Evaluation of Stream Benthic Macroinvertebrate Sampling Protocols: Comparison of 3 ft^2 and 8 ft^2

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Evaluation of Stream Benthic Macroinvertebrate Sampling Protocols: Comparison of 3 ft^2 and 8 ft^2

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[1] Reports, presentations, and relevant documents are available on the B-IBI Recalibration Documents and Materials project page of the PSSB: http://www.pugetsoundstreambenthos.org/Projects/BIBI-Recalibration-Documentation.aspx.

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EXECUTIVE SUMMARY

Numerous Puget Sound and state agencies collect stream benthic macroinvertebrate data to assess biological integrity. The benthic index of biotic integrity (B-IBI), a 10-metric index that includes measures of taxa richness, tolerance to disturbance, and feeding ecology, is the tool typically used to evaluate and assess biotic condition in the region. Different collection methodologies (for example, collecting samples from 3- and 8-ft^2 areas) have evolved over time throughout the region. The comparability of data collected from these different methods is not fully understood although it is presumed that sample collection from a larger surface area will generally result in collecting a larger number of unique taxa and an increase in B-IBI scores. The Washington State Department of Ecology (Ecology), the Pacific Northwest Aquatic Monitoring Partnership (PNAMP), and the United States Environmental Protection Agency (EPA) have been encouraging standardization towards an 8 ft^2 collection method; however, some local entities have been reluctant to shift sampling protocols due to the risk of orphaning their existing long-term data sets collected using the 3 ft^2 areas. Additionally, the designation of the B-IBI as a Puget Sound Partnership (PSP) freshwater ecosystem indicator necessitates evaluation throughout the region to evaluate biological condition. Therefore it is important to determine if samples collected from different surface areas are comparable, and whether establishing a ”cross-walk” between methods to allow data comparison is necessary.

In the summer of 2011, side-by-side samples were collected at 55 Puget Lowland sites with the help of 9 agency partners to evaluate the influence of sample collection surface area (3 ft^2 and 8 ft^2 sample areas) on B-IBI scores and individual component metrics. Samples were collected in riffle habitats using Surber or D-frame kicknets with 500 micron mesh. Taxa were sent to Rithron Associates, Inc., a certified taxonomic laboratory, subsampled to a 500-minimum organism count and identified to lowest practical level (generally genus or species). Taxonomic data were uploaded to the Puget Sound Stream Benthos (PSSB) data management system which enables data downloads of B-IBI scores and metrics or raw taxonomic composition.

The goal of this sampling effort was to collect sufficient data to (1) determine if data collected from 3 ft^2 and 8 ft^2 are comparable, and (2) if they are not comparable develop a conversion algorithm or “cross-walk” so that data (and associated B-IBI metrics) collected from both 3 ft^2 and 8 ft^2 at a given site can be readily compared.

B-IBI scores, individual B-IBI metrics, and taxonomic composition results were compared for samples collected from both 3 ft^2 and 8 ft^2. There were no significant differences in mean B-IBI or B-IBI metric scores between 3 ft^2 and 8 ft^2 method. 3 ft^2 and 8 ft^2 results were also highly correlated and fairly evenly scattered above and below a one-to-one line indicating no predictable shift. Similarly, taxonomic composition between 3 ft^2 and 8 ft^2 samples were not statistically different. Therefore it was concluded that no ”cross-walk” or scoring adjustment algorithm was necessary for data to be comparable.

Results support the conclusions that each collection method can be compared with confidence and reported interchangeably enabling the use of existing long-term and future data to evaluate trends in biological integrity over time. This has allowed some jurisdictions within the Puget Sound region to transition to collection of 8 ft^2 samples without losing the ability to track long-term trends based on historical data collected from 3 ft^2 areas. In addition, results of this work will enable direct comparison of a larger pool of regional data and in doing so will promote data integration to evaluate ecosystem conditions across jurisdictional boundaries, a PSP goal.

1.0. INTRODUCTION

A 2009 assessment of Puget Sound regional monitoring programs identified 21 local, state, and federal agencies, citizen and non-profit groups, and tribes that collect macroinvertebrate data to track biological integrity (King County 2009). Most of these groups evaluate these data using the Puget Lowland benthic index of biotic integrity (B-IBI), a 10-metric index that includes measures of taxa richness, tolerance to disturbance, and feeding ecology and describes biological condition. Historically, Puget Sound samples have typically been collected from a 3 ft^2 riffle-targeted habitat area, which is the methodology associated with the original B-IBI; however, 8 ft^2 and 9 ft^2 primarily riffle-targeted methods have emerged throughout Puget Sound (Figure 1, page 2).

