Estimating vertebrate, benthic macroinvertebrate, and diatom taxa richness in raftable Pacific Northwest rivers for bioassessment purposes

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Abstract The number of sites sampled must be considered when determining the effort necessary for adequately assessing taxa richness in an ecosystem for bioassessment purposes; however, there have been few studies concerning the number of sites necessary for bioassessment of large rivers. We evaluated the effect of sample size (i.e., number of sites) necessary to collect vertebrate (fish and aquatic amphibians), macroinvertebrate, and diatom taxa from seven large rivers in Oregon and Washington, USA during the summers of 2006–2008. We used Monte Carlo simulation to determine the number of sites needed to collect 90–95% of the taxa 75–95% of the time from 20 randomly located sites on each river. The river wetted widths varied from 27.8 to 126.0 m, mean substrate size varied from 1 to 10 cm, and mainstem distances sampled varied from 87 to 254 km. We sampled vertebrates at each site (i.e., 50 times the mean wetted channel width) by nearshore-raft electrofishing. We sampled benthic macroinvertebrates nearshore through the use of a 500-μm mesh kick net at 11 systematic stations. From each site composite sample, we identified a target of 500 macroinvertebrate individuals to the lowest possible taxon, usually genus. We sampled benthic diatoms nearshore at the same 11 stations from a 12-cm^2 area. At each station, we sucked diatoms from soft substrate into a 60-ml syringe or brushed them off a rock and rinsed them with river water into the same jar. We counted a minimum of 600 valves at 1,000× magnification for each site. We collected 120–211 diatom taxa, 98–128 macroinvertebrate taxa, and 14–33 vertebrate species per river. To collect 90-95% of the taxa 75-95% of the time that were collected at 20 sites, it was necessary to sample 11–16 randomly distributed sites for vertebrates, 13–17 sites for macroinvertebrates, and 16–18 sites for diatoms. We conclude that 12–16 randomly distributed sites are needed for cost-efficient sampling of vertebrate richness in the main stems of our study rivers, but 20 sites markedly underestimates the species richness of benthic macroinvertebrates and diatoms in those rivers.

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Introduction

Because of increased state and federal interest in biological assessments of large unwadeable rivers, a number of papers have recently been published documenting the level of sampling effort needed for adequately estimating the taxa richness of a river site, particularly for fish (Cao et al. 2001; Lyons et al. 2001; Hughes et al. 2002; Dußling et al. 2004; Utrup and Fisher 2006; Eros et al. 2008). Sampling effort studies have also been conducted for assessing the site-scale taxonomic richness of benthic macroinvertebrates in rivers (Li et al. 2001; Cao et al. 2002; Fesl 2002; Flotemersch et al. 2006). Fewer studies have assessed the site-scale sampling effort for diatoms (e. g., Weilhoefer and Pan 2007).

In addition to the need for assessing site-scale sampling effort sufficiency, managers and ecologists are interested in determining the number of sites needed for adequately assessing taxa richness at the extent of entire mainstem rivers or their catchments. This is an important issue because too few sites underestimate taxa richness and too many sites increase monitoring costs unnecessarily (Hughes and Peck 2008; Kanno et al. 2009; Ligeiro et al. 2010). Also, studies of entire rivers as landscapes with emergent properties changing with the spatial extent of the observations have received increased attention in recent years (Schlosser 1991; Ward 1998; Fausch et al. 2002). Smith and Jones (2005) estimated that electrofishing 17–49 sites, each 30 times the mean wetted channel width (MWCW), were needed to collect 90% of the fish species in wadeable streams of nine Michigan watersheds. Based on their research in four wadeable Great Plains streams, Fischer and Paukert (2009) concluded that six to ten electrofishing sites of 40 MWCW were needed to detect 90% of the species in segments 20–28 km long. Blocksom et al. (2009) found that 15 electrofishing sites, each 500 m long, were needed for detecting 90% of the species found in seven Ohio River reaches that were 58–153 km long. Discontinuously distributed, patchy or rare species are the major reason for the reported levels of sampling effort needed to assess fish species richness at site, basin or river spatial extents (Angermeier and Smogor 1995; Cao et al. 2001; Hughes et al. 2002; Eros et al. 2008; Kanno et al. 2009).

Possibly because of their high diversity, fewer studies are available concerning the number of sites needed to adequately assess macroinvertebrate richness in long river reaches or segments. Bartsch et al. (1998) concluded that 18–40 sites, depending on the indicator, were needed for assessing the upper Mississippi River. Raunio and Antilla-Huhtinen (2008) estimated that eight sites were necessary for assessing a large soft-bottomed Finnish river.

We located no rigorous studies of the number of sites need to assess periphyton (diatom) richness in mainstem rivers. Reavie et al. (2010) sampled over 100 sites in each of the Ohio (1,560 km), upper Mississippi (1,400 km), and undammed reaches of the Missouri (2,900 km) Rivers. The Delaware River Basin Commission (2007) samples 25 sites along the 320 km long river. But Miettinen (2007), Raunio and Soininen (2007), Sgro et al. (2007), and Taylor et al. (2007) sampled only 8–11 sites in rivers 115–400 km long.

