. The river is impounded by the Veazie Dam at the head of tide, the furthest point upstream affected by tidal fluctuations (Figure 1). Two other main-stem dams (Great Works and Milford) are also included in the study area, which is bounded upriver by the Stanford Dam.

METHODS

Sampling Designs

Fixed-station sampling design.—We sampled along established transects chosen and sampled previously by Kleinschmidt Associates (2009), using a design modeled after an Index of Biotic Integrity study. The purpose of the original Kleinschmidt study design was to evaluate river quality using fish-assemblage criteria. The fixed-station sampling design consisted of 11 transects on the main stem (Figure 1), all of which were approximately 1,000 m long. Six main-stem transects were concentrated in areas above and below dams scheduled for

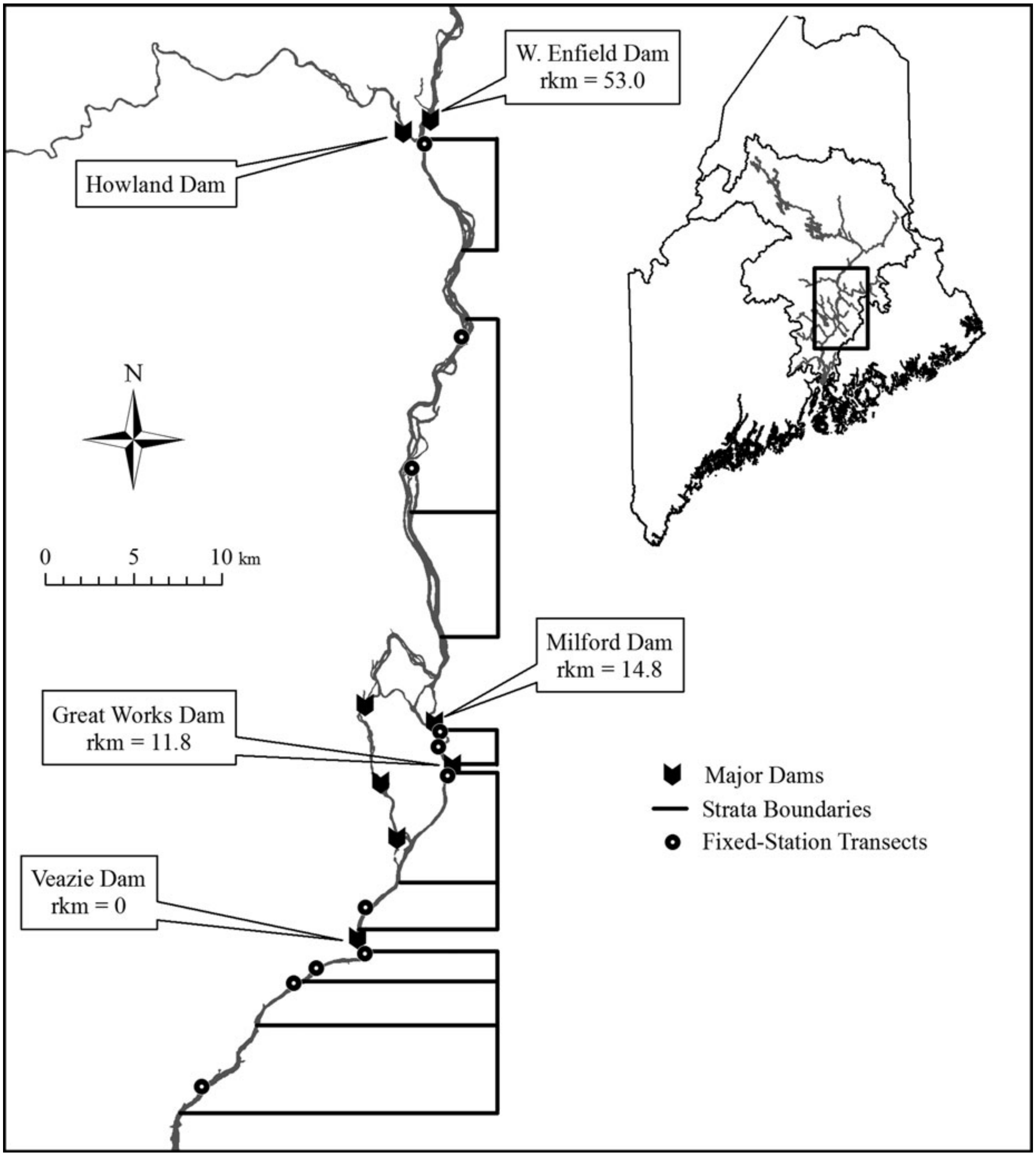

FIGURE 1. The Penobscot River and locations by river kilometer (rkm) of main-stem dams within our study area, as depicted by the inset, along with strata boudaries and fixed-station transects where fish were captured via boat electrofishing during 2010 and 2011.

removal, and all transects were chosen in a nonprobabilistic design. We made an effort to sample at all of these transects during each sampling event, although time restrictions precluded sampling of transects above Great Works Dam during fall 2011. During the summer 2011 sampling, we divided each fixed transect in half when feasible to collect data from a transect length consistent with the stratified random design (described below).