In recent years, the Washington State Department of Ecology (Ecology), the Pacific Northwest Aquatic Monitoring Partnership (PNAMP), and the United States Environmental Protection Agency (EPA) have encouraged standardization towards an 8 ft^2 collection method (Plotnikoff & Wiseman 2001, Cusimano et al. 2006, Hayslip 2007, Klemm et al. 2006) and Ecology requires a minimum collection area of 8 ft^2 for results to be considered for their water quality assessment (Ecology 2012). However, some local entities have been reluctant to shift sampling protocols due to the risk of orphaning their existing long-term data sets (> 4000 site visits) collected from 3 ft^2 or 9 ft^2 areas. Sample collection from a larger surface area is thought to result in collection of a greater variety of taxa and an increase in index values, regardless of analytical method used (Cazier 1993, Vinson & Hawkins 1996). In addition, the Puget Sound Partnership (PSP) has recently designated B-IBI as a freshwater ecosystem indicator requiring evaluations across programs throughout the region to evaluate biological condition (PSP 2012). Therefore it is important to determine if samples collected from different surface areas are comparable and whether establishing a "cross-walk" between methods to allow data comparison is necessary. This work builds on a 2009 pilot effort (Marconi 2010) to ensure that results reported from each method can be compared and reported interchangeably enabling the use of existing long-term and future data to evaluate trends in biological integrity over time.

In the summer of 2011, side-by-side samples were collected at 55 Puget Sound sites to evaluate the influence of surface area (3 ft^2 and 8 ft^2 sample areas) on B-IBI scores and component metric values. The goal of this sampling effort was to collect sufficient data to (1) determine if B-IBI scores from 3 ft^2 and 8 ft^2 are comparable, and (2) if they are not comparable develop a conversion algorithm or "cross-walk" so that data (and associated B-IBI metrics) collected from both 3 ft^2 and 8 ft^2 at a given site can be readily compared. A smaller scale effort (10 sites) also collected from 9 ft^2 areas. However, ten was not considered a sufficient sample size to evaluate the comparability of the 9 ft^2 samples and because of the large overlap between 8 ft^2 and 9 ft^2 areas differences in B-IBI or taxonomic composition are less likely than between 3 ft^2 and 8 ft^2 which is the focus of this report.

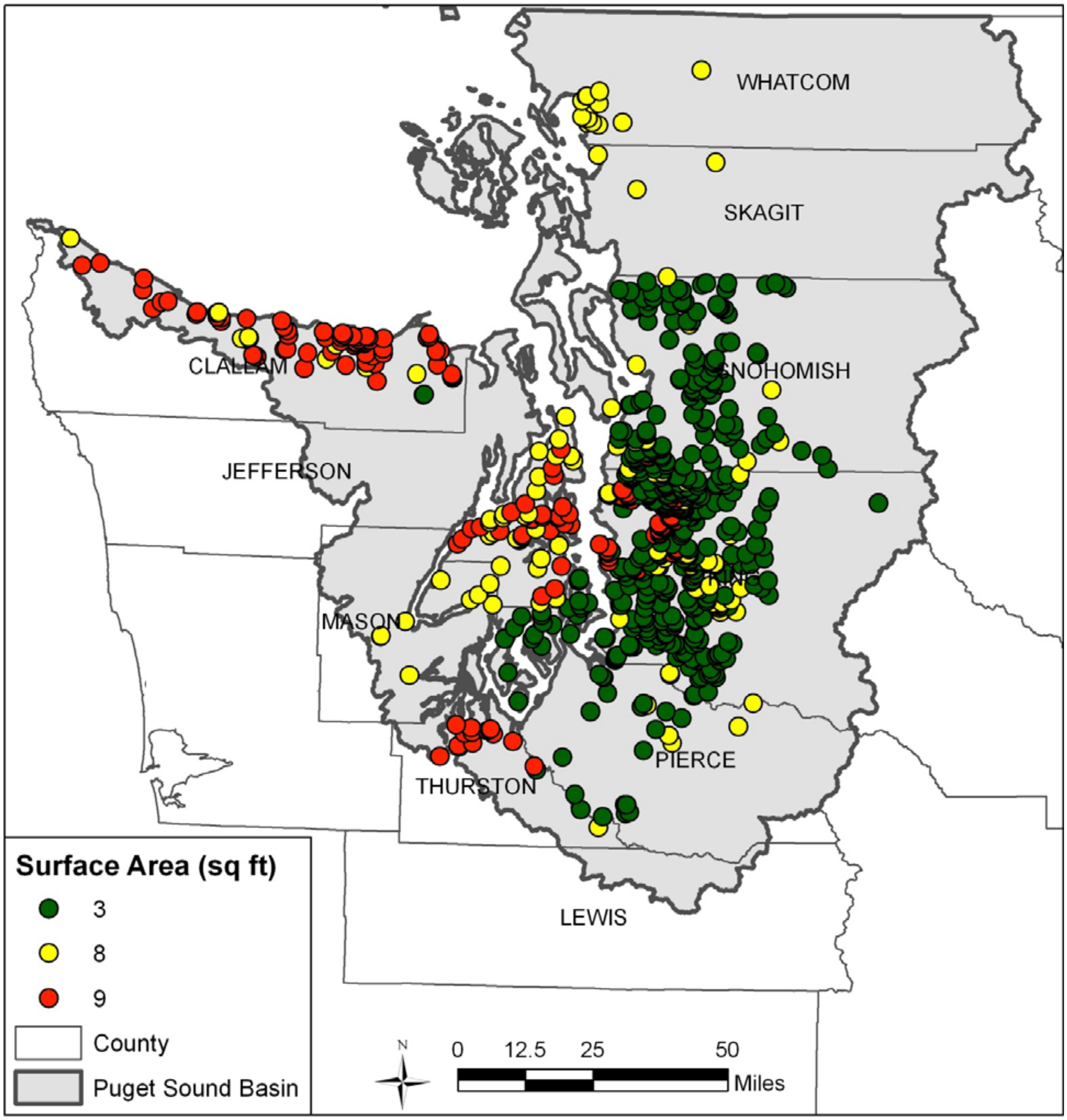


Figure 1. Map of benthic macroinvertebrate surface area collection methods. This map represents the method used for the most recent visit at a site as of June 2011 for monitoring programs with data in the PSSB[2].

