

# Effects of sampling effort, assemblage similarity, and habitat heterogeneity on estimates of species richness and relative abundance of stream fishes

Jesse R. Fischer and Craig P. Paukert

**Abstract:** We estimated the sampling effort required to accurately estimate species richness and to detect changes in catch-per-unit-effort (CPUE) in four Great Plains, USA, streams. The number of sampled reaches (i.e., <1 km) required to estimate stream-segment (i.e., 20–28 km) species richness decreased with increased sampled reach length (i.e., 10, 20, 40, or 60 mean stream widths, MSW), whereas total sampling effort decreased with a greater number of shorter sampled reaches. Collecting all species in a stream segment required all sampled reaches (i.e., 10) of a length equal to 40 or 60 MSW. The number of stream reaches sampled with lengths equal to 40 MSW required to detect a 50% change in CPUE of common species (i.e., total abundance > 1% of total catch) with  $\beta = 0.80$  ranged from 7 to 630 (mean = 99) and decreased with longer sampled reaches. A greater number of sampled reaches were needed to detect 90% of species richness and 25% changes in CPUE when Jaccard's similarity of samples of stream fish assemblages and habitat heterogeneity was lower within streams. Our results suggest that homogeneous stream segments require more sampled reaches to characterize fish assemblages and monitor trends in fish abundance.

**Résumé :** Nous avons estimé l'effort d'échantillonnage requis pour évaluer correctement la richesse spécifique et pour détecter les changements dans les prises par unité d'effort (CPUE) dans quatre cours d'eau de la région des Grandes Plaines, É.-U. Le nombre de sections échantillonnées (de <1 km) nécessaires pour estimer la richesse spécifique dans un segment (soit 20–28 km) de cours d'eau diminue en fonction de l'augmentation de la longueur des sections échantillonnées (largeurs moyennes des cours d'eau (MSW) de 10, 20, 40 ou 60 m), alors que l'effort total d'échantillonnage diminue avec l'augmentation du nombre de sections plus courtes échantillonnées. La récolte de toutes les espèces dans un segment de cours d'eau nécessite que toutes les sections échantillonnées (soit 10) soient d'une longueur égale à 40 ou 60 MSW. Le nombre de sections de cours d'eau de longueur égale à 40 MSW requis pour détecter un changement de 50 % de CPUE des espèces communes (avec une abondance totale > 1% des captures totales) avec  $\beta = 0,80$  varie de 7 à 630 (moyenne = 99) et décroît si les sections échantillonnées sont plus longues. Il faut échantillonner un plus grand nombre de sections afin de détecter 90 % de la richesse spécifique et des changements de 25 % de CPUE lorsque la similarité de Jaccard est plus faible dans les échantillons des peuplements de poissons et que l'hétérogénéité des habitats est réduite dans les cours d'eau. Nos résultats indiquent que, dans les segments homogènes de cours d'eau, il faut échantillonner un plus grand nombre de sections afin de caractériser les peuplements de poissons et de suivre les tendances d'abondance des poissons.

[Traduit par la Rédaction]

## Introduction

Stream fish assemblages are often sampled at a few locations to describe species richness and catch-per-unit-effort (CPUE) of individual species across a larger spatial scale. These data are then used to develop management and conservation decisions (e.g., conservation of areas of greatest diversity), evaluate ecosystem health (e.g., index of biotic integrity) (Karr 1981; Fausch et al. 1990), describe fish distributions (Rahel and Hubert 1991), and understand patterns

of fish assemblage structuring within streams (Angermeier and Winston 1999). The accuracy and quality of fish assemblage data are therefore critical to the validity of these decisions.

Fausch et al. (2002) proposed that the fundamental problem with the current conservation and management of stream fish is the lack of scientific research relevant over large spatial and temporal scales and argued that the current spatial gap of knowledge exists between reaches (i.e., <1 km) and segments (i.e., 1–100 km). Understanding

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relations between environmental and fish assemblage characteristics is often explored using one assemblage sample from a reach to characterize a segment (Gido et al. 2006) or entire catchment (Smith and Kraft 2005; Diana et al. 2006; Heitke et al. 2006). This increases the need for accurate estimates of assemblage attributes and to understand the sampling effort required to characterize stream segments. It is therefore important to understand the relationship between fish assemblage data collected at reach scales and that of entire stream segments.

Ideally, discrete samples of fish assemblages are representative of fish assemblages at larger spatial scales. However, it is often impossible to sample all species of a community (Krebs 1998), which creates difficulties in determining the amount of sampling effort required to describe an assemblage at a larger spatial scale. Second, the number of species collected increases as stream area or sample length and the number of samples increases (Angermeier and Schlosser 1989; Lyons 1992; Peterson and Rabeni 1995). Finally, species are not evenly distributed within the stream due to habitat heterogeneity (Gorman and Karr 1978; Angermeier and Smogor 1995), and variability in spatial distributions of rare species (Lyons 1992; Paller 1995) affects species detection. Therefore, the relationship of fish assemblages within a reach to those at greater spatial scales (e.g., segments, entire streams, catchments) is not well understood.

Many studies have attempted to determine the sampling effort or sampled length, based on mean stream width (MSW), required in wadeable streams reaches to collect a high percentage (i.e., 90%–100%) of the species present (Angermeier and Smogor 1995; Dauwalter and Pert 2003; Reynolds et al. 2003). Lyons (1992) found that the length of sampled reaches required to obtain asymptotic species richness ranged from 32 to 104 MSWs (mean = 29) in Wisconsin streams, whereas Paller (1995) determined sampled reach lengths of 35–158 MSWs (mean = 87) were required in South Carolina coastal plain streams. Therefore, the sampling effort required to obtain asymptotic species richness in streams may vary among regions and (or) stream size. However, the need to develop standardized methods for sampling fish assemblages has resulted in established protocols that recommend a sampling length of 40 MSW (Peck et al. 2006). These programs are often focused on determining conditions and monitoring trends over time, so fish assemblage sampling is typically conducted at a single reach (Moulton et al. 2002). It is therefore necessary to evaluate the natural variability in fish assemblage characteristics with multiple reaches sampled within stream segments using these standardized methods.

Most studies of sampling effort do not extensively sample multiple reaches at each stream. However, Matthews (1990) found that increasing the number of sampling sites better represented the fish assemblage in a Virginia, USA, river. Smith and Jones (2005) determined that first- to third-order streams of Great Lakes watersheds required 76–115 sampled reaches of lengths equal to 30 MSWs to collect 100% of estimated species richness. Additionally, Peterson and Rabeni (1995) determined that at least 24 sampled channel classification units (i.e., scour, slackwater, transition, and riffle) that ranged in size from 33 to 660 m<sup>2</sup> were needed to obtain species richness with a 10% level of precision in Missouri

streams and that spatial variation exceeded temporal variation. This suggests that more samples may be needed to accurately describe the fish assemblage structure in many streams because of habitat heterogeneity. For rare fishes, high variation in catch rates leads to low precision and thus an unobtainable number of samples needed to detect trends (Paukert 2004). Even monitoring trends in common species may require more sampling than is feasible (Quist et al. 2006). Therefore the evaluation of the effort needed in wadeable streams to describe assemblage structure and monitor fish populations is important to both local and regional ecosystem management.

Our objectives were to determine the sampling effort needed to describe fish assemblages in four midsized (i.e., 10–29 m mean width), wadeable Great Plains streams across large spatial extents (i.e., 20–28 km stream segments) and to attempt to relate differences in sampling effort required to estimate assemblage characteristic and habitat differences between streams. Specifically, we wanted to determine (i) the number and length of sampled reaches required to collect precise (75%, 90%, and 100%) estimates of all species and common species (i.e., comprising >1% of the cumulative catch for each stream) within stream segments and (ii) the number and length of sampled reaches required to detect 25%, 50%, and 75% changes in CPUE (with  $\beta = 0.80$ ) of common fish species. Differences in the number and length of sampled reaches required to describe species richness estimates and changes in CPUE between streams were evaluated using (i) species–area relationships to determine differences in species accumulation rates, (ii) fish assemblage similarity among sampled reaches to determine the effect of fish assemblage structure, and (iii) physical habitat data. Our findings increase the understanding of the relationship of fish assemblage data collected at individual reaches to those at larger spatial scales in midsized wadeable streams with varying species distributions, assemblage structure, and habitat structure. We provide researchers with a reference for the appropriate sampling effort needed to improve the development of wadeable stream protocols when estimating species richness and monitoring fish CPUE at large spatial scales (i.e., stream segments).