Following the goals of the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program, our objectives were to determine the single-gear sampling effort needed to collect 90–95% of the vertebrate, macroinvertebrate, and diatom taxa collected from intensive surveys of seven raftable river mainstems (each 87–254 km long) 75–95% of the time. Such percentages also have been reported sufficient for assessing fish assemblage condition through use of multimetric indices of biological integrity at individual sites (Reynolds et al. 2003; Hughes and Herlihy 2007; Maret et al. 2007). Those site-scale levels of sampling effort have also been found sufficient for assessing the dissimilarity of benthic macroinvertebrate assemblages among sites (Cao et al. 2002; Flotemersch et al. 2010). Based on our field experience and the literature, we hypothesized that seven to ten sites would be sufficient for collecting 90–95% of the vertebrate species collected from 20 sites 75–95% of the time, but 14–19 sites would be necessary for the more speciose benthic macroinvertebrate and diatom assemblages. We chose 20 sites as the upper limit for these rivers because our sample site lengths of 50 MWCW meant that with 20 samples we actually electrofished approximately half of the total length of each river mainstem. We also hypothesized that the highest and lowest vertebrate species richness would occur in the largest and smallest catchments, respectively, and that the highest and lowest benthic macroinvertebrate and diatom taxa richness would occur in

the rivers with largest and smallest mean substrate sizes, respectively.

Methods

We sampled seven large unwadeable rivers in Oregon and Washington by raft during the summers of 2006–2008 (Fig. 1; Table 1). We selected those rivers because of interests by tribes or federal or state agencies and because they lacked major dams in the mainstems. We randomly selected 20 sites on each river from the 1:100,000 scale NHDPlus digital line graph (USEPA and USGS 2006) to be unequally dispersed but not overlapping (Stevens and Olsen 2004). The rivers included three with largely forested catchments on the wet west side of the Cascade Mountains and four with largely steppe catchments on the dry east side of the Cascades. They varied in size from the Malheur (27.8 m mean width, 1.0 m mean thalweg depth, 7,847 km^2 catchment area) to the Willamette (126 m mean width, 2.9 m mean thalweg depth, 13,554 km^2 catchment area). Mean slopes of the rivers varied from 0.03% to 0.19%, percent snag incidence per site varied from 0% to 57.7%, percent catchment urbanized varied from 0.2% to 8.5% per site, and percent catchment agriculture varied from 0.4% to 21.7% per site. The mainstem distances sampled varied from 87 to 254 km for the Chehalis and Willamette, respectively (Fig. 1).

The site-scale sampling design followed that described for rivers in Hughes and Peck (2008) and Peck et al. (In Press). The site length was 50 times the MWCW, which was divided into ten equidistant sub-sites (each 5 MWCW long) and separated by 11 transects. We sampled vertebrate (fish and aquatic amphibian) assemblages by daytime raft electrofishing along alternating shorelines for two sub-sites (10 MWCW) to reduce potential bias from differentially shaded banks and to avoid excessive ferrying; we electrofished the thalweg when rapids or other obstacles necessitated it. One netter collected vertebrates as the rower maneuvered the raft downstream at a slightly greater velocity than the river. The electrofisher was a Smith-Root GPP 2.5 (Smith-Root, Vancouver, Washington, USA) operated at 30–60 pps pulsed DC and 400–1,000 V depending on conductivity. Voucher specimens were preserved in 10% formalin and confirmed at the Oregon State University Museum of Ichthyology (Corvallis, Oregon, USA).

At each of 11 nearshore systematic transects 5 MWCW apart (and also sampled at alternating shorelines every two transects), we sampled benthic macroinvertebrates with a 500-μm mesh kick net with a 30×30-cm opening and a bag length of 80 cm. The area sampled per transect was 0.09 m^2. We preserved the 11 macroinvertebrate sub-samples in 95% ethanol and, like the vertebrates, composited sub-samples into a single sample for each site, for a total sample area of 0.99 m^2. In our Oregon State University laboratory, we identified macroinvertebrates to the lowest practical taxonomic resolution, typically genus—except for Annelids, Arachnids, and Ostracods to class; Maxillipods to family; and Cnidaria, Platyhelminthes, Nemata, and Tardigrada to phylum—with a count goal of 500 individuals. Using an air-lift sampler in the Danube River, Schönbauer (1999) found that macroinvertebrates were distributed across the entire channel, but that diversities and abundances were greatest in the vicinity of protected areas nearshore. Cao et al. (2002) reported that for Oregon macroinvertebrates, ten composited sub-samples and 400–500 individuals were sufficient for distinguishing dissimilar assemblages when using Jaccard or Bray-Curtis similarity estimators.

We collected one benthic diatom sample from the littoral zone near each of the 11 macroinvertebrate transects. At each transect, we collected diatoms from a 12-cm^2 area through use of a 3.9-cm diameter PVC pipe as a template, and we combined all 11 samples into a single diatom composite sample per site, for a total sample area of 132 cm^2. In fine sediment locales, we sucked diatoms from the upper 1–2 mm of sediment into a 60-ml syringe and expelled the contents into a 500-ml jar. In coarse sediment areas, we brushed diatoms off rocks with a toothbrush and rinsed the sample with river water into the same jar. After sampling all 11 transects, we thoroughly mixed the contents of the jar, poured 50 ml into a centrifuge tube, and preserved the sample with 2 ml of 37% formalin.