[2] Several agencies sample macroinvertebrates from a 9 ft² surface area and 3, 8, and 9 ft² side by side samples were collected at ten of the 55 sites. However, ten was not considered a sufficient sample size to evaluate the comparability of the 9 ft² samples.

2.0. METHODS

Collection methods, sampling design, and quality assurance and quality control (QAQC) techniques for comparing side by side benthic macroinvertebrate samples collected from 3 ft^2 and 8 ft^2 were outlined in two previous documents (King County 2011a, 2011b), and are summarized briefly here.

2.1 Site Selection

Sites were selected from over 1,100 existing sample locations from the PSSB to limit property access issues and minimize the need for additional site reconnaissance. Site selection considered the following criteria:

1. Availability of regional groups interested in partnering;
2. Presence of suitable riffle habitat or other non-depositional, flowing aquatic habitat;
3. Inclusion of sites to represent a range of human disturbance (e.g., ranging from close to pristine to highly impacted); and
4. A history of sufficient organism counts (>350) where possible.

Analysis of pre-2011 macroinvertebrate data and natural features indicated that elevation, channel slope (gradient), and watershed area (up to at least 50 km^2 [19 mi^2]) do not have a consistent influence on B-IBI scores across a range of urbanization, and therefore the sampling design did not address these factors (Fore 2011)[3].

2.2 Field collection methods

Field collection methods are presented in detail in the Sampling and Analysis Plan (King County 2011a) and are summarized below. Field collections were completed by a minimum of two people in August and September. Sampling methods generally followed Ecology's sample collection protocol for regulatory purposes (Adams 2010a). At each sampling station, macroinvertebrate samples were collected from a total surface area of 8 ft^2 sampled across multiple riffles or fast-moving, non-depositional aquatic habitats using a Surber sampler or D-frame kick net with 500 µm mesh. These samples were collected 1 ft^2 at a time divided into two sample containers for each site: one collected from 3 ft^2 and one from 5 ft^2 (Figure 2). Sample reaches typically consisted of four distinct riffle habitats, with two 1 ft^2 collections from each riffle for a total sampled surface area of 8 ft^2. However, at sites where four distinct riffle habitats were not present, samples were collected from fewer riffles. For example, if only two riffle habitats were present, then four 1 ft^2 samples were collected from each of the two riffle habitats. If three riffle habitats were present, then the samples were distributed so that there were two to three 1 ft^2 samples collected in each riffle for a total sampled surface area of 8 ft^2. When streams were too narrow for simultaneous side-by-side sampling, samples were collected up- and downstream of one

[3] Subsequent analysis of natural factors supports the results of this initial analysis; see King County 2014a for more details.

after the other (Figure 3). Collections always started downstream and proceeded in an upstream direction.