## Materials and methods

### Site description

Four streams in Nebraska and Kansas, USA, were sampled from 15 May to 30 June 2006, with individual streams sampled in five days or less to minimize temporal variation among samples within streams. Streams were selected to represent stream characteristics found in the Great Plains and were located in three of the 12 major basins (i.e., Kansas, Niobrara, and Platte) of the Missouri River. Streams were also selected on the basis of access so that intensive longitudinal sampling (i.e., 20–28 km) could be conducted in a localized area to determine species presence for the stream segment. The Niobrara River, a National Scenic River, is located in north-central Nebraska and is a tributary to the Missouri River. Blue Creek is located in western Nebraska and is a tributary to the North Platte River. The North Loup River is a tributary to the Loup River that drains into the Platte River. The North Loup River is located in the

**Table 1.** Characteristics of 10 sampled reaches in each of four streams in Nebraska and Kansas sampled in the summer of 2006.

	Blue Creek	Niobrara River	North Loup River	West Branch Mill Creek
Species richness ( <i>N</i> )	13	21	19	32
Stream-segment length (m)	28 416	20 125	25 232	25 719
Mean distance between sampled reaches (m)	3 157 (330)	2 236 (202)	2 804 (409)	2 858 (436)
Mean width (m)	9.94 (27.1)	28.89 (19.2)	10.95 (23.8)	11.39 (43.7)
Mean depth (m)	0.45 (13.4)	0.16 (26.7)	0.33 (17.4)	0.36 (69.6)
Filamentous algae (% cover)	6.39 (26.3)	2.15 (53.2)	0.54 (152.8)	21.54 (100.0)
Macrophytes (% cover)	30.59 (56.9)	0.59 (130.4)	13.06 (74.0)	0.00 (0.0)
Large woody debris (% cover)	0.00 (0.0)	0.03 (144.1)	0.00 (0.0)	0.33 (64.0)
Small woody debris (% cover)	0.28 (64.7)	0.08 (203.7)	0.00 (0.0)	0.32 (200.3)
Overhanging vegetation (% cover)	13.12 (76.3)	1.10 (48.6)	1.63 (46.3)	0.10 (122.5)
Undercut bank (% cover)	0.06 (185.4)	0.03 (39.8)	0.06 (100.3)	0.12 (81.6)
Boulder (% cover)	0.00 (0.0)	0.60 (82.0)	1.12 (128.8)	0.18 (124.1)

**Note:** Standard error of mean distance between sampled reaches and coefficient of variation of habitat variables is given in parentheses.

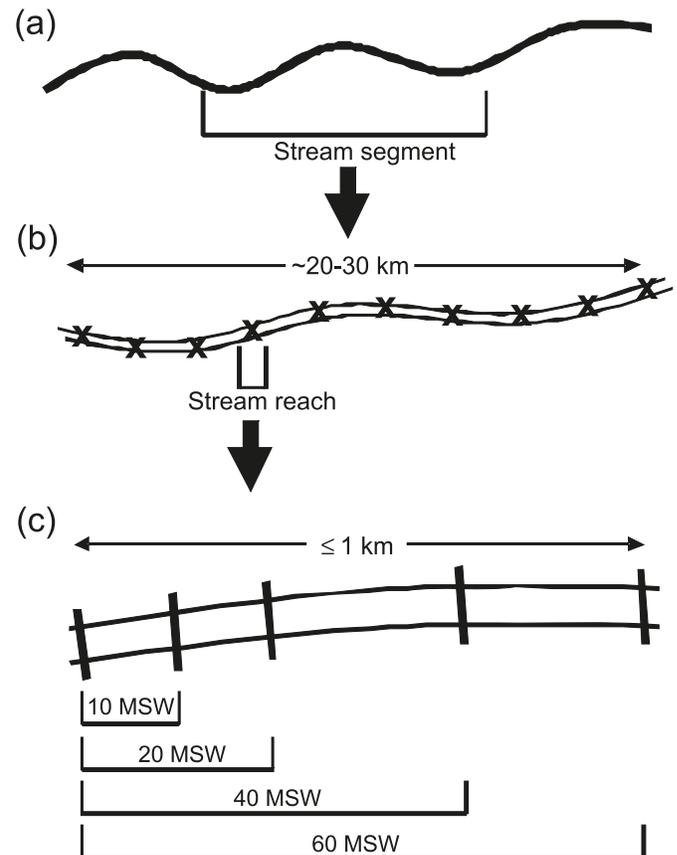
central Nebraska Sandhills region. The West Branch Mill Creek is located in the Flint Hills region of eastern Kansas and is a tributary to the Kansas River. A summary of the physical characteristics of each stream is provided (Table 1).

#### Data collection

Stream-segment estimates of fish assemblage characteristics were determined with 10 systematically sampled reaches with a minimum of 1 km between reaches (Fig. 1). Each sampled reach was at least 60 MSW or 1 km in total length. Sampled reaches were subdivided into sampling lengths of 10, 20, and 40 times the MSW. The four MSW sampled reaches were adjacent to each other (i.e., the 10 MSW reach was included in the 20 MSW reach, etc.). Fish were sampled using a towed, pulsed DC electrofisher with two anode poles. Electrofishing was conducted by two people with anodes and two netters in a zigzag pattern with an emphasis to sample all available habitats (Lazorchak et al. 1998). Fish from each sampled reach were held in fish cages for identification after electrofishing for that entire sampled reach (i.e., 60 MSWs) was complete. Species were identified, counted, and released in the field. Unidentifiable and voucher specimens were preserved in 10% formalin and identified to species and counted in the laboratory.

Physical habitat was surveyed at six reaches on each stream using procedures adapted from the Environmental Protection Agency's (EPA) protocol for sampling wadeable streams (Peck et al. 2006) to determine the effect of habitat on sampling effort required to accurately estimate species richness. The only modification to these techniques was that individual sampling reaches were characterized with six transects (i.e., reduced from 11) with five sections between transects. Wetted width (m) was measured at each transect, and depth (m) was measured at three equally spaced points along the transect (i.e., one-quarter, one-half, and three-quarters of the wetted width distance) and divided by four to calculate transect mean depth (Arend 1999). Stream width and depth were calculated from the mean of transect depths and widths. Instream fish cover categories (filamentous algae, aquatic macrophytes, large woody debris, small woody debris, overhanging vegetation, undercut banks, and

**Fig. 1.** (a–b) Stream segments of approximately 20–30 km were systematically sampled with 10 reaches and a minimum of 1 km between sampled reaches. (c) Sampled reaches were at least 60 times the mean wetted stream width (MSW) in length, with a maximum length of 1 km. Each sampled reach was subdivided into four sampling lengths (10, 20, 40, and 60 MSWs) for fish assemblage sampling.



boulders) were estimated individually using five cover classes (absent, 0%; sparse, <10%; moderate, 10% to 40%; heavy, 40% to 75%; and very heavy, >75%) at each transect (Peck et al. 2006). The midpoint of each percentage

class was used to calculate the mean percent cover for each reach. Coefficients of variation (CV) were calculated for each habitat parameter for each surveyed reach.

### Sampling effort simulations

A Monte Carlo simulation that randomly selected sampled reaches (without replacement) from each stream was conducted to determine the number of sampled reaches needed to detect 75%, 90%, and 100% of all species collected in all samplings within each stream. The simulation methods used were similar to those explained by Quist et al. (2007). Total segment species richness was calculated as all species encountered with all 10 sampled reaches of length equal to 60 MSW or 1 km for each stream. The probability of sampling precise estimates of the total segment species richness was calculated by counting the number of 1000 simulations for each given number of sampled reaches in which species richness met or exceeded 75%, 90%, and 100% of the segment species richness (e.g., proportion of total segment richness with one sampled reach, two sampled reaches, three sampled reaches). The number of sampled reaches needed to detect 75%, 90%, and 100% of the collected species within a stream segment was determined when probability of detecting 75%, 90%, and 100% of segment richness exceeded 95%. Simulation results were plotted for each stream and sampled reach length (i.e., 10, 20, 40, and 60 MSW). All simulations were run using all species collected and then again for common species, defined as species comprising <1% of the cumulative catch for each stream (Paller 1995; Reynolds et al. 2003). Total sampling effort (i.e., cumulative number of MSWs or total sampling length for all sampled reaches) was determined for each combination of reach length and number of reaches required from simulation results (e.g., four sampled reaches of length equal to 20 MSW would equal a total sampling effort of 80 MSW).