Stratified random sampling design.—The stratified random sampling design was implemented to better account for spatial heterogeneity within the river and to provide unbiased design data that would be comparable over time (Cochran 1977). We divided the river longitudinally into nine strata, the bounds of which were based on dam locations, broad-scale habitat types, and boat access. Using ArcGIS 9.3, we delineated accessible river shoreline, including shoreline around large islands, into

219 transects approximately 500 m long. We selected multiple transects at random from within each stratum; a prioritized list was created to select alternative transects if that area of river was inaccessible by boat. Different transects were sampled during each season and year. The stratified random design offered flexibility in the number of transects required for sampling within each stratum, the only requirement being that at least two were required per stratum. If the field season duration became restricted, fewer transects were selected for sampling, whereas if we were presented with more time to sample, a greater number of transects were selected within a given stratum (as determined in the field). The purpose of this was to improve precision by increasing the number of replicates whenever possible, but also to balance the amount of sampling with time constraints.

Fish Collection

Single-pass daytime boat electrofishing surveys (Curry et al. 2009) were conducted in the summer (June) and the fall (September and October) during 2010 and 2011, for a total of four sampling events. We electrofished only if discharge was less than 425 m^3/s at West Enfield, Maine (USGS gauge 01034500) and when water temperatures were below 22°C, as measured at the start of each transect. We used a 5.5-m-long aluminum boat (Lowe, Roughneck, Lebanon, Missouri) equipped with Smith–Root (Vancouver, Washington) electrofishing equipment, including two booms with 6-dropper anode arrays, and a GPP 5.0 electrofishing system. We installed custom cathode dropper arrays near and along the bow of the boat. Metal conduit encased half of the droppers to increase the cathode surface area (about 30,755 cm^2); the purpose of this design was to reduce fish injury and mortality. The electrofishing unit was operated using pulsed DC at 60-Hz and 30–40% of power, as required to capture fish successfully while limiting injury; settings were chosen to maximize power transfer. Two netters captured stunned fish with Duraframe (Viola, Wisconsin) dip nets; all net bags were constructed of 4.8-mm mesh.

Surveys were conducted by maneuvering the boat parallel and close to shore and fishing in a downstream direction, at a speed equal to or slightly greater than the current. Pockets, eddies, and shoreline were sampled by maneuvering the boat perpendicular or at an angle to shore. Habitat structure (e.g., boulders, large woody debris, and vegetation) were fished thoroughly as well. All fish that were captured were identified to species and measured (1 mm, 0.1 g). If age-0 or small fish (length <80 mm) of any species were captured in high abundance (>50), these fish were separated by size-class, counted, and mass was measured for batches, and lengths were taken (mm) for the smallest and the largest specimens in a batch. This method was implemented to collect required data from these specimens while reducing mortality and processing time. Due to endangered species permitting restrictions, we did not attempt to net adult Atlantic Salmon, or sturgeon (i.e., Atlantic Sturgeon *Acipenser oxyrinchus* and Shortnose Sturgeon *Acipenser brevirostrum*) but rather noted their occurrence visually and considered each encounter as a capture for data analysis below. Estimated mass for Atlantic Salmon observed in 2010 was calculated by approximating size and year-class (Dube et al. 2010) along with historical (Baum 1997) and recent (Bacon et al. 2009) length-mass data. Similar methods were used to estimate mass of Atlantic Salmon during 2011, but mass data were available from fish that were captured in the Penobscot River (O. Cox, Maine Department of Marine Resources, unpublished data). Sturgeon mass was estimated from length-frequency and length–mass data (G. Zydlewski and M. Altenritter, University of Maine, unpublished data).

Data Analysis

Data set.—Catch per unit effort and biomass per unit effort (BPUE) for each species (Table 1) were calculated for each stratum and for each fixed-station transect by dividing the total catch or biomass by the total length of shoreline electrofished, as measured between start and end GPS coordinates using orthoimagery in ArcGIS 9.3 (Redland, California). Age-0 Smallmouth Bass (<30 mm) and White Suckers (<40 mm) were removed from the summer sampling data prior to analyses because the growth of these specimens necessary to recruit to our gear (>25 mm) appeared to be variable among strata for the duration of the summer sampling; by fall, these fish were large enough to be captured reliably within all strata.