At our Portland State University laboratory, we cleaned diatom valves with concentrated nitric acid using the Microwave Accelerated Reaction System (Model MARS® 5, CEM Corporation, Matthews, North Carolina, USA) following a pre-programmed

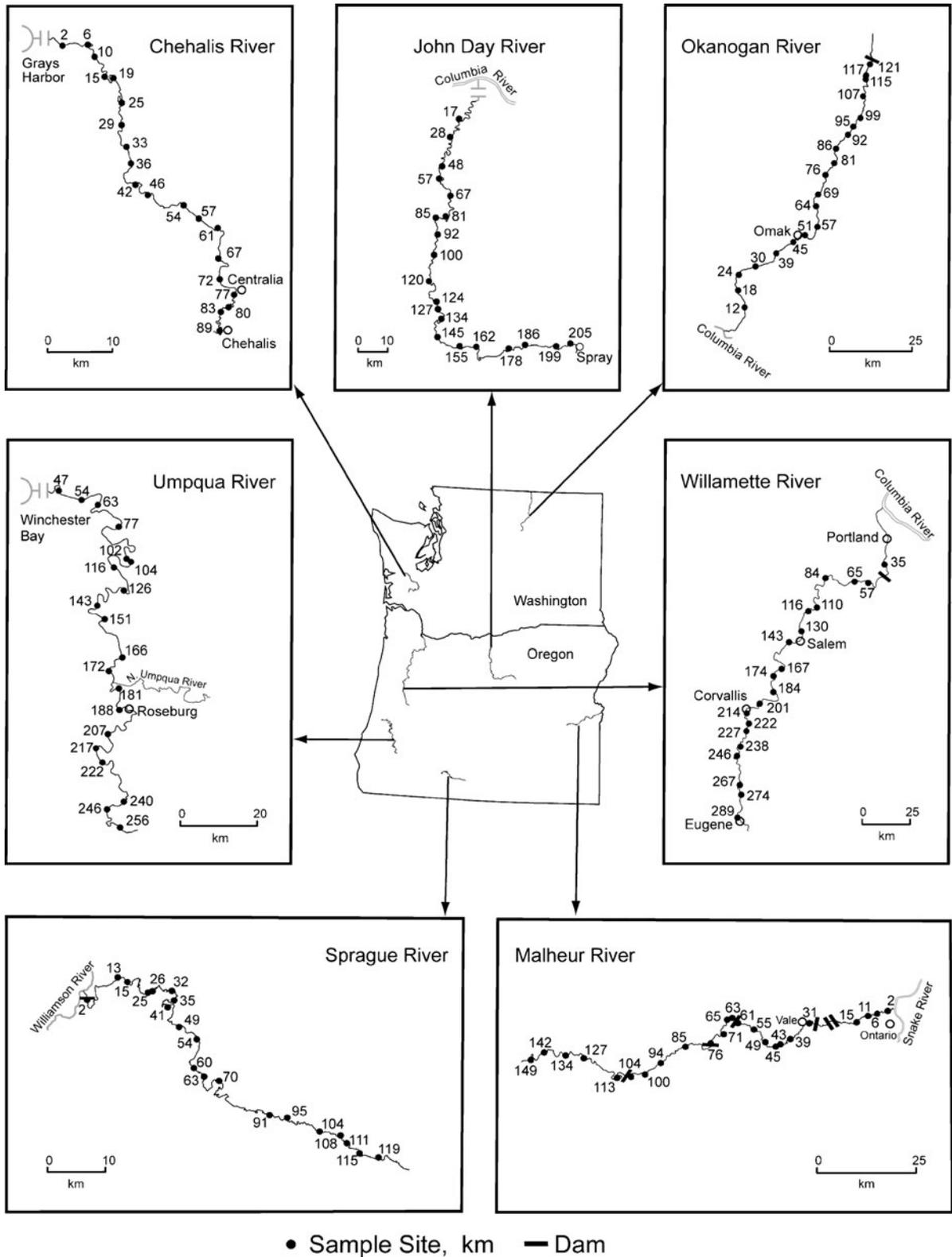Fig. 1 River locations and survey design for seven Pacific Northwest rivers, USA

Table 1 Habitat characteristics (medians and ranges) for 20 sites in Pacific Northwest rivers, USA

Variable	Chehalis	Willamette	Umpqua	Sprague	Malheur	John Day	Okanogan
Mean width (m)	47.7	126.2	84.5	30.9	27.8	51.3	79.5
	29–65	88–236	33–134	15–55	18–47	42–72	40–104
Mean thalweg depth (m)	1.8	2.9	2.3	1.2	1.0	1.4	1.3
	0.9–4.8	1.7–18.0	0.6–4.0	0.6–2.5	0.5–1.3	0.9–1.9	0.3–2.3
Mean littoral depth (m)	0.3	1.0	0.2	0.2	0.6	0.2	0.7
	0.2–0.7	0.7–1.5	0.1–1.0	0.2–0.5	0.2–1.1	0.1–0.4	0.4–1.5
Catchment area (km^2)	2,738	13,554	8,637	3,637	7,847	15,214	18,890
	1,586–3,554	5,318–28,912	1,950–10,491	1,346–4,169	6,278–9,053	12,871–17,819	17,392–21,115
Map sinuosity	1.3	1.3	1.7	1.4	1.2	1.5	1.1
	1.0–2.6	1.1–2.6	1.0–3.6	1.1–2.5	1.0–4.1	1.0–17.8	1.0–2.7
Map slope (%)	0.04	0.04	0.08	0.03	0.14	0.19	0.03
	0.03–0.2	0.01–0.1	0.04–0.4	0.01–0.4	0.01–0.3	0.06–0.4	0.02–0.08
Log mean substrate (mm)	1.4	1.6	2.3	0.7	1.2	2.1	0.9
	−2.0–2.7	−0.4–2.9	1.3–3.4	−0.2–2.4	0.3–2.4	1.6–2.4	−0.1–1.5
Littoral sand dominant (%)	9.1	18.2	27.3	18.2	27.3	9.1	9.1
	0.0–27.3	0.0–36.4	9.1–45.5	4.5–63.6	9.1–54.	0.0–45.5	0.0–36.4
% site with snags present	57.7	47.5	20.1	3.9	2.0	0.0	39.0
	18–78	20–88	0–59	0–58	0–28	0–7	26–64
Bank canopy cover (%)	39.2	36.2	29.9	7.0	27.7	2.3	37.7
	14–70	16–58	6–44	0–34	11–54	0–9	13–64
Water temp. (°C)	20.6	19.9	23.1	21.8	21.3	22.5	20.8
	19–25	16–25	22–26	20–27	15–29	20–24	15–24
Max. July air temp. (°C)	25.4	27.2	28.7	28.8	34.0	32.3	31.3
	25–26	27–28	26–29	28–30	33–35	30–34	30–33
Total N (μg/L)	550	355	160	250	465	215	160
	240–920	90–570	130–890	200–320	270–4,830	180–260	120–240
Total P (μg/L)	69.5	59.0	49.0	77.5	283.5	31.0	26.0
	48–113	45–104	21–168	53–118	223–458	23–37	19–52
Sulfate (μeq/L)	70.6	87.4	44.7	15.0	371.9	112.3	733.0
	51–88	14–124	41–174	12–21	158–4,531	85–140	476–801
Chloride (μeq/L)	174.5	82.2	109.0	34.8	142.7	39.1	99.4
	162–209	30–127	85–403	30–46	67–787	27–46	84–122
Conductivity (μS/cm)	104.0	66.5	79.0	113.0	269.5	200.0	301.0
	92–115	43–79	67–173	101–132	131–1,120	150–220	236–332
Riparian disturb./transect	1.4	2.2	1.4	1.4	1.3	0.9	2.2
	0.4–2.3	0.6–3.6	0.8–3.2	0.2–2.4	0.5–2.6	0.3–1.7	1.5–3.0
% catchment urban	7.9	4.3	2.2	0.4	0.3	0.8	1.8
	6.7–8.5	2.2–7.0	0.6–2.8	0.3–0.4	0.2–0.7	0.8–0.9	1.4–2.4
% catchment agriculture	8.6	14.7	5.8	2.2	0.9	0.5	2.0
	7.7–9.1	3.5–21.7	1.9–6.1	1.4–2.3	0.5–3.8	0.4–1.0	0.7–2.6