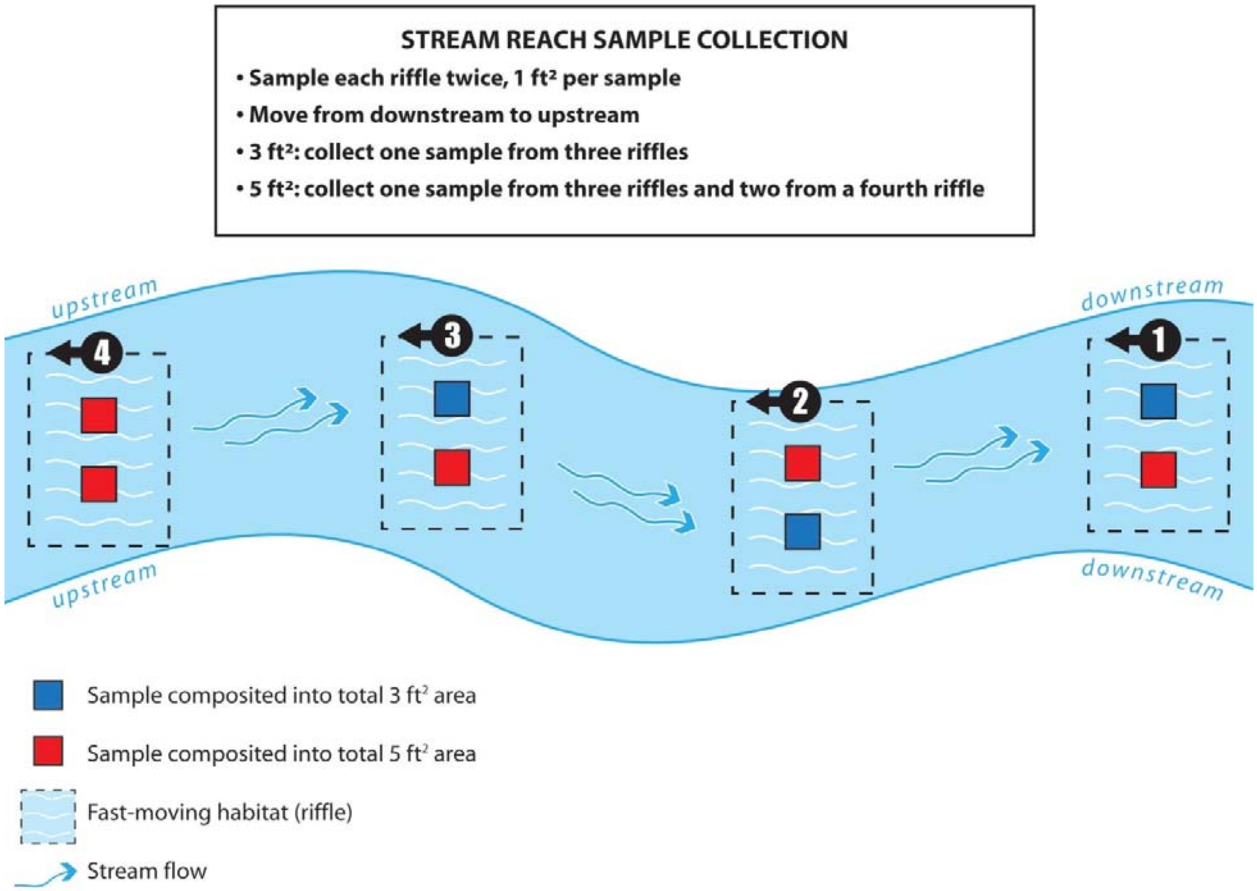


Figure 2. Stream reach sample collection procedures.

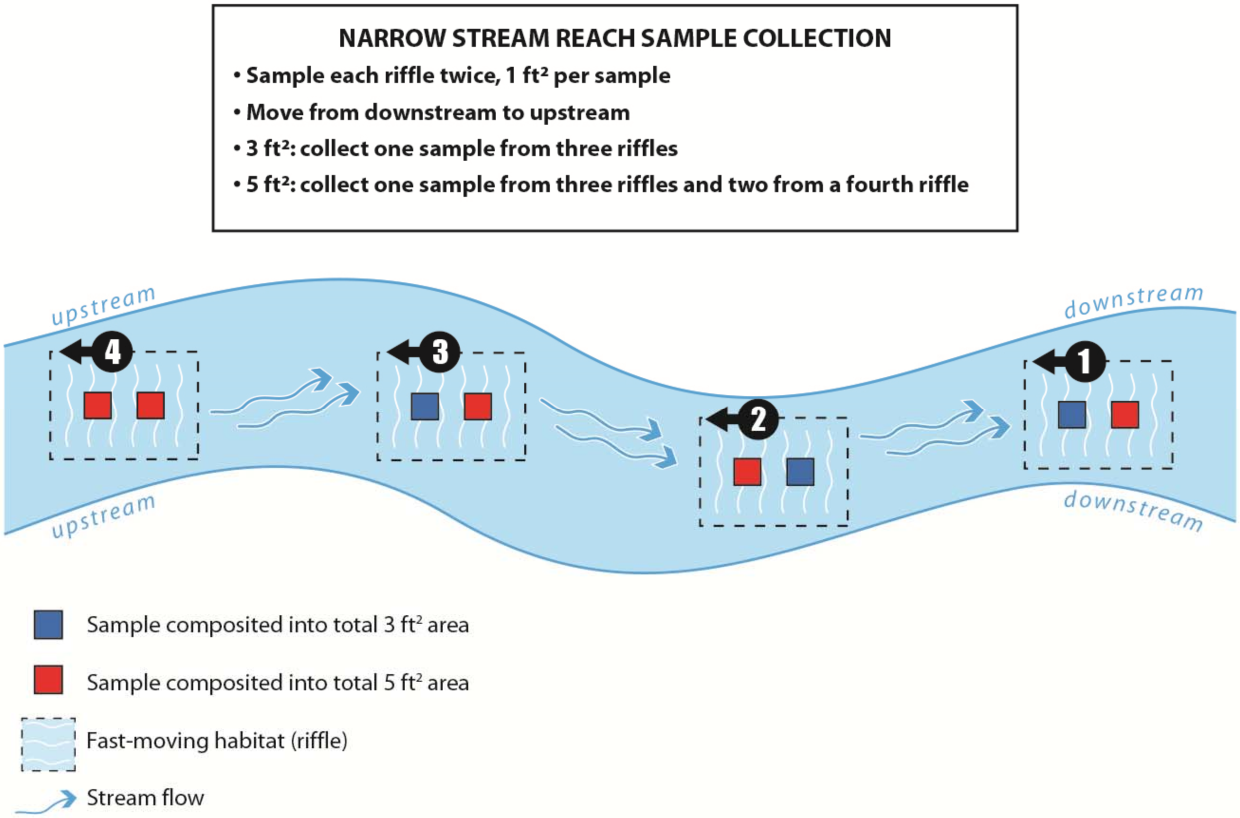


Figure 3. Stream reach sample collection procedures for a narrow stream.