Species-accumulation curves.—Sample-based species-accumulation curves show the average species richness that is calculated for all subsets of total site effort (the number of transects), as opposed to individual-based species-accumulation curves, which show the average species richness calculated for all subsets of the total number of individuals encountered (Kindt and Coe 2005). We constructed sample-based species-accumulation curves using the exact method, which uses analytical formulae to calculate average species richness at each level of effort (Colwell et al. 2004; Kindt and Coe 2005; Oksanen et al. 2011). Standard error calculations for estimated species richness estimates were not conditional on the empirical data. The exact method replaces random resampling methods often used to create sample-based species accumulation curves, and provides useful confidence intervals (Colwell et al. 2004). Transect length differed between sampling designs; therefore, all species accumulation curves were scaled to kilometers of electrofishing in order to facilitate direct comparisons of effort (Kindt and Coe 2005). All curves were constructed using the statistical package R (R Development Core Team 2010) and the BiodiversityR and Vegan libraries (Kindt and Coe 2005; Oksanen et al. 2011). Curves were inspected visually for asymptotic behavior. Error bars representing 95% confidence intervals were also plotted and inspected visually across different species-accumulation curves; curves and locations along curves with confidence interval overlap were considered similar (Colwell et al. 2004).

Dissimilarity curves.—Species-accumulation curves utilize presence data and do not incorporate abundance of each species.

TABLE 1. Fish captured on the Penobscot River by boat electrofishing for stratified random and fixed-station sampling designs, 2010–2011. Biomass per unit effort (BPUE; g/km) and CPUE; number/km) were calculated as the mean catch and mass per kilometer among four discrete sampling events.

	Mean CPUE		Mean BPUE	
Species	Stratified random	Fixed station	Stratified random	Fixed station
Common Shiner *Luxilus cornutus*	258.3	64.3	380	96
Fallfish *Semotilus corporalis*	239.9	65.0	687	269
Redbreast Sunfish *Lepomis auritus*	58.0	29.7	1,011	534
White Sucker *Catostomus commersonii*	34.6	18.5	824	3,510
Smallmouth Bass *Micropterus dolomieu*	85.6	45.9	2,928	3,253
Pumpkinseed *Lepomis gibbosus*	17.1	14.5	116	139
Golden Shiner *Notemigonus crysoleucas*	15.7	6.5	32	18
American Eel *Anguilla rostrata*	15.0	9.4	1,528	1,096
Chain Pickerel *Esox niger*	8.1	1.9	582	234
Sea Lamprey *Petromyzon marinus*	5.1	2.5	83	10
Yellow Perch *Perca flavescens*	4.7	3.0	105	63
Brown Bullhead *Ameiurus nebulosus*	3.0	2.0	449	322
Banded Killifish *Fundulus diaphanus*	7.3	1.0	18	5
Alewife *Alosa pseudoharengus*	3.2	2.3	440	51
Burbot *Lota lota*	1.2	0.7	71	28
Blueback Herring *Alosa aestivalis*	1.6	2.0	47	111
Mummichog *Fundulus heteroclitus*	0.2	0.1	<1	<1
Creek Chub *Semotilus atromaculatus*	0.2	0	<1	0
Eastern Silvery Minnow *Hybognathus regius*	0.7	0.2	1	0
Black Crappie *Pomoxis nigromaculatus*	0.2	0.2	2	1
White Perch *Morone americana*	0.7	<0.1	7	1
Largemouth Bass *Micropterus salmoides*	0.1	0.2	3	1
Longnose Sucker *Catostomus catostomus*	0	<0.1	0	37
Atlantic Salmon *Salmo salar*	<0.1	0.5	271	1,446
Brook Trout *Salvelinus fontinalis*	<0.1	0	1	0
Blacknose Dace *Rhinichthys atratulus*	<0.1	0	<1	0
Blacknose Shiner *Notropis heterolepis*	<0.1	<0.1	<1	<1
Finescale Dace *Chrosomus neogaeus*	<0.1	0	<1	0
Northern Redbelly Dace *Chrosomus eos*	<0.1	<0.1	<1	<1
American Shad *Alosa sapidissima*	<0.1	<0.1	24	24
Threespine Stickleback *Gasterosteus aculeatus*	<0.1	0	<1	0
Ninespine Stickleback *Pungitius pungitius*	<0.1	<0.1	<1	<1
Acipenser spp.	0	<0.1	0	287
Striped Bass *Morone saxatilis*	0	<0.1	0	94