### Sample size estimation

The number of reaches needed to detect changes in CPUE (i.e., fish·100 m<sup>-1</sup> of electrofishing) at various statistical power levels was determined using simple interactive statistical analysis (SISA; Uitenbroek 1997), which has been commonly used to determine needed sample sizes in fish studies (Allen et al. 1999; Tate et al. 2003; Paukert 2004). We estimated the number of reaches needed to detect 25%, 50%, and 75% changes in CPUE at four levels of statistical power (i.e.,  $\beta = 0.60, 0.70, 0.80, \text{ and } 0.90$ ) for each species that accounted for greater than 1% of the cumulative catch for each stream. The mean number of sampled reaches needed for all common species was calculated for each stream and sampled reach length among all streams. An analysis of variance (ANOVA) was used to determine if the mean number of sampled reaches required to detect changes in CPUE of common species differed with a level of statistical power of  $\beta = 0.80$  among streams and sampled reach lengths separately. A significance level of  $\alpha = 0.05$  was used for all sample size estimates. Comparisons among the number of required sampled reaches were conducted using pairwise comparisons with significance levels adjusted with a Bonferroni correction ( $\alpha = 0.05/6$  or  $\alpha = 0.008$ ).

### Species–area relationship

The species–area relationship for each stream was calcu-

lated to determine if species accumulation variability among streams would provide insight into differences in the number of sampled reaches required for precise estimates of species richness and to detect changes in CPUE. The cumulative number of species collected and sampled area were log<sub>10</sub>-transformed and plotted for each stream and sampled reach length to determine the rate of species accumulation ( $z$ ) (Ricklefs 2000). Linear regression was used to estimate the slope ( $z$ ) across all sampled reach lengths for each stream. Statistical comparisons of species–area relationships were not conducted due to lack of independence of observations.

### Fish assemblage structure

We evaluated the effect of longitudinal variation of sampled fish assemblages within streams on estimates of species richness among sampled reaches. Species presence or absence within sampled reaches was measured using Jaccard's similarity coefficient, a common metric of community similarity (Krebs 1998), and is useful in determining sampling sufficiency in stream fish assemblage surveys (Cao et al. 2001). Linear regression was used to describe the relationship between the mean Jaccard's similarity coefficient and the distance between sampled reaches to determine the overall fish assemblage variability for each stream. We also evaluated the ability of distance between sampled reaches to predict species similarity with a Mantel test (Mantel 1967). We compared distance matrices of Jaccard's similarity coefficients and distance between sampled reaches with a Mantel test to determine the statistical significances ( $\alpha = 0.05$ ) and strength of the association (Mantel 1967).

### Habitat associations

The effect of instream habitat structure on differences in number of sampled reaches required for precise estimates of species richness and to detect changes in CPUE among streams were evaluated. First, a stepwise discriminant analysis was used to reduce the number of habitat variables and determine the subset of uncorrelated variables that best discriminated among streams (i.e., greatest amount of variation among measured habitat variables) from the set of nine habitat variable means and CVs (see earlier section Data collection). Next, a canonical discriminant analysis (CDA) was conducted with the new subset of significant habitat variables (Johnson 1998) to quantify the greatest amount of variability between streams. Linear regression was used to quantify the relationship between the number of sampled reaches with lengths equal to 40 MSW needed to collect 75%, 90%, and 100% estimates of the total and common species richness and the mean canonical axis scores for each stream.

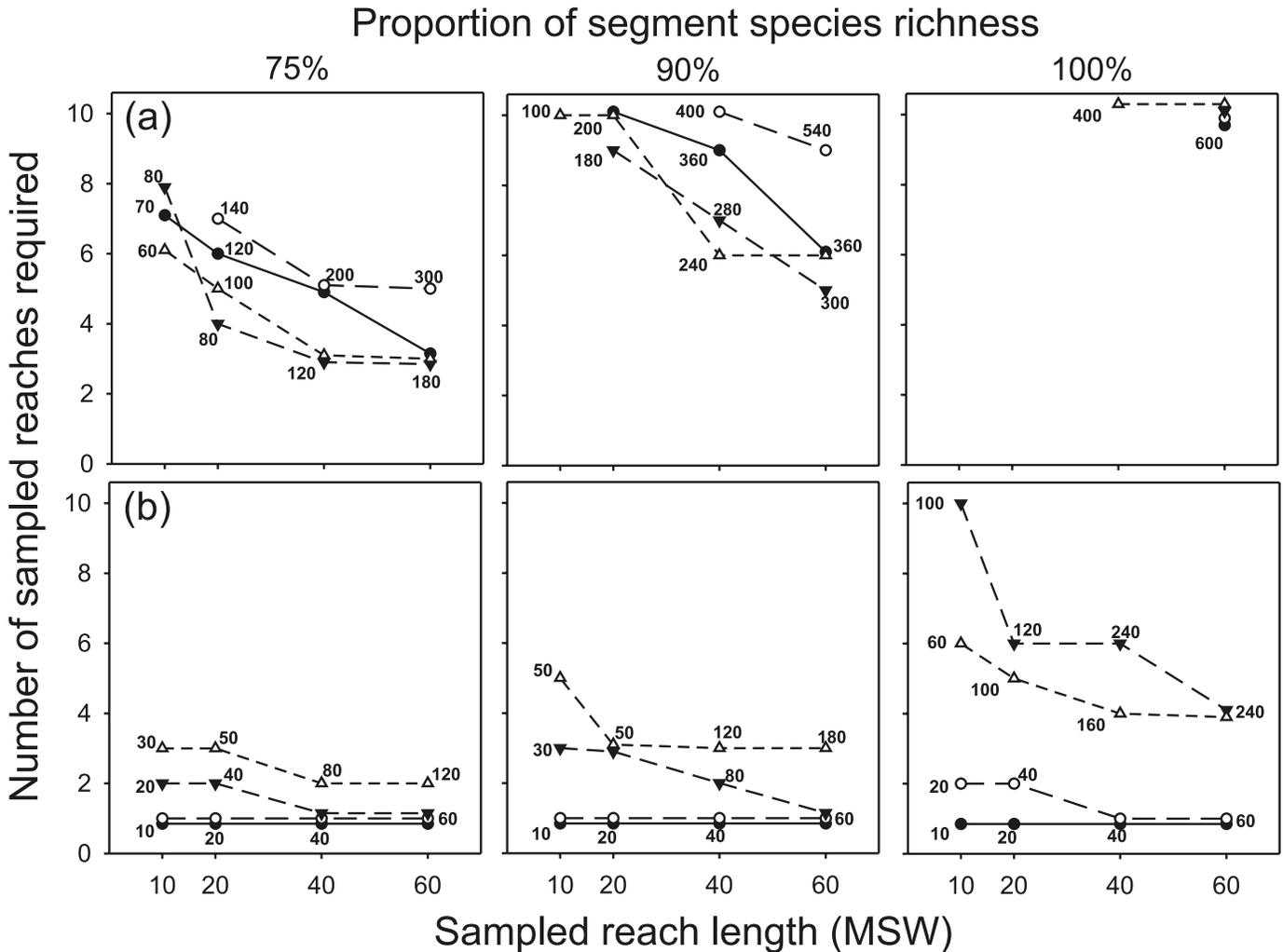
### Results

Overall fish assemblage structure varied among streams. Total species richness ranged from 13 to 32 in the 20.1 to 28.4 km segments for the four streams sampled (Table 2). The number of fish sampled in each stream segment ranged from 2660 in Blue Creek to 27 894 in the Niobrara River (Table 2). Individual species CPUE ranged from 0 to 81.1 ( $\bar{x} = 4.6$ ) for Blue Creek, from 0 to 259.2 ( $\bar{x} = 15.5$ ) for the Niobrara River, 0 to 83.6 ( $\bar{x} = 5.6$ ) for the North Loup

**Table 2.** Percent abundance of fishes collected from four midsized streams of Nebraska and Kansas sampled using a towed electrofishing unit, summer 2006.