For each 1 ft^2 sample collection, the Surber sampler or D-net opening was placed in the riffle so that the net opening faced into the stream flow. The net was secured on the stream bottom to eliminate any gaps under the frame and all large material (e.g., large gravel, cobble, and woody debris) within the 1 ft^2 sampling area was scrubbed by hand so that dislodged organisms were washed into the collection net. These materials were examined for any remaining invertebrates before being placed outside of the sample area[4]. After removal and processing of any large stones or debris, the remaining substrate within the 1 ft^2 sampling area was agitated to a depth of approximately 10 cm for 60 seconds[5] to suspend the substrate and any associated macroinvertebrates into the water column, allowing the water to carry the macroinvertebrates into the net.

The net was then moved to the next upstream collection location (i.e. riffle), and this process was repeated until the appropriate number of individual 1 ft^2 samples (3 or 5) were cumulatively sampled into one net. Once the desired area (3 ft^2 or 5 ft^2) was collected, the net was removed from the stream and the contents were carefully transferred to a sample container and preserved in the field with 95% denatured ethanol.

[4] Some partners kept scrubbed substrate separate for visual checks in rinse bins including City of Seattle, Pierce County, and Lake Forest Park Streamkeepers.
[5] There was some variability in agitation time amongst partnering agencies. Five agitated the substrate for 60 seconds, 1 didn't keep time, 1 agitated for 30 seconds, and 1 did a 60 second rub followed by a 60 second agitation.

Site information and minimal habitat data were collected at each sampling location and were recorded on a data sheet along with a hand-drawn sketch of the sampling reach and collection locations (Appendix A).

2.3 Laboratory analysis methods

Laboratory analysis methods are presented in detail in King County 2011a and are summarized here. All samples were submitted to Rhithron Associates, Inc. (hereafter Rhithron) in October 2011. Rhithron processed the two samples from each site into two fixed-count 500 minimum subsamples: one from the 3 ft^2 and one from 8 ft^2 (which was composited from the 3 ft^2 and 5 ft^2 samples after the 3 ft^2 sample was fully processed; Figure 4). Standard sorting protocols (Plotnikoff and Wiseman 2001) using Caton subsampling devices (Caton 1991) were applied to achieve representative subsamples of a minimum of 500 organisms followed by a scan to find any large or rare taxa that were missed during the subsampling procedures.