Dissimilarity curves provide similar insights to species accumulation curves by plotting the amount of compositional change that occurs with increased sampling effort (McCune and Grace 2002). Dissimilarity curves were created by randomly resampling subsets of the data and calculating the Bray–Curtis dissimilarity between the centroid of each subset and the centroid of the total sample (McCune and Grace 2002), thereby incorporating proportional abundance of each species, which can be valuable for evaluating sampling effort for entire assemblages (e.g., Schneck and Melo 2010). High dissimilarity values indicate that subsamples are dissimilar in the abundance of species compared with the whole sample. Dissimilarity curves for each sampling event and sampling design were constructed using PC ORD software (McCune and Mefford 1999). We calculated dissimilarity curves using CPUE data for each species from each sampling design. These curves were then plotted along an x-axis scaled to kilometers of electrofishing.

Estimation of total species richness.—A variety of methods exist for estimating total species richness (S_{est}) within an area (Colwell and Coddington 1994). Because evaluating S_{est} through extrapolation using asymptotic values of models is unreliable (Palmer 1990; Hortal et al. 2006), we estimated total species richness for both sampling designs from every sampling event with an incidence-based, nonparametric, first-order jackknife estimator. This method estimates the total species richness based on the number of species present at only one site within a sample (Palmer 1990; McCune and Grace 2002; Kindt and Coe 2005) and has been shown to be a relatively unbiased and precise estimator using both simulated and real data (Smith and Van Belle 1984; Palmer 1990; Walther and Morand 1998). There are other suitable nonparametric estimators of total species richness, but the purpose of analyses here was to compare sampling designs rather than the accuracy of richness estimators; thus, multiple estimators were not examined. All calculations of S_{est} were performed using the statistical package R with the Vegan library (R Development Core Team 2010; Oksanen et al. 2011).

Abundance and biomass estimates.—We estimated mean abundance (N/km) and biomass (g/km) for each species and all fish combined with the statistical package R (R Development Core Team 2010) and the survey library (Lumley 2011) for each sampling design and event. Estimates derived from stratified random sampling were calculated using inverse-probability weights for each stratum (Lumley 2004); we calculated weights as the inverse of the number of transects surveyed within a stratum divided by the total number of transects located within that stratum. Estimates derived from fixed-station sampling were also calculated using sampling weights similar to the stratified random design, under the assumption that the fixed-station transects were representative of the river segments that they were within.

Comparison of proportional CPUE.—Dissimilarity among sampling designs was evaluated by using multi-response permutation procedures (MRPP) complemented with indicator species analysis (McCune and Grace 2002; Fischer et al. 2010; Gubiani et al. 2010; Jacquemin and Pyron 2011). The MRPP is a nonparametric method that tests for differences in assemblages among groups and is similar to analysis of variance (ANOVA) in that it compares dissimilarities within and among groups. We performed MRPP computations with PC ORD software (McCune and Mefford 1999) after rank-transforming the distance matrix. Because of our focus on the stratum scale (i.e., analyzing differences among strata), as opposed to the transect scale (i.e., analyzing differences among transects), we combined catch data from transects within each stratum and standardized by kilometers of electrofishing; this was performed for both sampling designs. Calculations for MRPP analyses were based on Bray–Curtis dissimilarity, the same measure that was used to create our dissimilarity curves. We conducted indicator species analysis (ISA) (Dufrene and Legendre 1997) on significant ($\alpha = 0.05$) MRPP comparisons to identify potential bias within our sampling designs. Indicator species analysis can identify the species associated with each sampling design by incorporating relative abundance and frequency of a given species; strong indicator species are found mostly within a single group and are also present at the majority of sites (Dufrene and Legendre 1997). The *P*-values for ISA were calculated using a Monte Carlo test of significance based on 1,000 permutations.

RESULTS

Fish Collection

Over all sampling events, 88 km of shoreline was surveyed and 45,874 fish were captured that were suitable for analysis. Sampling effort for each event within each design ranged from 9.0 to 15.7 km of electrofishing, except for fall 2011 fixed-station sampling when only 3.0 km of shoreline was electrofished. We encountered 34 species total: 31 species in stratified random sampling and 30 species in fixed-station sampling (Table 1). Four species were encountered that were unique to the stratified random sampling; three were encountered in fixed-station sampling. All such design-unique species were encountered in very low abundances (1–10 individuals) and at few transects. One of these species, Creek Chub, was the most abundant (10) and was found along seven stratified random transects.