digestion scheme (temperature, 180°C; pressure, 350 PSI; ramp, 15 min; hold, 15 min). We repeatedly rinsed the digested diatoms with distilled water until the sample pH approximated 7. We mounted the

cleaned valves in NAPHRAX® to make permanent slides for taxa identification and counted a minimum of 600 valves at 1,000× magnification using a compound microscope with differential interference contrast optics. Diatom taxonomy mainly followed Krammer and Lange-Bertalot (1986, 1988, 1991a, b) and Patrick and Reimer (1966, 1975).

We measured physical habitat from a second raft in the thalweg and at the 11 systematic transects. Depths, dominant substrate, and snag incidence were measured at ten systematic points in the thalweg of each sub-site (100 total measurements per site). We measured additional physical habitat variables at each transect in a 100-m^2 area. The transect measurements included nearshore depths and substrates, wetted and bank full widths, bank full and incision heights, bank angle, vertebrate and benthos cover, large woody debris count, canopy density, riparian vegetation structure, and human disturbance counts.

We measured water conductivity and temperature at each transect, and at the end of the sample site, a water sample was taken, sealed, iced, and transported to the laboratory for analysis of total nitrogen and phosphorus by persulfate digestion and colorimetry, and sulfate and chloride by ion chromatography (USEPA 1987).

We estimated the minimum number of sites needed to estimate 75%, 90%, and 95% of the total river taxa richness by comparing the taxa richness of all 20 sites sampled against an increasingly greater number of sites, beginning with one randomly selected site, then two randomly selected sites, and so on. We assumed that the taxa richness of all 20 sites provided an adequate estimate of total river richness of the entire mainstem for purposes of our analyses—but they do not represent true taxa richness, especially for diatoms and macroinvertebrates (Cao et al. 2001, 2007; LaVigne et al. 2008b). We analyzed the taxa richness data through use of 1,000-run Monte Carlo analyses for each site number to obtain random samples without replacement of 1–20 study site composites. This technique is unbiased by the initial starting site, which may occur when field sampling until no new taxa are encountered (e.g., Gammon 1976; Lyons et al. 2001) or when subjectively choosing a starting site in a data set (Angermeier and Smogor 1995; Reynolds et al. 2003). For example, for one site, we randomly selected a single site without replacement from the 20 candidates and repeated this 999 times to determine an average and range of taxa richness for one site. For two sites, we randomly selected two sites without replacement and repeated this 999 times to determine an average and range of taxa richness for two sites. This process was repeated for 1–20 site composites for each river for all three assemblages. The Monte Carlo approach avoids most of the shortcomings of rarefaction and statistical estimators (varying results with different models) as reported by Cao et al. (2001, 2007) and Hughes et al. (2002).

For most purposes, we used the mean of the 1,000 Monte Carlo simulations as the best estimate of the taxa richness in each of the 1–20 sample size composites and constructed individual river taxa accumulation curves for each biotic assemblage. We made box and whisker plots that combined the results of the seven rivers into one taxa accumulation curve per assemblage after normalizing richness among rivers by calculating it as a percent of total river richness. We also reported on the variability in the Monte Carlo analysis by presenting results for the taxa richness in each sample size composite based on the 5th and 95th percentiles of the 1,000 Monte Carlo simulations instead of the mean. These results show a type of 90% confidence interval for the taxa accumulation curves based on the random accumulation of sample sites that was expressed in the Monte Carlo analysis.