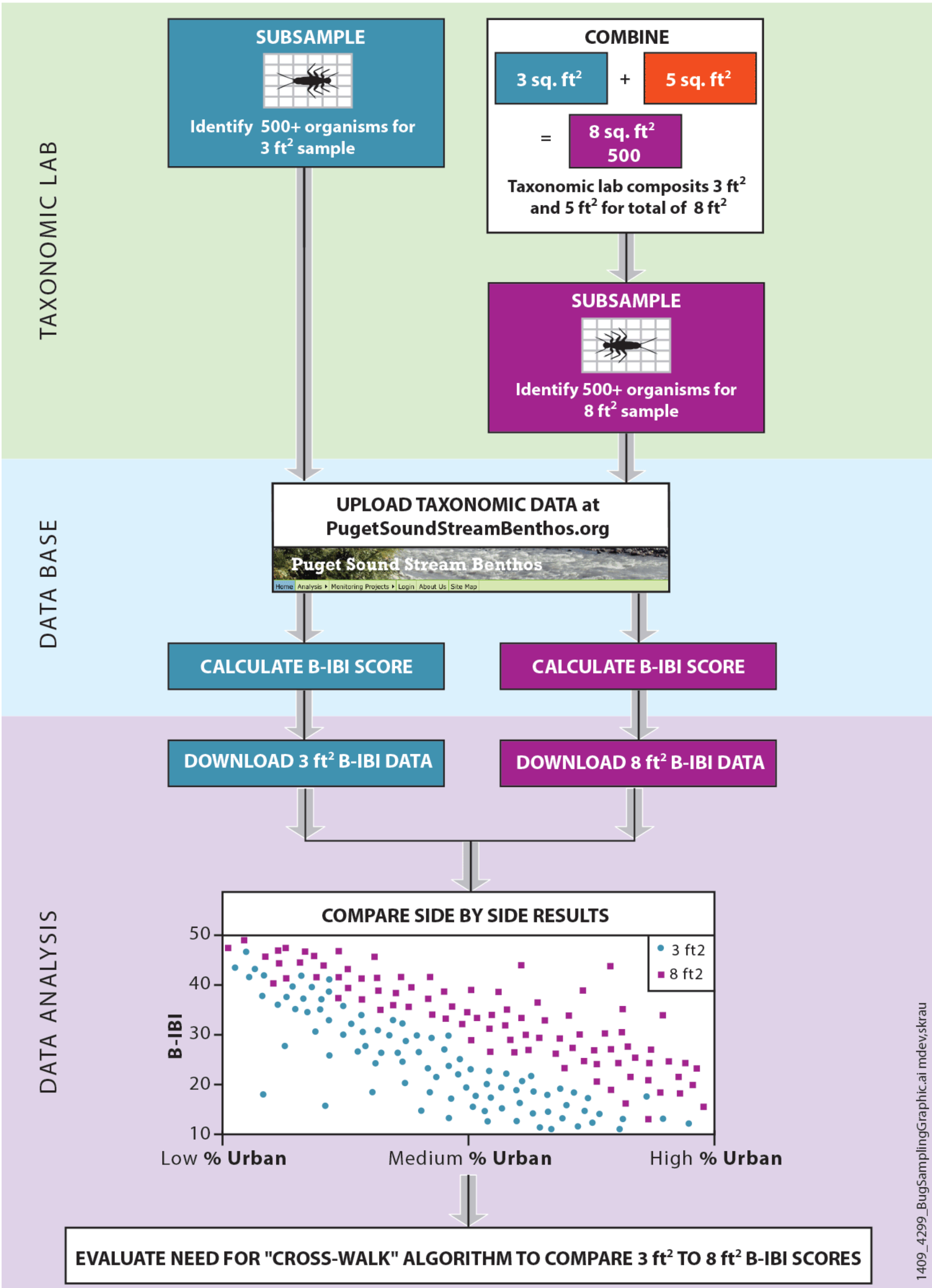


Figure 4. Laboratory procedures and subsequent data management and analysis.

Organisms were identified to the lowest practical taxonomic level[6] (Adams 2010b) which is generally genus or species using appropriate published taxonomic references and keys. Identification, counts, life stages, and information about the condition of specimens were recorded.

2.4 Data management: PSSB

All project data are stored on the PSSB as "King-DNRP: B-IBI Recalibration Data" [7]. B-IBI can be calculated using different scoring systems on the PSSB. B-IBI_{10-50} was first developed in the 1990s and includes ten metrics scored on a 1, 3, or 5 rank or discrete scale and summed for a total score ranging from 10 to 50 (Fore et al. 1996, Fore et al. 2001, Karr and Chu 1999, Kerans 1994, Karr 1998, May et al. 2000, Morley and Karr 2002, Kleindl 1995). B-IBI_{0-100} utilizes the same 10 metrics, but utilizes taxa attributes updated in 2012 (e.g., clinger, predator, long-lived, tolerant, and intolerant) and re-calibrated scoring (King County 2014b, King County 2013b). The ten metrics are scored on a continuous scale from 0 to 10 for a total B-IBI score ranging from 0-100.

Three different data sets were downloaded from the PSSB for analysis[7]:

1. B-IBI scores based on 1998 attributes and B-IBI_{10-50} metric calculations
2. B-IBI scores based on 2012 attributes and B-IBI_{0-100} metric calculations
3. Raw taxonomic data with scientific nomenclature and organism counts.

2.5 Basin delineation and landcover metrics

Geographic information systems (GIS) analysis was conducted for all sampling locations to delineate contributing watersheds and calculate landcover metrics at buffer and watershed scales. Contributing watersheds were delineated[8] following the methods laid out by Leinenbach (2011a, 2011b) and King County (2013a) based on the 30 meter National Elevation Dataset (Gesch 2007, Gesch et al. 2002). The following metrics were calculated for each of the 55 sampling locations: (1) percent watershed urbanization, defined as the sum of high-, medium-, and low-intensity development from the Coastal Change Analysis Program 2011 data (NOAA 2011), (2) level III ecoregion (Omernik 1987, EPA 2013), (3) watershed area for the upstream contributing watershed, and (4) elevation above sea level of the downstream sampling location.

[6] Taxonomic identification in 2011 matched the resolution used for Ecology samples in 2010 (lowest practical for all organisms including Chironomidae, Acari, and Oligochaetes). See Appendices G and H in Adams 2010b.
[7] The PSSB has several user-defined options for determining how the B-IBI scores are calculated. For this project data were downloaded twice: once for the B-IBI_{10-50} with the Wisseman 1998 attributes and again for the B-IBI_{0-100} with the Fore and Wisseman 2012 attributes with the following additional user-defined options. selected: (1) project King-DNRP: B-IBI Recalibration Data, (2) keyword filter: sample-tag: 3sf or 8sf, (3) replicates combined, (4) taxonomic resolution as defined by project metadata, (5) at most 500 organisms, subsampled when over.[7] The watershed shapefiles and spatial data summarized in a spreadsheet are available for download on the PSSB under the subheadings GIS Resources/Shapefiles.
[8] The watershed shapefiles and spatial data summarized in a spreadsheet are available for download on the PSSB under the subheadings GIS Resources/Shapefiles.

2.6 Analysis methods

The analysis methods for comparing results from 3 ft^2 and 8 ft^2 surface areas are described below. Results were vetted by stakeholders at a regional meeting in May 2012.

2.6.1 B-IBI scores: comparison of 3 ft^2 versus 8 ft^2

B-IBI scores for benthic macroinvertebrate samples collected from 3 ft^2 and 8 ft^2 surface areas were calculated for both $B\text{-}IBI_{10\text{-}50}$ and $B\text{-}IBI_{0\text{-}100}$ to verify that the results were similar regardless of the B-IBI scoring system used. Mean B-IBI scores for 3 ft^2 and 8 ft^2 results were compared and assessed using a t-test and the distribution of values was graphed using box plots. Visual exploratory analyses were conducted using figures including regression and correlation of 3 ft^2 versus 8 ft^2, histograms of residuals (8 ft^2 results minus 3 ft^2 results), and graphs of B-IBI versus percent urbanization in the watershed.