Scientific name	Common name	Blue Creek (N = 2660)	Niobrara River (N = 27 894)	North Loup River (N = 5273)	West Branch Mill Creek (N = 23 600)
<i>Lepisosteus osseus</i>	Longnose gar				0.068
<i>Campostoma anomalum</i>	Central stoneroller		0.183		36.78
<i>Cyprinella lutrensis</i>	Red shiner		11.325	3.717	6.475
<i>Cyprinus carpio</i>	Common carp		0.018	0.133	0.992
<i>Hybognathus hankinsoni</i>	Brassy minnow		0.09	5.385	
<i>Luxilus cornutus</i>	Common shiner				2.674
<i>Lythrurus umbratilis</i>	Redfin shiner				0.106
<i>Notropis dorsalis</i>	Bigmouth shiner	0.226	25.79	7.225	
<i>Notropis rubellus</i>	Rosyface shiner				9.737
<i>Notropis stramineus</i>	Sand shiner	0.414	47.58	15.172	1.479
<i>Notropis topeka</i>	Topeka shiner				0.017
<i>Phenacobius mirabilis</i>	Suckermouth minnow				6.869
<i>Phoxinus eos</i>	Northern redbelly dace			4.077	
<i>Phoxinus erythrogaster</i>	Southern redbelly dace				1.742
<i>Pimephales notatus</i>	Bluntnose minnow				11.051
<i>Pimephales promelas</i>	Fathead minnow	0.226	1.391	0.778	0.068
<i>Pimephales vigilax</i>	Bullhead minnow				0.034
<i>Platygobio gracilis</i>	Flathead chub			5.765	
<i>Semotilus atromaculatus</i>	Creek chub	72.782	2.416	2.333	1.326
<i>Rhinichthys cataractae</i>	Longnose dace	1.466	0.549	36.45	
<i>Carpionodes carpio</i>	River carpsucker			0.91	0.03
<i>Catostomus commersonii</i>	White sucker	10.301	6.586	9.71	4.742
<i>Moxostoma erythrum</i>	Golden redbreast				1.114
<i>Moxostoma macrolepidotum</i>	Shorthead redbreast		3.09	1.346	
<i>Ameiurus melas</i>	Black bullhead			0.057	0.047
<i>Ameiurus natalis</i>	Yellow bullhead				0.225
<i>Ictalurus punctatus</i>	Channel catfish			0.114	
<i>Pylodictis olivaris</i>	Flathead catfish				0.14
<i>Noturus exilis</i>	Slender madtom				2.415
<i>Noturus flavus</i>	Stonecat	0.639	0.312	5.86	0.004
<i>Esox lucius</i>	Northern pike		0.007	0.019	
<i>Oncorhynchus mykiss</i>	Rainbow trout	0.038	0.004		
<i>Salmo trutta</i>	Brown trout	0.376			
<i>Fundulus sciadicus</i>	Plains topminnow	0.902	0.484		
<i>Fundulus zebrinus</i>	Plains killifish		0.004	0.645	
<i>Culaea inconstans</i>	Brook stickleback	0.301	0.004		
<i>Ambloplites rupestris</i>	Rock bass		0.054		
<i>Lepomis cyanellus</i>	Green sunfish	0.564		0.303	2.161
<i>Lepomis humilis</i>	Orangespotted sunfish				0.008
<i>Lepomis macrochirus</i>	Bluegill		0.004		0.318
<i>Lepomis megalotis</i>	Longear sunfish				3.106
<i>Micropterus salmoides</i>	Largemouth bass		0.004		0.288
<i>Etheostoma nigrum</i>	Johnny darter				0.585
<i>Etheostoma spectabile</i>	Orangethroat darter	11.767			5.174
<i>Perca flavescens</i>	Yellow perch		0.108		
<i>Percina caprodes</i>	Logperch				0.216
<i>Percina maculata</i>	Blackside darter				0.013

**Fig. 2.** Number of sampled reaches required to detect 75%, 90%, and 100% of segment species richness for four sampling lengths (i.e., 10, 20, 40, and 60 mean stream widths, MSW) of four wadeable streams in Nebraska and Kansas based on 1000 Monte Carlo simulations. Values are (a) for all species and (b) after the removal of rare species (i.e., species accounting for <1% of the cumulative catch for a stream). Symbols: Blue Creek, ●; Niobrara River, ○; North Loup River, ▼; West Branch Mill Creek, △. Total sampling effort (i.e., cumulative number of MSW) is indicated adjacent to symbols. Missing values indicate that the number of reaches required was greater than our maximum number of reaches. The numbers of reaches required represent whole numbers and symbols are offset for clarity.



River, and 0 to 536.0 ( $\bar{x} = 14.0$ ) for the West Branch Mill Creek. The number of rare species ranged from eight in the North Loup River to 17 in West Branch Mill Creek. These species cumulatively represented 1.8% to 3.7% of the total fish collected for each stream. The proportion of rare species in the North Loup River (42.1%) and West Branch Mill Creek (53.1%) was less than that in the Niobrara River (66.7%) and Blue Creek (69.2%) (Table 2).

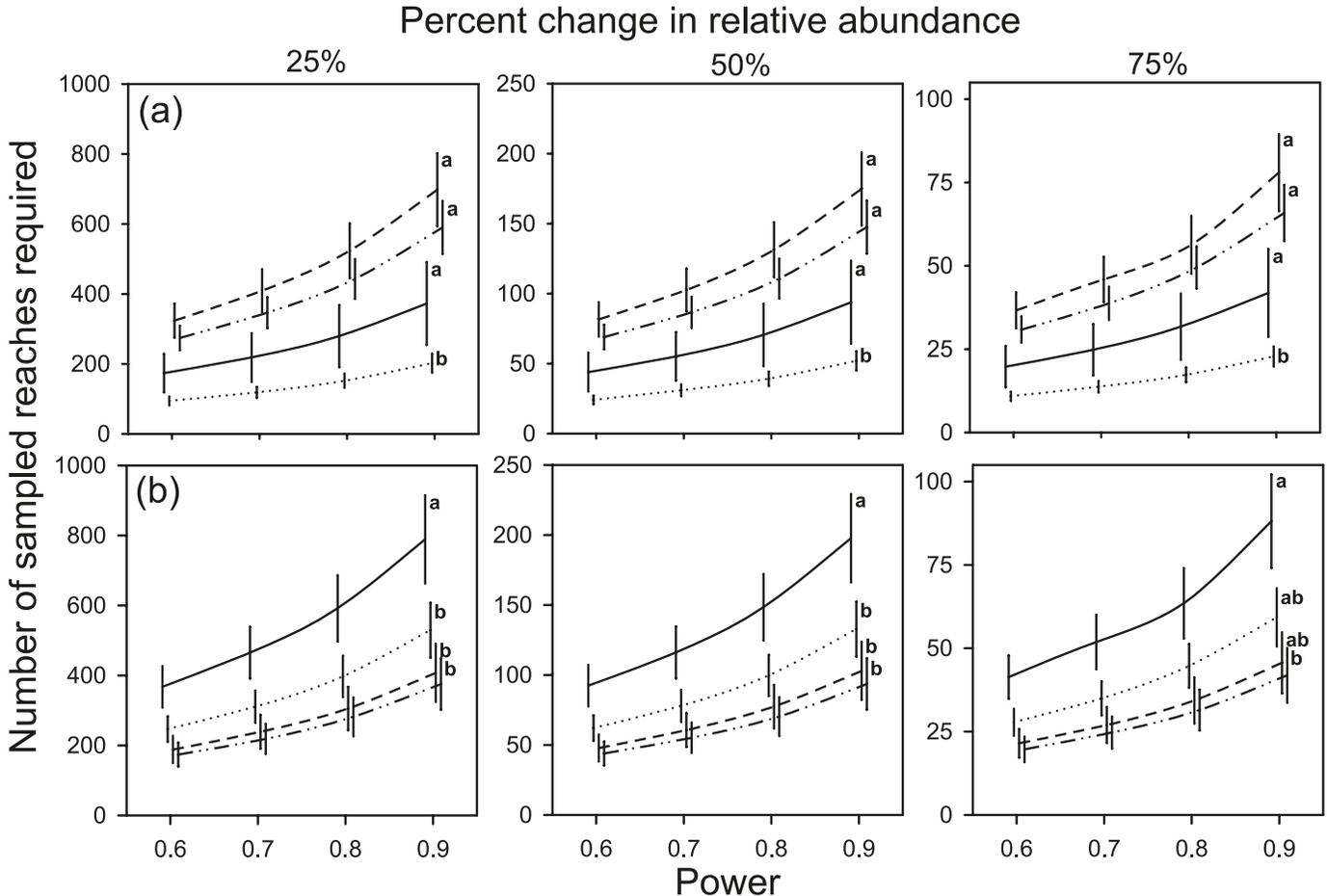
Habitat structure differed greatly between streams. Niobrara River was the shallowest (0.16 m) and widest (28.9 m) stream sampled with the least variability in width. Blue Creek was the narrowest (9.9 m) and deepest (0.45 m) stream with the least variability in depth, whereas North Loup River and West Branch Mill Creek were similar in mean width (11.0 and 11.4 m). West Branch Mill Creek exhibited the most variability in mean width and mean depth (Table 1). Mean percent cover of instream habitat variables and CVs varied considerably among streams (Table 1). For example, Niobrara River and West Branch Mill Creek had