We explored the correlations between mean river environmental data and the shape of the individual river taxa accumulation curves using the mean Monte Carlo simulation value for the five sample composite expressed as percent of total river richness. We chose five sites because they represent the steepest part of the accumulation curves but were a large enough sample to be meaningful (markedly fewer sites would have little meaning and using all 20 sites has the same 100% of total value for each river). The environmental data used were means of the 20 sites at each river for field data and whole watershed values for landscape data because we were seeking to produce a single value for each assemblage in each of the seven rivers.

Results

Cumulative taxa richness continued to increase with effort for all three assemblages in all seven rivers

(Figs. 2, 3, and 4), especially for macroinvertebrates and diatoms, suggesting greater taxa richness than we were able to sample adequately with 20 sites.

Diatom taxa richness varied from 120–210 in the John Day and Sprague, respectively, and the Sprague had the highest individual site richness (62 taxa), whereas the John Day, Malheur, and Willamette had the lowest site richness (40 taxa; Table 2). Contrary to what we hypothesized, diatom taxa richness was highest in the two rivers (Sprague and Okanogan) with the smallest mean substrate size (Tables 1 and 2). Maximum sampled richness varied from 98–128 macroinvertebrate taxa for the Okanogan and Umpqua, respectively; but the Umpqua and Chehalis had the lowest and highest richness at individual sites with 23 and 62 taxa, respectively (Table 2). As hypothesized, there was a weak tendency for macroinvertebrate richness to be highest and lowest in the rivers with the largest and smallest mean substrate sizes, respectively (Tables 1 and 2). For vertebrates, maxima varied from 14–33 species in the John Day and Willamette Rivers, respectively; those two rivers also had the lowest and highest species richness at a site (3 and 24 species, respectively). Although the highest vertebrate species richness was associated with the largest catchment area, that was not the case for the lowest

Fig. 3 The mean macroinvertebrate species richness from 1,000 Monte Carlo simulations for cumulative sample sizes of 1 to 20 samples collected from seven Pacific Northwest rivers, USA

richness because the John Day catchment was the third largest (Tables 1 and 2).
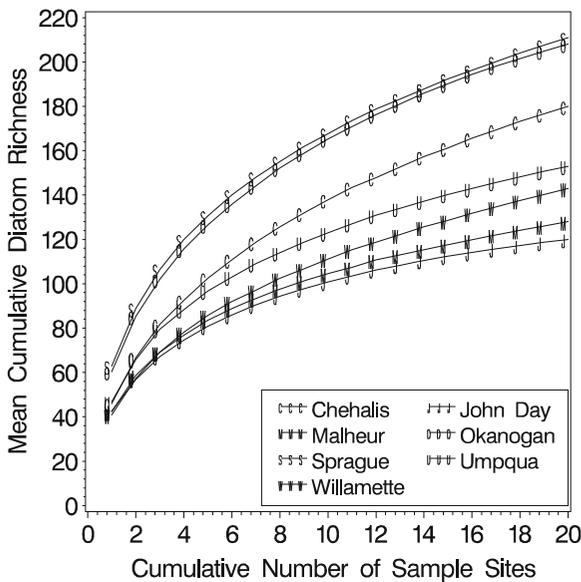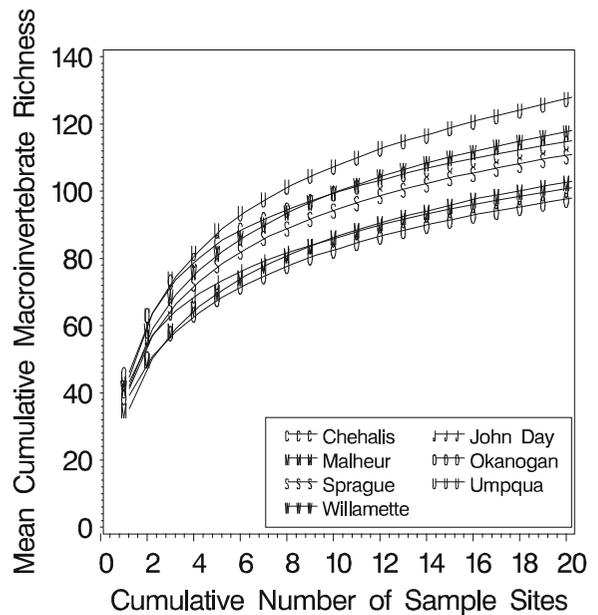

Fig. 2 The mean diatom species richness from 1,000 Monte Carlo simulations for cumulative sample sizes of 1 to 20 samples collected from seven Pacific Northwest rivers, USA
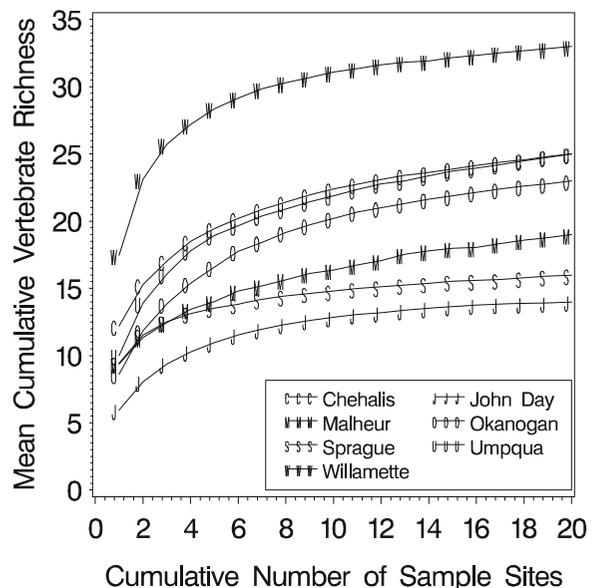