Curve Comparisons

None of the species-accumulation curves were fully asymptotic, but all curves approached an asymptote, except for fall 2011 fixed-station sampling (Figure 2). Curves begin to approach an asymptote after about 5 km of electrofishing. The 95% confidence intervals of stratified random and fixed-station sampling for each event and at all levels of effort for the species-accumulation curve overlapped considerably.

The fixed-station design during fall 2011 also produced the only nonasymptotic dissimilarity curve (Figure 2). Dissimilarity curves declined slightly more rapidly and leveled out more completely under the stratified random design than under the fixed-station sampling design during most sampling events.

Total Species Richness, Abundance, and Biomass

Observed species richness (S_{obs}) for each sampling design within each sampling event ranged from 15 to 27 species, whereas S_{est} ranged from 22.0 to 31.8 species (Figure 3). Estimated abundance for the most numerous species such as Common Shiner and Fallfish was consistently higher for the stratified random design (Table 2), although uncommon ($0.1 <$ number/km < 2) or rare (< 0.1/km) species estimates were comparable between the sampling designs. Estimated biomass was considerably higher in the fixed-station design for White Suckers and Atlantic Salmon. Total estimated abundance for all species combined was higher for the stratified random design, especially during fall 2010 sampling (Figure 4), whereas total estimated biomass was similar between designs for all sampling events.

Proportional Abundance

The MRPP analysis indicated no significant difference ($\alpha = 0.05$) in proportional CPUE between our sampling designs during summer 2010, fall 2010, and summer 2011 seasons (Table 3). A significant difference ($P = 0.04$) between sampling designs was present during fall 2011 sampling, which was the only sampling event where our species-accumulation and