greater CVs for small woody debris, whereas Blue Creek and North Loup River had higher CVs for undercut banks.

**Sample effort simulations**

Total sampling effort to obtain 75% of the segment species richness was lowest with sampled reaches 10 MSW in length, which required six to eight sampled reaches and ranged from 60 to 80 MSWs of total sampling effort (Fig. 2a). However, 75% of all species were only obtained within three of the four streams with sampled reach lengths of 10 MSW (i.e., 75% of all species were not collected with all 10 sampled reaches in Niobrara River). Increasing reach length typically decreased the number of sampled reaches needed to collect 75% of the species. However, total effort either increased or remained constant with fewer sampled reaches of lengths greater than 10 MSW. For example, only three sampled reaches of 40 MSW were needed to collect 75% of all species in North Loup River, but this required a total effort of 120 MSWs (i.e., three sampled reaches of 40

**Fig. 3.** Mean number of sampled reaches required to detect a 25%, 50%, and 75% change in catch-per-unit-effort (i.e., fish·100 m<sup>-1</sup>) of common fish species (i.e., after removal of rare species, <1% cumulative catch) for (a) four streams of Nebraska and Kansas and (b) four sampled reach lengths (mean stream width, MSW) at different levels of statistical power. In a, the solid line represents Blue Creek, the dotted line represents Niobrara River, the broken line represents North Loup River, and the dashed-dotted line represents West Branch Mill Creek. In b, the solid line represents 10 MSW, the dotted line represents 20 MSW, the broken line represents 40 MSW, and the dashed-dotted line represents 60 MSW. Bars represent one standard error. The mean number of sampled reaches needed with the same letter did not differ among (a) streams or (b) sampled reach length (analysis of variance (ANOVA), *P* > 0.008) at a 0.8 level of statistical power.



MSW). To achieve the same proportion of species richness, four sampled reaches with lengths of 20 MSW were needed (i.e., 80 MSWs of total sampling effort), which was equal to eight sampled reaches with lengths of 10 MSW (Fig. 2a). There was a greater amount of total sampling effort required to collect 90% of the species (i.e., 100–540 MSWs total sampling effort; Fig. 2a) in all streams, but total sampling effort was still lowest with a greater number of shorter sampled reaches. To estimate 100% of the segment species richness, all 10 sampled reaches with lengths of 60 MSWs were required for three streams (Fig. 2a), the maximum amount of sampling conducted in our study. All species were collected with 10 sampled reaches with lengths of 40 MSW for West Branch of Mill Creek. No combination of sampled reaches with lengths of 10 or 20 MSW collected 100% of the species (Fig. 2a).

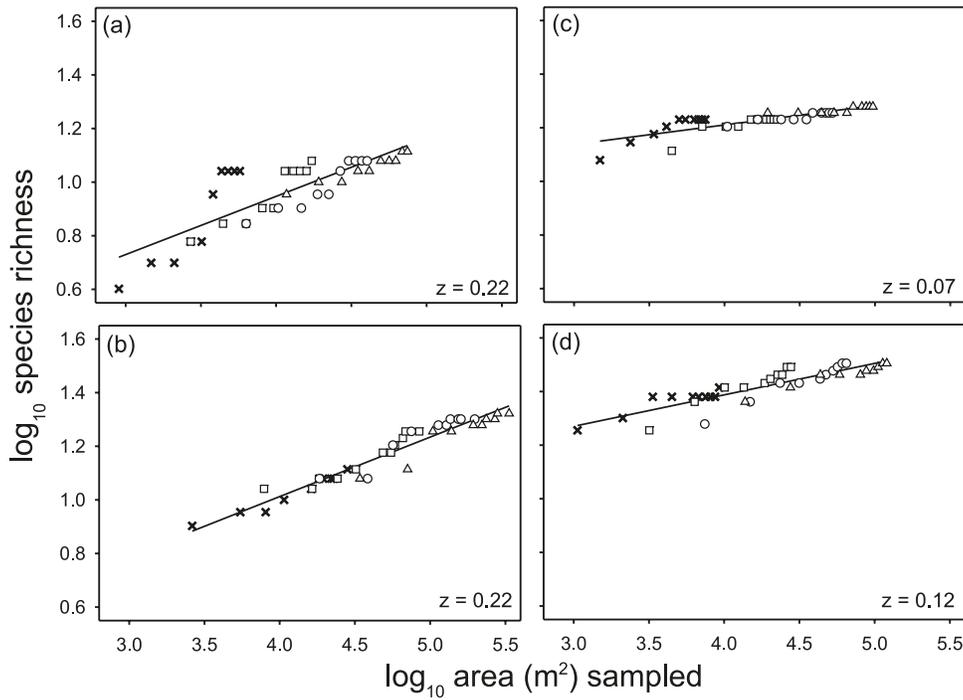
The number of sampled reaches required to obtain similar estimates of common species (compared with that required for all species) decreased substantially for all streams (e.g., all common species were collected with just one sampled reach with a length of 10 MSW in Blue Creek) (Fig. 2b).

The number of sampled reaches required to estimate 75% of the segment common species richness ranged from one to three sampled reaches with lengths of 10 MSW. Total sampling effort was less with sampled reaches of 10 MSW lengths for all streams (i.e., 10–30 MSWs of total sampling effort). One to five sampled reaches with lengths of 10 MSW were required to obtain 90% of the common species for all streams (Fig. 2b). Total sampling effort required to obtain 100% of common species ranged from 10 to 100 MSWs of total sampling effort between all streams and sampled reach lengths. Despite a greater number of sampled reaches, total sampling effort was minimized when sampled reach lengths were shorter (i.e., 10 MSWs). Overall, less effort was required to obtain common species than all species between all streams.