Fig. 4 The mean vertebrate species richness (fish and aquatic amphibians) from 1,000 Monte Carlo simulations for cumulative sample sizes of 1 to 20 samples collected from seven Pacific Northwest rivers, USA

Table 2 Means and ranges of summer vertebrate, macroinvertebrate, and diatom richness of seven Pacific Northwest rivers, USA

River	Vertebrate mainstem richness	Vertebrate site richness mean (range)	Macroinvertebrate mainstem richness	Macroinvertebrate site richness mean (range)	Diatom mainstem richness	Diatom site richness mean (range)
Chehalis	25	12 (7–16)	115	46 (31–62)	180	46 (29–65)
Willamette	33	18 (7–24)	118	42 (32–58)	143	40 (31–62)
Umpqua	25	10 (6–16)	128	45 (23–60)	153	46 (37–62)
Sprague	16	10 (7–12)	111	41 (29–51)	211	62 (52–71)
Malheur	19	10 (7–18)	103	35 (28–43)	128	40 (30–54)
John Day	14	6 (3–8)	101	44 (31–52)	120	40 (34–53)
Okanogan	23	9 (5–12)	98	39 (33–48)	208	60 (53–68)

The percent of total river taxa richness increased with number of samples at a faster rate, but with greater variability, for vertebrates than for benthic macroinvertebrates and diatoms, as expected for a less speciose assemblage (Fig. 5). We found that 75% of the time, 90% of all vertebrate species encountered in all 20 sites could be encountered at 11 sites; to collect 95% of the total river vertebrate species, 16 sites would be needed. To collect 90% of the total river

Fig. 5 Box and whisker plot of the combined results for the seven rivers showing percent of total river richness versus cumulative sample size based on the mean of 1,000 Monte Carlo simulations for each sample size in each river. The *line in the box* represents the median among the seven sample rivers and the boxes are the 1st and 3rd quartile and the whiskers are the minimum and maximum value. *Horizontal lines* show 75%, 90%, and 95% of total river richness

benthic macroinvertebrate taxa 75% of the time, 13 sites were required; to collect 95% of the total river benthic macroinverteabrate taxa 75% of the time, 17 sites were needed. To collect 90% of the benthic diatom taxa collected at 20 sites 75% of the time, 16 sites were required; and to collect 95% of the diatom taxa collected at 20 sites 75% of the time, 18 sites were needed.

In examining variability associated with the Monte Carlo simulations and using the 95th percentile of the simulations instead of the mean, we collected 90% of the vertebrate species after accumulating two sites (Fig. 6). At the other extreme with the 5th percentile of the simulations, we collected 90% of the vertebrate species only after compositing 11 sites. For macroinvertebrates, in 95% of the Monte Carlo simulations we collected 90% of the total river taxa by accumulating nine sites, but in 5% of the simulations we collected 90% of the total taxa only after accumulating 15 sites. Similarly, in 95% of the Monte Carlo simulations, we collected 90% of the total river diatom taxa by compositing 11 sites, but in 5% of the simulations we collected 90% of the river diatom taxa only after compositing 15 sites (Fig. 6). These numbers of sites approximated the ranges of the numbers that we hypothesized: seven to ten for vertebrates and 14–19 for benthic macroinvertebrates and diatoms.

Several patterns are evident in comparing taxa richness versus physical and chemical habitat variables (Table 3). Malheur River taxa richness was associated with the greatest number of predictor variables and demonstrated the most significant correlations. No single predictor variable was highly correlated with taxa richness in all rivers for all 3
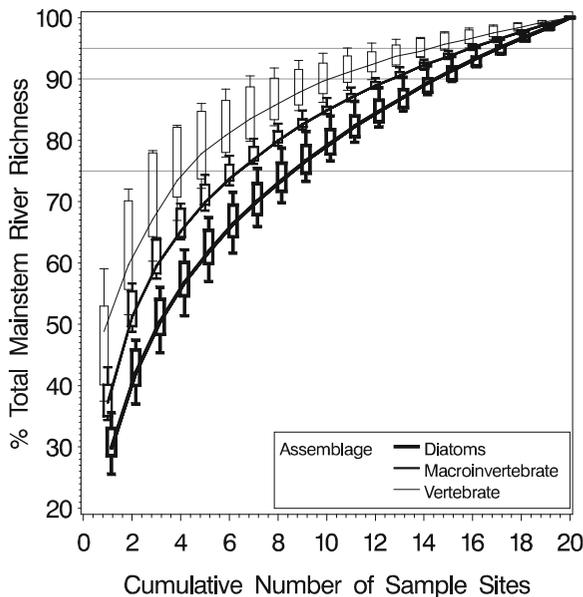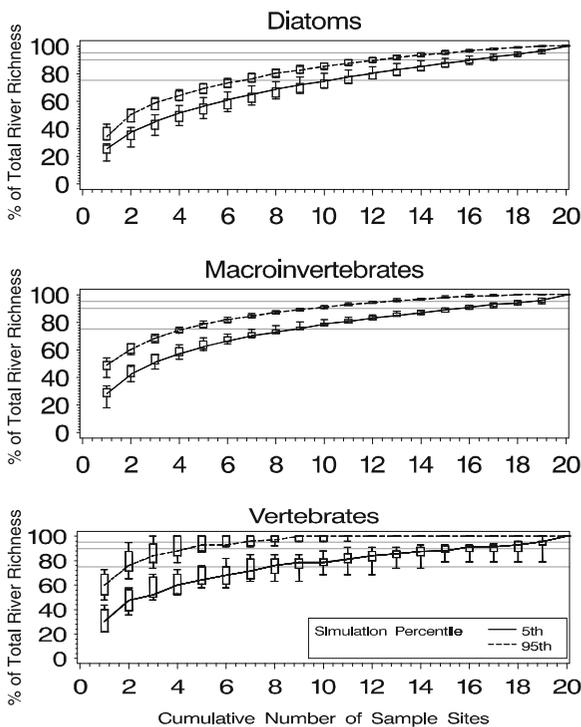
Fig. 6 Same plot as Fig. 5, but instead of plotting the mean of the 1,000 Monte Carlo simulations both the 5th and 95th percentiles of the 1,000 simulations are shown for each assemblage