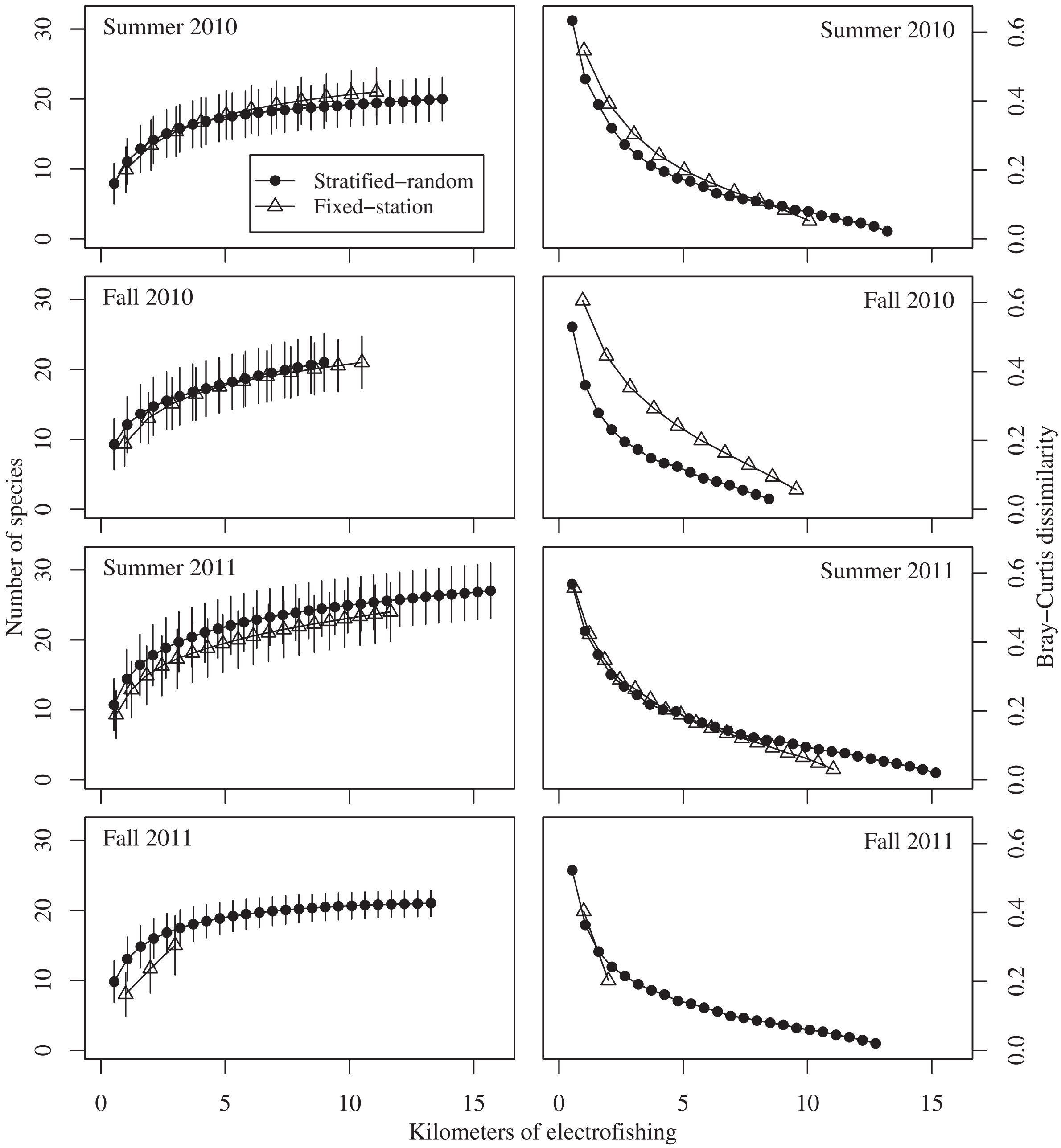

FIGURE 2. Species-accumulation and dissimilarity curves for data derived from stratified random and fixed-station sampling designs on the Penobscot River, 2010–2011. Error bars in species-accumulation plots represent 95% confidence intervals.

dissimilarity curves indicated that effort for the fixed-station design did not reach the minimum effort required for repeatable results. No significant differences were present for BPUE.

Indicator species analysis revealed three significant ($\alpha = 0.05$) indicator species, all from the fall 2011 sampling: Fallfish (indicator value [IV] = 97, $P = 0.05$), Common Shiner (IV = 94, $P = 0.023$), and Smallmouth Bass (IV = 81, $P = 0.035$). These indicator values are relatively high and are associated with the stratified random sampling; Fallfish and Smallmouth Bass were present in all strata within both sampling designs, but the proportional CPUE of those species was much higher within the stratified random design. Additionally, Common Shiners were present in all strata within the stratified random design but were captured within only one stratum of the fixed-station design; the proportional CPUE of Common Shiners was also much higher within the stratified random design.

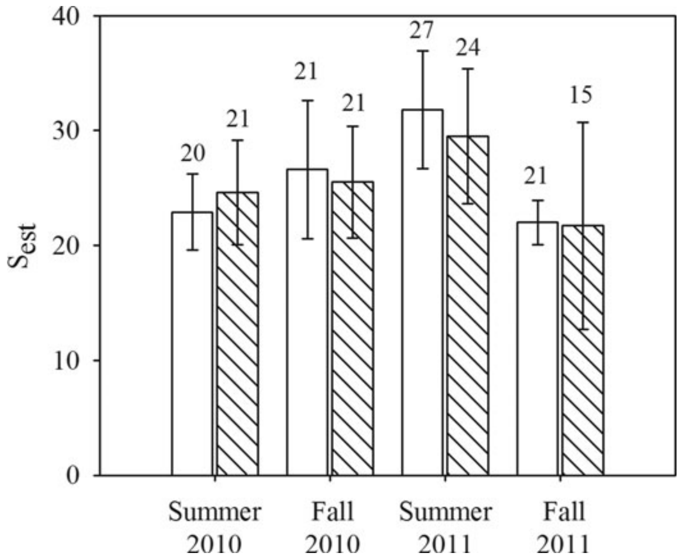

FIGURE 3. Total estimated species richness (S_{est} ± 2 SE; calculated using the first-order jackknife) derived from data collected by boat electrofishing using stratified random (clear) and fixed-station (cross-hatched) sampling designs on the Penobscot River. Estimates are for all four sampling seasons during 2010 and 2011. The number of species captured is shown above each bar.

TABLE 2. Mean summer fish abundance (number/km) and biomass (g/km) estimates for all species captured within the Penobscot River by boat electrofishing for stratified random and fixed-station designs, 2010–2011. Estimates are the mean of weighted abundance and biomass calculations between two summer sampling events.