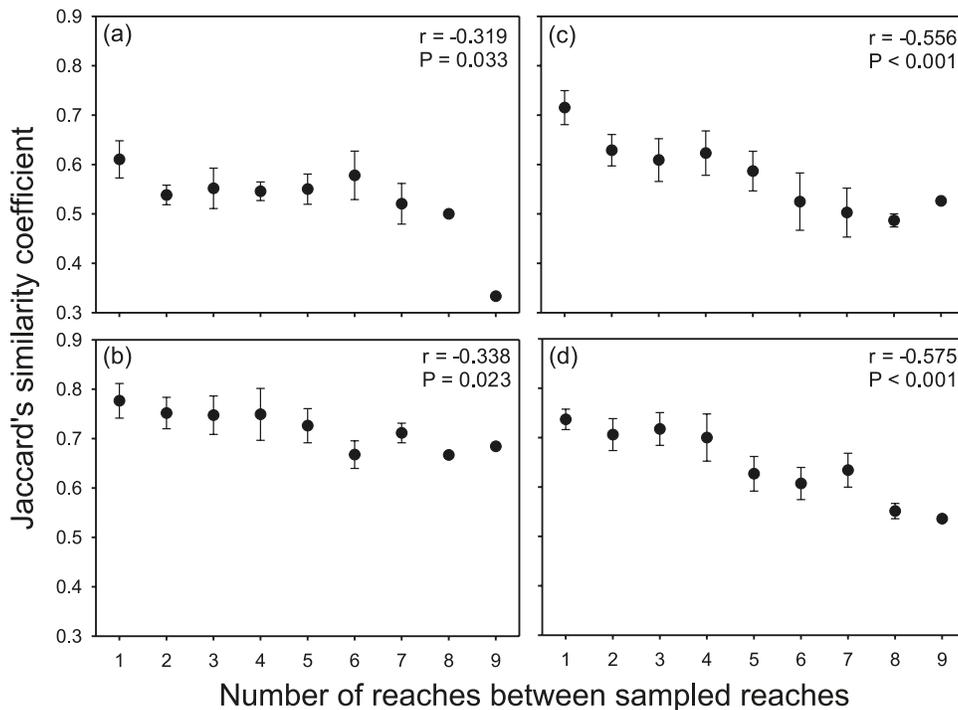
**Sample size estimation**

The mean number of sampled reaches required to detect 25%, 50%, and 75% changes in CPUE of common species ranged from 63 to 994, 17 to 249, and 8 to 111, respectively, among all levels of power for all streams (Fig. 3).

**Fig. 4.** Species–area relationships for four mid-sized streams in Nebraska and Kansas sampled in May–June 2006: (a) Blue Creek; (b) Niobrara River; (c) North Loup River; and (d) West Branch Mill Creek. Symbols represent length of sampled reaches for 10 (×), 20 (□), 40 (○), and 60 (△) mean stream widths. Lines represent linear regressions and  $z$  is the slope.



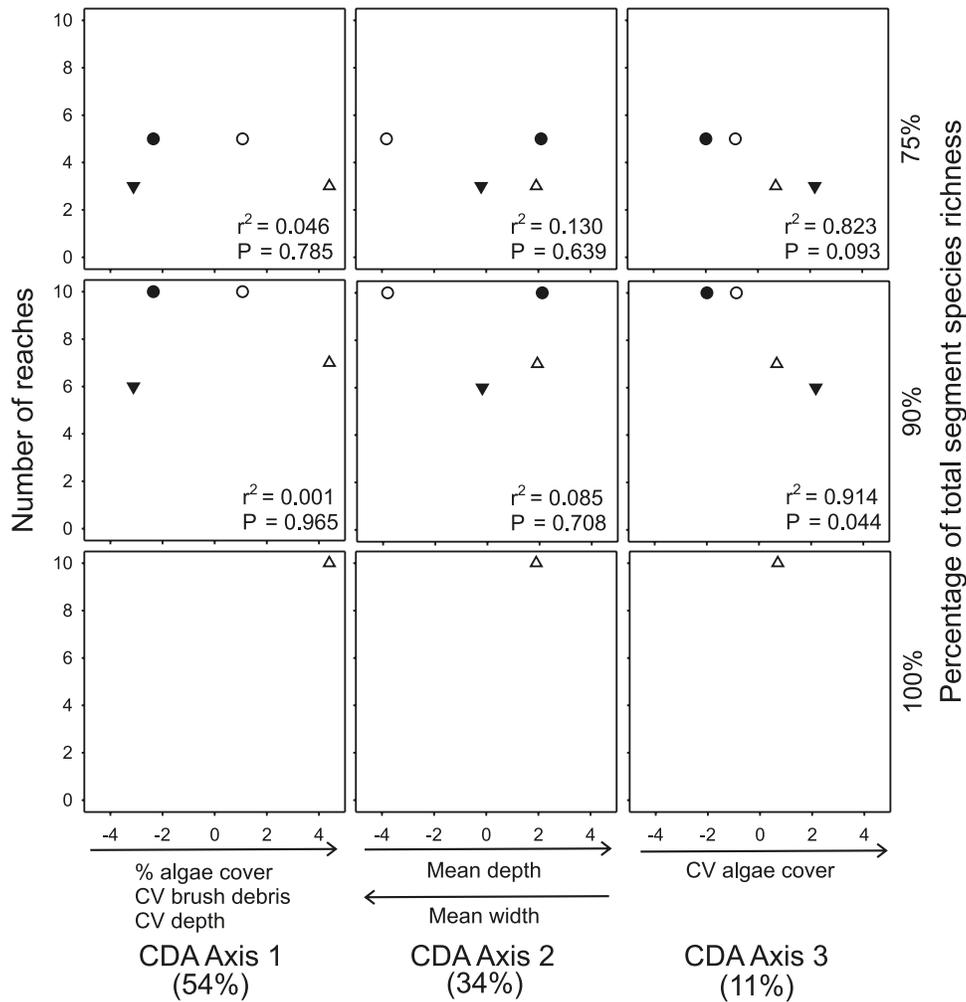
**Fig. 5.** Relationship between distance between samples (i.e., number of sampled reaches between selected 40 mean stream width reaches sampled) and mean Jaccard's similarity coefficients of sampled fish assemblages for four mid-sized streams in Nebraska and Kansas sampled with a pulsed DC, towed electrofisher: (a) Blue Creek; (b) Niobrara River; (c) North Loup River; and (d) West Branch Mill Creek. Error bars represent one standard error.



The number of sampled reaches needed varied among streams ( $F_{[3,144]} = 5.20, P < 0.01$ ) and sampled reach lengths ( $F_{[3,144]} = 4.10, P < 0.01$ ) for 25% changes in CPUE, among

streams ( $F_{[3,144]} = 5.07, P < 0.01$ ) and sampled reach lengths ( $F_{[3,144]} = 4.10, P < 0.01$ ) for 50% changes in CPUE, and among streams ( $F_{[3,144]} = 4.74, P < 0.01$ ) and sampled reach

**Fig. 6.** Relationship between the number of sampled reaches of lengths equal to 40 mean stream widths required to detect 75%, 90%, and 100% of segment species richness with a 95% probability and the mean canonical coefficients from the canonical discriminant analysis (CDA) of 24 habitat surveys from four midsized streams in Nebraska and Kansas: Blue Creek, ●; Niobrara River, ○; North Loup River, ▼; and West Branch Mill Creek, △. Note that the number of sampled reaches required to detect 100% of total segment richness was greater than 10 (i.e., unknown and missing from plot) for all streams except West Branch Mill Creek.



lengths ( $F_{[3,144]} = 3.50, P = 0.02$ ) for 75% changes in CPUE. The number of sampled reaches (at  $\beta = 0.80$ ) needed to detect 25%, 50%, and 75% changes in CPUE for North Loup River, West Branch Mill Creek, and Blue Creek did not differ ( $P > 0.008$  after Bonferroni correction) and were greater than the number of sampled reaches required to detect changes for Niobrara River (Fig. 3). The number of sampled reaches needed to detect changes decreased as the length of sampled reach increased. However, the number of sampled reaches required to detect 25% and 50% changes in CPUE did not differ ( $P > 0.008$ ) between reach lengths of 20, 40, and 60 MSWs and were less than the number of sampled reaches required for sampled reaches of lengths of 10 MSWs (Fig. 3). Overall, the number of sampled reaches needed to detect changes was high. Even for common species, the number of sampled reaches of lengths of 60 MSW required to detect a 75% change in CPUE of common species (at  $\beta = 0.80$ ) was still greater than 25% for stream segments of 20–28 km in length.