assemblages. Greater mean thalweg depth and greater mean littoral depth were associated with lower taxa richness. In most cases increased thalweg substrate size and increased littoral sand tended to decrease taxa richness. Increased air or water temperature was occasionally correlated with increased taxa richness, but so was increased canopy cover (for diatoms). Increased nutrients (total nitrogen, total phosphorus) were correlated with increased vertebrate species richness, but decreased diatom and macroinvertebrate richness. Similarly, vertebrate species richness increased with increased riparian or catchment disturbance in three rivers.

Most correlations were insignificant between mean river environmental variables and the steepness of the taxa accumulation curves as indicated by the percent of total river taxa accumulated after five samples. Because the sample size was only 7 (the seven rivers) and we examined 30 correlations per assemblage, r had to be >0.75 to be significant at $p < 0.05$ (Harrell 2001; Tabachnick and Fidell 2001). All correlations were insignificant between environmental variables

and the percent of total river macroinvertebrate taxa observed after sampling any five sites. However, there were significant correlations between percent of total river vertebrate species in five samples and watershed road density ($r=+0.79$). There were also significant correlations between percent total river diatom taxa in five samples and bank canopy density ($r=-0.86$), maximum July air temperature ($r=+0.82$), and watershed population density ($r=-0.79$). These associations suggest a relationship between river-scale disturbance and within-river sample taxa homogeneity for vertebrates and diatoms. The more homogeneous the samples are within a river, the fewer the number of samples required to accumulate all the taxa in the river.

Discussion

There is no absolute number of sample sites needed for assessing taxa richness or biological condition in a river. We found that 75% of the time, we needed 11–16 randomly selected sites to assess 90–95% of the vertebrate species collected at 20 sites. Beyond those numbers of sites, each additional site typically only added a single individual of a rarely occurring species. Kanno et al. (2009) also reported that singletons and doubletons governed the recommended sampling distance for stream and river sites. These site numbers are slightly greater than the six to ten sites found appropriate by Fischer and Paukert (2009) for wadeable Great Plains rivers and the nine to ten sites typically used by Ohio EPA for assessing point sources along nonwadeable Ohio rivers (Yoder and Smith 1999). The range in our site numbers encompass the 15 sites reported by Blocksom et al. (2009) for navigation pools on the Ohio River and the 10–56 sites used by Gammon and Simon (2000) on the Wabash River, but their site lengths were shorter than ours and they sampled less heterogeneous rivers. Our baselines of 50 MWCW of site length and 20 sampling sites per river may seem insufficient to some readers. However, LaVigne et al. (2008a, b) documented that they sufficed for detecting significantly more vertebrate species than previous surveys of the Willamette and Malheur Rivers, and we detected a previously unrecognized species in the Umpqua River (Kettratad and Markle 2010).

Table 3 Correlations between taxa richness and physical and chemical habitat variables for seven Pacific Northwest rivers, USA

Variable	Chehalis	Willamette	Umpqua	Sprague	Malheur	J. Day	Okanogan	ALL (seven)
Mean width	**0.52***							
Mean thalweg depth	**−0.49***	**−0.47***			*0.52**			**0.36*****
Mean littoral depth		**−0.47*** −0.47*			**−0.58***		−0.46*	
Catchment area				**0.44***	**0.89*****			
Map sinuosity	−0.44*		**0.60***					
Map slope	**0.51***					0.45*		*0.50****
Log mean size substrate		*−0.49**			**−0.68****	−0.44*		*0.41****
Littoral sand dominant		−0.43*				0.57*	**−0.48***	**0.45*****
% snags							**−0.44*** 0.64*	**0.52*****
Bank canopy cover	*0.58**	*0.69***					*0.44**	**0.44*****
Water temp.				−0.44*	**0.53*** *−0.45**			
Max. July air temp.		0.65*			**0.51***			**−0.59***** *−0.33****
Total N	*−0.56**			**0.46***	**0.67****			**0.35***** *−0.35****
Total P			**0.46*** *−0.45**		**0.88*****		−0.60*	
Sulfate				*0.60**	**0.83***** −0.47*	**−0.49***		
Chloride			**−0.46***		**0.86*****			
Conductivity					**0.85***** −0.47*	**−0.51*** *0.45**		**−0.56*****
Riparian disturbance		−0.53*				*0.46**		
% urban	*−0.46**		−0.54* *−0.47**	**−0.51***	**0.89*****			**0.48*****
% agriculture				**0.48***	**0.76*****			**0.71*****

The bold text refers to vertebrates, the underlined refers to macroinvertebrates, and the italicized refers to diatoms. Significance: 0.05–0.002*; <0.002–0.0001**; <0.0001***; less significant correlations not shown

Much greater sampling effort than the 11 composite samples from 20 sites that we employed is needed for estimating true taxa richness of benthic macroinvertebrates in large rivers. Similar to our results from each of seven rivers from which we collected 98 to 128 taxa, Fesl (2002) reported a log-normal distribution of Chironomid species that continued increasing even after 120 grab samples and 80 species at nearshore sites in the Danube River. Because of the greater alpha and beta diversity and available microhabitats for macroinvertebrates versus vertebrates, the number of sites out of 20 that are needed to collect 90-95% of the benthic macroinvertebrate taxa 75% of the time is 13–17, respectively. Bartsch et al. (1998)

estimated 18–40 sites were needed for the upper Mississippi River and Raunio and Antilla-Huhtinen (2008) recommended eight sites, but they were studying soft-bottomed systems compared to our typically hard bottomed rivers (Table 1). Collier and Lill (2008) collected 63 macroinvertebrate taxa from the Waikato River, New Zealand, by using a kick net with the same mesh size as ours and by sampling all available habitats, but they only sampled 47 stations (versus our 220 per river) and only counted 200 individuals per sample (versus our 500 per composite sample).