	Abundance		Biomass	
Species	Stratified random	Fixed station	Stratified random	Fixed station
Common Shiner	192.9	65.5	378	217
Fallfish	189.0	64.8	812	485
Redbreast Sunfish	38.0	12.8	1,424	744
White Sucker	35.2	20.7	1,314	3,822
Smallmouth Bass	27.2	28.5	2,577	2,405
Pumpkinseed	19.3	19.9	75	76
Golden Shiner	18.1	9.2	24	17
American Eel	14.0	9.2	1,758	1,185
Chain Pickerel	9.4	3.0	574	353
Sea Lamprey	8.5	2.3	125	21
Yellow Perch	3.7	4.8	114	78
Brown Bullhead	3.5	2.6	724	812
Banded Killifish	3.0	0.9	7	1
Alewife	2.1	0.8	298	43
Burbot	1.6	0.6	114	41
Blueback Herring	1.6	4.0	38	75
Mummichog	0.3	<0.1	<1	<1
Creek Chub	0.3	0	<1	0
Eastern Silvery Minnow	0.2	0	<1	0
Black Crappie	0.2	0.3	1	2
White Perch	0.2	<0.1	2	<1
Atlantic Salmon	<0.1	0.9	2	1,810
Brook Trout	<0.1	0.0	<1	0
Blacknose Dace	<0.1	0	<1	0
Blacknose Shiner	<0.1	<0.1	<1	<1
Finescale Dace	<0.1	0	<1	0
Northern Redbelly Dace	<0.1	<0.1	<1	<1
American Shad	<0.1	<0.1	30	14
Threespine Stickleback	<0.1	0	<1	<1
Largemouth Bass	0	<0.1	0	<1
Longnose Sucker	0	<0.1	0	45
Ninespine Stickleback	0	<0.1	0	<1
Acipenser spp.	0	0	0	0
Striped Bass	0	<0.1	0	55

DISCUSSION

Sampling Effort and Precision

We suggest 5 km of electrofishing as the minimum level of effort to produce repeatable estimates of fish assemblage structure in the Penobscot River, which is lower than total sampling distance suggested for western U.S. rivers (Hughes et al. 2002, 2012). However, Hughes et al. (2012) concluded that at least 11 randomly selected sampling sites were required for sampling 90% of vertebrate species; this is similar to results from our stratified random design, for which 10 transects would meet our 5-km minimum. High river discharge and adverse sampling conditions on the Penobscot River during fall 2011 constrained the field season such that sampling with only one design could be completed; effort during fall 2011 was focused on completion of sampling within the entire study area using stratified random sampling. Results from fixed-station sampling during fall 2011, when only 3 km of shoreline were sampled, illustrate how sampling below this minimum results in lower precision. Low effort during fall 2011, which was restricted to transects below Great Works Dam for the fixed-station design due to time constraints, resulted in species-accumulation curves and dissimilarity curves that did not approach an asymptote, wide confidence intervals around estimates, and MRPP and ISA values that differed significantly (but as an artifact of the spatially restricted sampling). During all other sampling events, effort beyond 5 km per sampling event did not increase precision considerably, but this does not imply that increasing sampling effort beyond this minimum is not useful. Additional project objectives such as capturing rare species would necessitate sampling effort far past the minimum required for repeatable whole-assemblage estimates.

Logistics

Fixed-station sampling designs have been recommended as a logistical alternative to randomized designs (King et al. 1981). In our case, the total time spent traveling between transects for fixed-station sampling would have been slightly lower than for stratified random sampling due to longer and fewer transects.

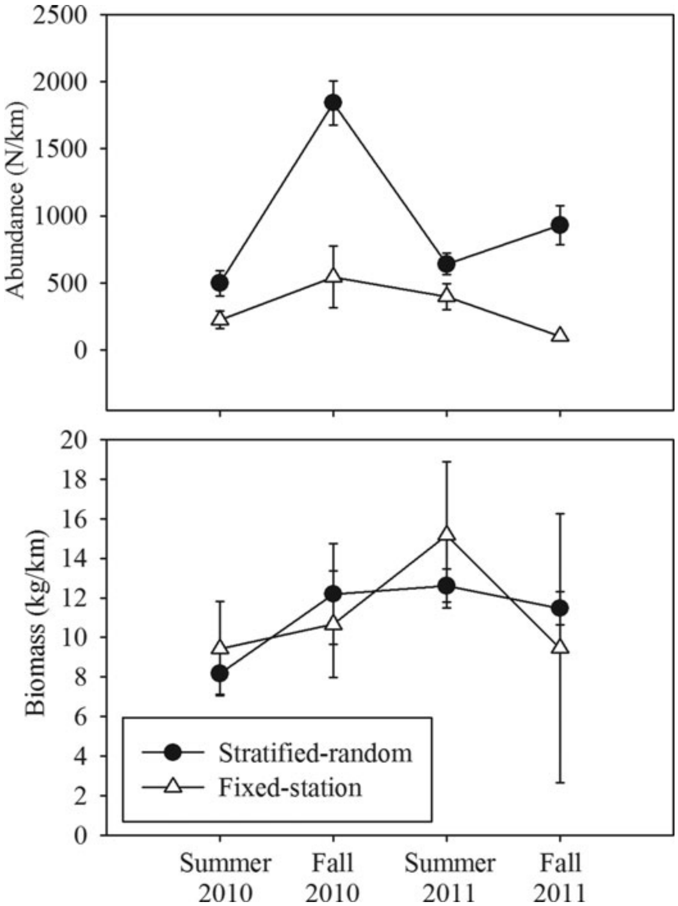

FIGURE 4. Estimates of total abundance (± SE) and biomass (± SE) of fish in the Penobscot River, 2010–2011, for stratified random and fixed-station sampling designs.