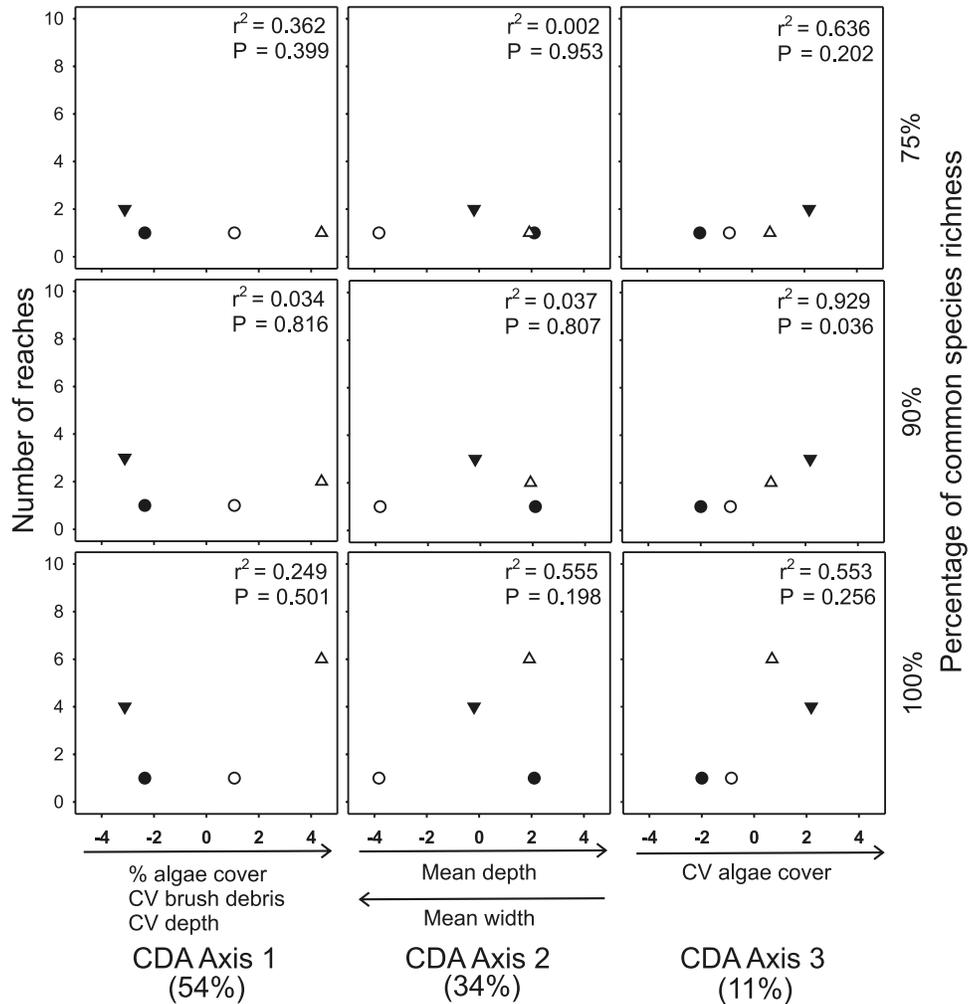
**Species–area relationship**

The observed rate of species accumulation ( $z$ ) was calculated for all streams with sampled reach lengths combined (Fig. 4). The rate of species accumulation was lowest from sampled fish assemblages of North Loup River ( $z = 0.07$ ), followed by West Branch Mill Creek ( $z = 0.12$ ). The rate was similar for samples from Blue Creek and Niobrara River ( $z_s = 0.22$ ) and was greater than those observed from North Loup River and West Branch of Mill Creek.

**Fish assemblage structure**

Jaccard’s similarity of sampled fish assemblages decreased as the distance between sampled reaches increased for all streams (Fig. 5). The relationship between Jaccard’s similarity coefficients and the number of reaches between sampled reaches was more variable in Blue Creek ( $r^2 = 0.10$ ) and Niobrara River ( $r^2 = 0.11$ ) than in North Loup River ( $r^2 = 0.31$ ) and West Branch Mill Creek ( $r^2 = 0.33$ ). There was no difference in the rates of assemblage similar-

**Fig. 7.** Relationship between the number of sampled reaches of lengths equal to 40 mean stream widths required to detect 75%, 90%, and 100% of common segment species richness (i.e., species accounting for <1% of the cumulative catch for a stream segment removed) with a 95% probability and the mean canonical coefficients from the canonical discriminant analysis (CDA) of 24 habitat surveys from four mid-sized streams in Nebraska and Kansas: Blue Creek, ●; Niobrara River, ○; North Loup River, ▼; and West Branch Mill Creek, △.



ity decrease by distance among streams ( $F_{[3,172]} = 1.72$ ,  $P = 0.16$ ), but rates of similarity decrease tended to be greater for sampled fish assemblages of North Loup River ( $-0.03$ ) and West Branch Mill Creek ( $-0.02$ ) than for sampled fish assemblages of Blue Creek ( $-0.01$ ) and Niobrara River ( $-0.01$ ). A negative correlation of sampled fish assemblage similarity and distance between reaches was observed in all streams except the Niobrara River (Mantel test,  $r = -0.26$ ,  $P = 0.06$ ). A weak correlation existed for the Blue Creek assemblage (Mantel test,  $r = -0.37$ ,  $P < 0.01$ ), whereas assemblages of North Loup River (Mantel test,  $r = -0.55$ ,  $P < 0.01$ ) and West Branch Mill Creek (Mantel test,  $r = -0.66$ ,  $P < 0.01$ ) had greater decrease in fish assemblage similarity as distance between sampled reaches increased. Greater fish assemblage similarity existed among sampled reaches of Blue Creek and Niobrara River, whereas North Loup River and West Branch Mill Creek exhibited less fish assemblage similarity among all sampled reaches.

#### Habitat associations

Stream width, depth, and percent cover of filamentous al-

gae, depth CV, percent cover of filamentous algae CV, and percent cover of brushy debris CV discriminated among streams ( $P_s < 0.05$ ). Three axes of the CDA were significant ( $P_s < 0.05$ ) (Figs. 6 and 7). The first canonical axis accounted for 54% of total variability and was a gradient of percent cover of filamentous algae, CV of small woody debris percent cover, and CV of depth. Mean width and mean depth had the highest loadings on the second canonical axis and explained 34% of the total variability. The CV of filamentous algae percent cover was the only variable with a high loading on the third axis, which explained 11% of the total variation, and separated North Loup River and West Branch Mill Creek from streams with less variability in the percent cover of filamentous algae. The relationship of the mean standardized canonical axis scores of the first and second canonical axes and the number of sampled reaches needed to obtain 75%, 90%, and 100% of total (Fig. 6) and common species (Fig. 7) richness for each stream were not significant ( $P_s > 0.10$ ). The relationships of the mean canonical axis scores of the third canonical axis and the number of sampled reaches needed to detect 75% and 90% of total spe-

cies richness were significant ( $P = 0.09$  and  $0.04$ , respectively) and negative ( $\beta = -1.43$  and  $-0.85$ , respectively), indicating that as CV of filamentous algae increased, the number of sampled reaches needed to detect 75% and 90% of segment species richness decreased. After removal of rare species, the relationship between the third mean canonical axis scores and the number of sampled reaches needed to detect 75% of common species was the only significant relationship (Fig. 7;  $P = 0.04$ ). This relationship was positive ( $\beta = 1.83$ ), which indicates that by removing rare species, fewer sampled reaches were required with increased variation of filamentous algae cover.

## Discussion

The ability to precisely estimate species richness at stream-segment spatial scales and detect changes in abundances of species over time has important implications for the conservation and management of stream fish assemblages. Overall, the four Great Plains streams sampled in our study represented a diversity of species richness, habitat, and assemblage structure. Moreover, the sampling effort needed for precise estimates of segment species richness and abundance differed among streams and habitat complexity. Despite these differences, our results suggested that consistent patterns of required sampling effort were influenced by fish assemblage and habitat structure within streams. We believe that our results provide a framework for researchers to develop and revise protocols for wadeable stream fish assemblage sampling across stream-segment spatial scales.

Our results suggested that moderate levels (i.e., 75%) of segment species richness are only obtainable after sampling three to five reaches with lengths equal to the widely accepted protocol of 40 MSWs (Peck et al. 2006) and more accurate levels (i.e., 90%) of segment species richness were only obtained after an extensive number of sampled reaches (i.e., six to 10 sampled reaches of lengths equal to 40 MSW) in relatively small stream segments of 20–28 km. However, because the total sampling effort required (cumulative MSWs) was consistently greater for longer sampled reaches, an increased number of shorter sample reaches would characterize stream segments with less total effort. The effort required to obtain segment species richness was consistently higher in Niobrara River and Blue Creek and consistently lower in West Branch Mill Creek and North Loup River, suggesting that stream characteristics (e.g., habitat complexity) affected sampling variability. Our results from Great Plains streams indicated that more homogeneous streams require more sampling effort to characterize fish assemblage structure, which is consistent with findings in streams from Illinois and Virginia (Angermeier and Smogor 1995). The failure of all four stream simulations to reach asymptotic levels of segment species richness suggests that there are discontinuous distributions or low densities for some species (Angermeier and Smogor 1995; Paller 1995), which was minimized after the removal of rare species. A species probability of occurrence in the available habitat is strongly correlated to the species relative abundance (Angermeier and Smogor 1995), suggesting that rare species are less likely to occupy all available habitats, which decreases the chance of being collected in a single sample.