2.6.2 Metric: comparison of 3 ft^2 versus 8 ft^2

The ten B-IBI component metrics, plus EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa richness were compared for 3 ft^2 and 8 ft^2 results. Component metrics were assessed using the raw metric value rather than the metric score. For example, for taxa richness, the count of the number of taxa in a sample was used instead of the metric score of 1, 3, 5 or 0-10. By doing this, the results for the six metrics that do not rely on taxa attributes (taxa richness, EPT richness, Ephemeroptera richness, Plecoptera richness, Trichoptera richness, and percent dominant) are applicable across B-IBI scoring types (e.g., $B\text{-}IBI_{10\text{-}50}$ and $B\text{-}IBI_{0\text{-}100}$). The attribute dependent metrics (percent tolerant, intolerant richness, clinger richness, percent predator, and long-lived taxa richness) are variable between B-IBI scoring types because different attribute lists were used resulting in minor shifts.

Mean B-IBI metric values for 3 ft^2 and 8 ft^2 were compared using the same methods described for comparing B-IBI scores.

2.6.3 Taxonomic composition: comparison of 3 ft^2 versus 8 ft^2

The Mantel test was used to test for community-level taxonomic differences between 3 ft^2 and 8 ft^2 collection methods from the 55 Puget Sound sampling locations. This regression-based analysis is useful for comparing model pair-wise dissimilarities in communities as a function of pair-wise spatial, temporal, or environmental distances (Anderson et al. 2011). This analysis compares and calculates a correlation coefficient between two independent dissimilarity matrices and assesses its significance from randomized permutations of the data.

Abundance matrices of both sampling methods were transformed into presence or absence datasets. The Jaccard dissimilarity coefficient was used to generate a distance matrix for both datasets and the Mantel test was run using Spearman's rank correlation coefficient method. The Mantel test assumes that the variances in the different sampling method groups of the design are identical; this is called the homogeneity of variances assumption. Since the Mantel test is known to be sensitive to data heterogeneity (Anderson and Walsh

2013), a multi-response permutation procedure (MRPP) was performed based on 999 permutations of the data using the Jaccard dissimilarity index.

3.0. RESULTS

Sample collection was conducted with the help of nine partnering agencies at 55 sites in August and September 2011 (Figure 5) from 9 Puget Sound water resource inventory areas (WRIAs: 1, 5, 8, 9, 10, 15, 17, 18, and 20), 6 counties (Whatcom, Snohomish, King, Pierce, Kitsap, and Clallam), and four ecoregions (Puget Lowland, Cascades, Coastal Range, and North Cascades) (Table 1). Sites ranged from 4 to 328 m in elevation and had between 0 and 86% watershed urbanization in basins ranging in size from 17 to 19,740 hectares. See Appendix B for watershed characteristics and B-IBI scores for all 55 sites.