Despite using site-scale sampling and processing methods similar to ours, diatom taxa richness may

have been underestimated by the 8–11 sites in rivers 115–400 km long sampled by Miettinen (2007), Raunio and Soininen (2007), Sgro et al. (2007), and Taylor et al. (2007). Miettinen (2007) did not report taxa richness, but Sgro et al. only reported 80 taxa and a site mean of 20 taxa; however, their study river was affected by metal mining. Raunio and Soininen (2007) and Taylor et al. (2007) recorded 161 and 245 diatom taxa in their studies (which exceeded the taxa richness we recorded), despite sampling half the number of sites that we did. Reavie et al. (2010) used the same site-scale periphyton protocol as we did, plus phyto-plankton sampling, at over 100 sites in three great rivers and collected 410 algae taxa (diatoms and soft algae).

Because natural characteristics such as catchment area and substrate are known to affect local fish, macroinvertebrate, and diatom taxa richness in large rivers (e.g., McGarvey and Hughes 2008; Angradi et al. 2009; Biggs and Kilroy 2000), sampling effort may also need to be adjusted for such factors. However, a sample size of only seven rivers is insufficient for rigorously evaluating the relative importance of various natural variables. For example, discharge or water volume may be more important than catchment area (Oberdorff et al. 1995; McGarvey and Hughes 2008). Nonetheless, the John Day River, with the lowest vertebrate species richness, lacks dams and has a greater volume and catchment area than other rivers in this study that drain semi-arid landscapes (Fig. 1; Table 1). Clearly other factors than size and fragmentation are involved. Macroinverte-brate taxa richness followed the expected pattern of being highest in the river with the coarsest substrate. Substrate size has long been considered a key determinant of benthos richness (Hynes 1970; Allan 1995), and it incorporates aspects of current velocity, as well as temperature and oxygen concentration, which are also associated with increased invertebrate richness. In addition, we found that it was important to distribute the sampling effort along the entire mainstems of our study rivers because of taxa additions and subtractions associated with differences in temperature, nutrients, and substrate size between the upper and lower segments of each river. Ligeiro et al. (2010), working in a neotropical catchment, also reported that macroinvertebrate diversity was maxi-mized by sampling many sites in many segments over a large area, and across diverse substrate types. Y. Pan, Portland State University, Portland, OR. unpublished

data concluded that there was a substantial tributary effect on diatom assemblages at sampling sites of some rivers. Lane et al. (2007) and Leland et al. (2001) reported significant micro- and macro-habitat effects on benthic diatoms collected from large river sites in the Ohio and San Joaquin River Basins.

Although beyond the intent of this paper, the correlations between taxa richness and environmental variables (Table 3) stimulate additional thought concerning site-scale and catchment-scale determi-nants of richness. Most importantly, the lack of a single set of taxa richness predictor variables for any or all three assemblages in any or all seven rivers indicates that there is no key variable for predicting riverine richness or the necessary sampling effort, at least for these rivers and at the spatial resolution at which we studied them. This is one reason we support measuring a large and diverse set of environmental variables (Hughes and Peck 2008). We believe that the greater number of higher correlations between taxa richness and predictor variables in the Malheur River was associated with the stronger disturbance gradient in that river, which also covaried with its size (LaVigne et al. 2008a; Y. Pan, Portland State University, Portland, OR. unpublished data). The negative correla-tion between taxa richness and mean thalweg and littoral depth may have been an artifact of decreased sampling effectiveness because deeper sites are more difficult to sample effectively (Flotemersch et al. 2010). Increased temperature, nutrients, and anthropogenic disturbance were occasionally highly significantly correlated with increased taxa richness, particularly of vertebrates. As stated above, this is partly a result of those variables covarying with river size because river size is strongly correlated with fish species richness (e.g., Fausch et al. 1984; McGarvey and Hughes 2008). The three rivers (Umpqua, Sprague, and Malheur) for which these correlations were strongest also varied markedly in size from up- to down-river (Table 1). But for all these reasons, one must be cautious when attempting to use simple taxa richness when assessing anthropogenic impacts on rivers (Moya et al. 2011; Stoddard et al. 2008; Whittier et al. 2007).

We conclude that randomization analytical proce-dures, such as multiple-run Monte Carlo analyses, as recommended by Flotemersch et al. (2010) and Schneck and Melo (2010) are useful for estimating sufficient sampling effort for assessing vertebrate, macroinvertebrate, and diatom taxa richness in rivers.

We encourage others to undertake similar studies of large river sampling effort because such systems are rapidly losing their vertebrate and macroinvertebrate taxa as a result of water projects and water pollution (Allan and Flecker 1993; Dynesius and Nilsson 1994; Hughes et al. 2005; Dudgeon et al. 2006) generated by increased human population and economic growth (Czech et al. 2004; Perkins 2004; Leprieur et al. 2008; Limburg et al. 2011).

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