However, fragmentation of our study area by dams created river sections within which boat travel was relatively easy, but where travel between sections required loading, towing, and relaunching the boat. Time and effort for traveling within each river section was small compared with travel costs between river sections, thereby leading to similar travel requirements for both designs. A substantial advantage of the stratified random design is flexibility in the amount of effort spent sampling. This is particularly important for situations where weather or water level conditions limit the capacity to reach sampling goals. In these cases, fewer random transects could be selected for sampling within each stratum, leading to reduced precision but retaining the property of being unbiased in design. The stratified random design also allows for additional random transects to be selected quickly and easily, even in the field, if circumstances permit. In contrast, fixed-sampling designs are sensitive to missing data points as illustrated by our fall 2011 sampling. The potential for biases occurring due to missing data are an under-appreciated liability of such a design.

TABLE 3. Pair-wise comparisons of fish assemblage CPUE (number/km) and BPUE (g/km) data on the Penobscot River, 2010–2011, derived from boat electrofishing under stratified random and fixed sampling designs. An asterisk denotes a significant result (MRPP, $P \leq 0.05$). The change corrected within-group agreement (A) from MRPP analyses is also listed.

	CPUE		BPUE	
Sampling Event	A	*P*	A	*P*
Summer 2010	−0.019	0.59	0.025	0.15
Fall 2010	−0.001	0.42	0.004	0.38
Summer 2011	−0.034	0.78	−0.003	0.47
Fall 2011	0.143	0.04*	−0.052	0.81

Probabilistic and Nonprobabilistic Design Comparisons

Our overall findings are comparable to those from McClelland and Sass (2012), in that fish assemblage collections were similar between probabilistic and nonprobabilistic designs. However, McClelland and Sass (2012) determined that higher species richness and diversity could be captured by sampling with a fixed-station design while expending slightly less effort than with a random design. We found either no difference or that slightly less effort was required by the stratified random design relative to the fixed-station design, which probably resulted from stratifying our study area; this improved our sampling precision by distributing our transects throughout a large river containing considerable longitudinal variability.

Basic sample theory (e.g., Cochran 1977) demonstrates that stratified random sampling is design unbiased; therefore, our comparisons between stratified random and fixed-station estimates show potential for bias within the fixed-station design. Any bias observed for the fixed-station design is probably due to transect location; 4 of 11 fixed-station transects were near the tailrace of dams, where migratory adult fish are more likely to be captured, and where habitat for small or juvenile fish may be lacking. Total estimated abundance for stratified random sampling was consistently higher than fixed-station sampling, especially during fall sampling, when we captured many young-of-the-year fish. This is particularly interesting, given that nonprobabilistic station choice is often intended to provide higher CPUE (McClelland and Sass 2012).

Differences in transect length and method of site selection probably resulted in species-accumulation and dissimilarity curves with different shapes (Scheiner 2003; Chapman and Underwood 2009). Within the stratified random design, slightly less sampling effort was necessary to characterize proportional abundance than to determine species richness, which is similar to findings by Angermeier and Smogor (1995) and Terra et al. (2013), and probably resulted from distribution patterns of rare species within the study area. For the fixed-station design, the opposite pattern was observed due to steeper ending slopes of dissimilarity curves, indicating that distribution patterns of the fish assemblage may not be accurately represented when using fixed-station sampling. This could have considerable consequences for evaluating success of dam removal on the

Penobscot River, where distribution patterns of fish species is expected to change. Additionally, biased results associated with a nonprobabilistic design from this study and others could affect important fisheries policy decisions negatively (Hughes et al. 2000).

CONCLUSION

Our findings are helpful for the development and modification of assemblage-based assessment and monitoring programs. We found that a fixed-station, nonprobabilistic sampling design did not provide benefits (i.e., higher CPUE, logistical ease) that are often used to justify the use of such a design. Authors (e.g., Hughes et al. 2000) have suggested that nonprobabilistic sampling cannot be used to infer results to a reach or study-area scale because data collected only represent the chosen sites and are not a statistical sample from a population; we concur with this view. Probabilistic designs such as stratified random sampling provide unbiased results that can be used to make inferences regarding assemblages within river reaches, throughout the study area, and through time. As such, we conclude that stratified random sampling is generally preferable for quantifying fish assemblages in large rivers like the Penobscot River.

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