The number of sampled reaches required to obtain precise estimates of common species richness for the entire segment were consistently lower for Niobrara River and Blue Creek and higher for North Loup River and West Branch Mill Creek simulations. The increased number of sampled reaches needed to collect estimates of species richness within Niobrara River and Blue Creek when all species were included was likely caused by rare species, which were not sampled at a majority of sampled reaches. Although total segment species richness may not be a feasible objective, sampling effort decreased considerably when only common species were considered and would be much more reasonable to most managers and researchers of similar systems if common species were the focus of the study objectives. Our findings suggested that well-established standardized protocols used by agencies for monitoring fish assemblages and their responses to environmental change may not accurately characterize fish assemblages at larger spatial scales and may fail to catch species of low relative abundance within an individual stream segment.

Overall, a large number of sampled reaches would be needed to detect trends in the relative abundance of common stream fishes in the four Great Plains streams sampled, which is similar to other studies in rivers and streams (Peterson and Rabeni 1995; Paukert 2004; Quist et al. 2006). However, unlike other studies of sample size estimation, we determined that increasing sampled reach length decreased the number of sampled reaches needed to detect trends in relative abundance of fish species. This suggests that longer sampled reaches decreased variability in CPUE for common species. However, increasing the length of sampled reaches beyond 20 MSWs did not decrease the number of sampled reaches required to detect changes in CPUE. A similar pattern in the number of sampled reaches needed to obtain varying levels of total segment species richness estimates was observed in the estimated number of sampled reaches needed to detect trends in CPUE. Streams with greater habitat complexity and less overall assemblage similarity between sampled reaches required more sampled reaches to detect trends in CPUE than streams with less habitat complexity and greater similarity of sampled fish assemblages. Because only common species were used in the sample size estimates, our results indicated that greater variability in CPUE may be influenced by increased habitat complexity and were not a result of discontinuous distributions of rare species, which is in contrast to findings by Angermeier and Smogor (1995).

The species–area relationship has been well established over the last century (Arrhenius 1921; Williams 1964; Rosenzweig 1995) and has been the foundation of many studies focused on determining the balance between inaccurately characterizing assemblages by undersampling and cost-prohibitive oversampling. Although increasing sampling area and encountering more species may be the result of more effectively sampling the available species (Connor and McCoy 1979), increasing sampling area may also increase the habitat diversity sampled, which can support a greater number of species (Williams 1964; Cam et al. 2002). Within our study streams, the rate of species accumulation differed among streams but apparently was not affected by stream species richness. This may suggest that

the relationship between species and area may not be attributable strictly to sampling phenomena but is likely a combination of habitat complexity, species distributions, and sampling (Angermeier and Schlosser 1989). Additionally, our results from the species–area relationships were consistent with the effort required to obtain various levels of segment richness among streams. For example, Niobrara River and Blue Creek required consistently less effort than North Loup River and West Branch Mill Creek, further suggesting that habitat complexity and species distributions contributed to the differing rates of species accumulation, which is similar to findings of Angermeier and Smogor (1995). In contrast, other studies of species accumulations and sampling effort have found varying effects of habitat. Weak or uncorrelated habitat effects were found by Lyons (1992), Hughes et al. (2002), and Smith and Jones (2005), whereas Paller (1995) and Dauwalter and Pert (2003) found stream size best explained differences in sampling efforts.

Fish assemblage similarity increases with decreased distances between samples and is related to the physical habitat that changes as streams increase in size (Platanía 1991; Rachel and Hubert 1991; Edds 1993). Based on our data, assemblage similarity of sampled reaches was related to the number of sampled reaches required for precise estimates of species richness and the ability to detect trends in CPUE. Niobrara River and Blue Creek exhibited lower rates of dissimilarity between samples and greater variation among all sampled reaches than North Loup River and West Branch Mill Creek. Greater rates in these relationships indicate an increased rate of species turnover (i.e., increased dissimilarity as the distance between sampled reaches increases) among all sampled reaches. Additionally, greater variability in Jaccard's similarity of sampled fish assemblages indicated that samples from reaches with a greater likelihood for increased similarity (i.e., sampled reaches in closer proximity to each other) were less likely to be similar, whereas sampled reaches with greater distances between samples had greater assemblage similarity. This is likely due to species (e.g., rare or habitat specialists) that were not consistently sampled at all reaches within a segment. Our results suggested that stream segments with less similar assemblages among all reaches (i.e., North Loup River and West Branch Mill Creek) required fewer sampled reaches to achieve the same proportion of species richness than streams that had more similar assemblages among sampled reaches within the entire segment (i.e., Blue Creek and Niobrara River). Therefore, the random selection of sampled reaches with less similar fish assemblages represented a greater proportion of the stream-segment species richness (i.e., auto-similarity; Cao et al. 2002). Streams with higher species turnover (e.g., low similarity between sampled reaches) should require fewer systematically sampled reaches to characterize a greater proportion of species in the segment than streams with more similar assemblages (Cao et al. 2001). Overall, our results of Jaccard's similarity of fish assemblage samples within streams suggested that given a fixed number of samples to characterize a stream segment, a greater distance between sampled reaches would represent a greater percentage of the segment assemblage.

The increased number of sampled reaches needed to ob-

tain a given proportion of segment species richness in streams with higher variation in percent cover of filamentous algae is likely due to rare species discontinuous distributions and habitat selectivity (Angermeier and Smogor 1995). Because habitat complexity was lower in the streams that required a greater number of sampled reaches to obtain similar estimates of total segment species richness, rare species may be selective to specific habitat types, thus resulting in low overall relative abundances and infrequent collection throughout sampling segments. Stream segments with greater habitat homogeneity present fewer opportunities for rare species to be collected, which in turn may increase the number of sampled reaches needed to collect all species present. Additionally, Angermeier and Schlosser (1989) found that habitat complexity was correlated to species richness in Panama streams but not in streams in Minnesota and Illinois and suggested that the greater stability of streams in Panama may facilitate this relationship. Larger streams with greater habitat heterogeneity and less temporal variation typically have stable fish assemblages (Schlosser 1987). The four midsized Great Plains streams sampled in our study may be better suited for evaluating assemblage variability as related to habitat within stream segments because of their larger and more stable flow regimes relative to smaller streams.

Although a number of studies have described the length of sampled reach needed (number of MSWs) to describe stream fish assemblages within reaches (e.g., Lyons 1992; Patton et al. 2000; Reynolds et al. 2003), our results indicated that multiple sampled reaches within a segment are needed to characterize stream fish assemblages. To our knowledge, only one other study (i.e., Smith and Jones 2005) has evaluated the sampling effort (i.e., with multiple reaches) required to estimate species richness at large spatial extents. Results from Smith and Jones (2005) suggest that 15–119 sampled reaches of lengths equal to 30 MSW were needed to characterize Great Lakes watersheds but found no environmental or assemblage correlates to the required sampling effort. Our results suggested that the number of sampled stream reaches required in Great Plains streams to estimate species richness may not be cost effective because of discontinuous species distributions or low species abundance (40% of the species in our study streams represented less than 4% of the total number of individuals collected in each stream). However, 75% of all fish species were obtained with the least amount of total effort with five to 10 sampled reaches with lengths equal to 20 MSW for all four streams. Monitoring changes in common species relative abundance in Great Plains streams may require an even greater increase in sampling effort. The number of sampled reaches needed to detect presences and monitor changes in relative abundances of stream fishes will ultimately depend on individual study objectives and the scale to which stream fish assemblages are to be characterized. Our results suggested that the sampling effort needed to characterize streams could be decreased by evaluating instream habitat characteristics. Furthermore, previous knowledge of stream fish assemblage structure and species abundances may assist in the establishment of required sampling effort and in determining areas needing additional sampling effort to characterize a greater proportion of species present.

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