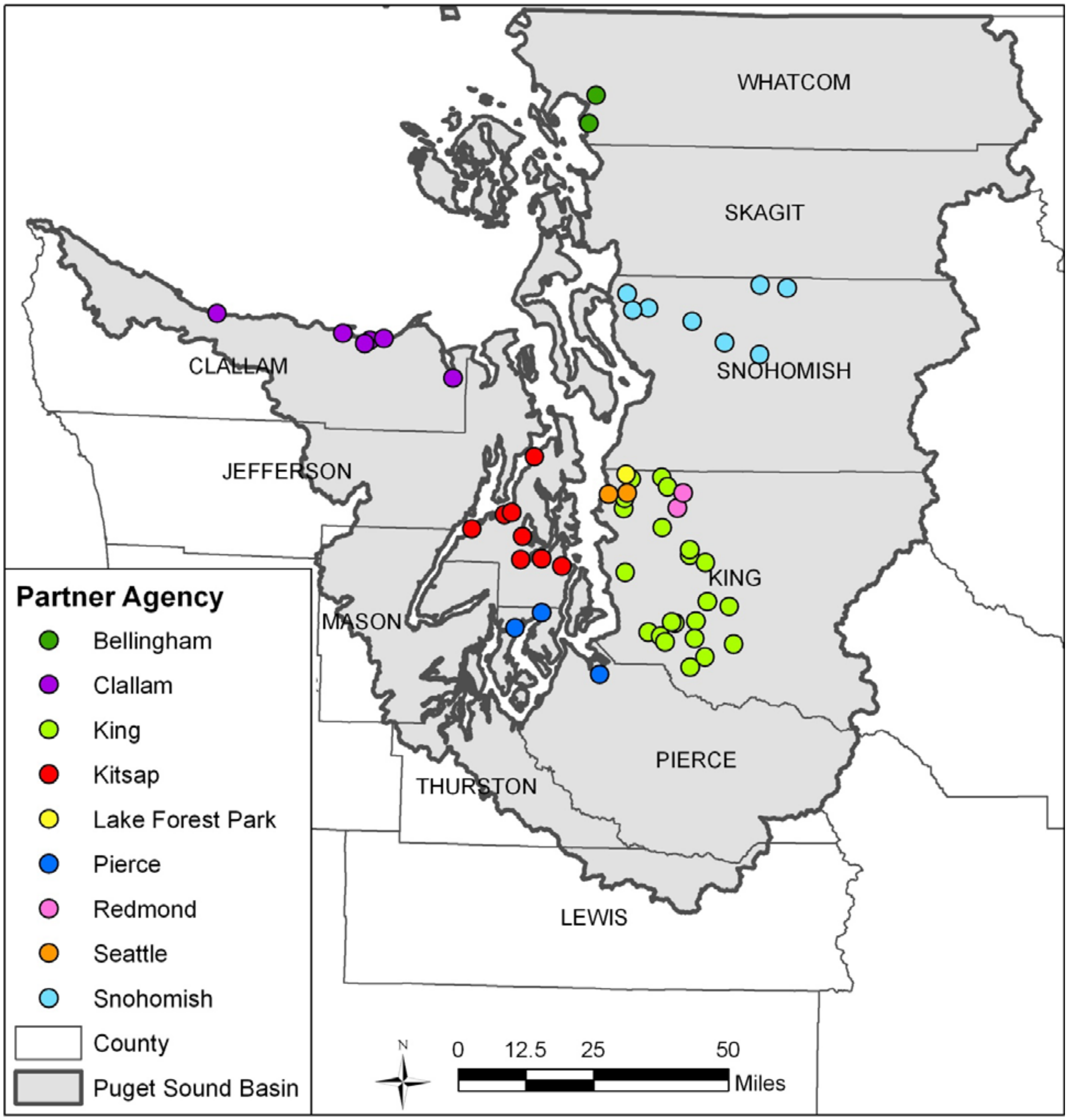


Figure 5. 2011 sampling at 55 Puget Sound locations.

Table 1. Summary statistics for watershed characteristics and B-IBI scores.

Statistic	Elevation (m)	WS Area (Hectares)	Watershed Urbanization	B-IBI$_{0\text{-}100}$ (3 ft^2)	B-IBI$_{0\text{-}100}$ (8 ft^2)	B-IBI$_{10\text{-}50}$ (3 ft^2)	B-IBI$_{10\text{-}50}$ (8 ft^2)
Min	4	17	0.0%	5.1	6.9	14	16
Max	328	19,740	86.4%	91.5	93.2	46	46
Median	34	1,698	12.5%	53.1	57.5	28	30
Average	62	3,449	23.2%	53.1	54.2	29.1	30.0
St Dev	75	4,632	27.5%	20.8	21.3	7.3	7.4

3.1 Site characteristics

Weather conditions were dry during sample collection with 49% of the sites sampled during sunny conditions, 38% mostly cloudy and 13% partly cloudy. Water clarity for all sample locations was "clear" and sample collection was never conducted within 24 hours of a heavy rain (defined as greater than ½ an inch in 24 hours). Surber nets were used at 93% of the sites (51) and D-frame kicknets were used at 7% of the sites (4).

Summary statistics for site characteristics are presented in Table 2. Samples were collected from four riffles at over 75% of the sites (41), but samples were collected from only 1 riffle at one site and from 2 riffles at 2 sites. Sampled habitat was classified as fast turbulent 98% of the time and fast non-turbulent 2% of the time. The dominant substrate at sampled riffle locations was coarse, cobble or larger substrate (> 64 mm diameter) 56% of the time; gravel (2-64 mm) just under 44% of the time; and sand (< 2 mm) at one riffle (0.2%). See Appendix C for individual site data.

Table 2. Summary statistics for site characteristics including temperature, reach length, wetted width, and riffle depth.

Statistic	Air Temp (°C)	Water Temp (°C)	Reach Length (ft)	Wetted Width (ft)	# Riffles Sampled	Riffle Depth (in)
Min	9	8	41	3	1	1
Max	25	16	1555	228	4	12
Median	16.5	13	234	12.2	4	4
Average	16.3	13	307.3	21.5	3.7	4.2
Std Dev	3.0	1.9	283.6	32.4	0.6	1.9

3.2 Abundance and density of organisms

Some Puget Sound sampling agencies have shifted to a larger surface area (>3 ft²) to try to ensure collection of a minimum of 500 organisms. When possible, sites with a history of sufficient organism counts were selected for this study, which may have biased the abundance analysis. All but three (94.5%) 3-ft² samples and all (100%) 8-ft² samples contained at least 500 organisms. As expected, it was necessary to sort a higher proportion of the sample from 3-ft² compared to 8-ft² to achieve the 500-minimum subsampling target and if extrapolated to the total surface area more organisms were present in 8-ft² samples than in 3-ft² samples (Figure 6). However, when standardized by surface area sampled, density per square foot was comparable between the 3-ft² and 8-ft² samples. These results indicate that subsampling is an essential consideration (see section 4.1 for additional discussion). To conduct analyses of B-IBI scores and metrics, data were downloaded for a 500-count subsample in all but the three 3-ft² samples with fewer than 500 organisms[9].

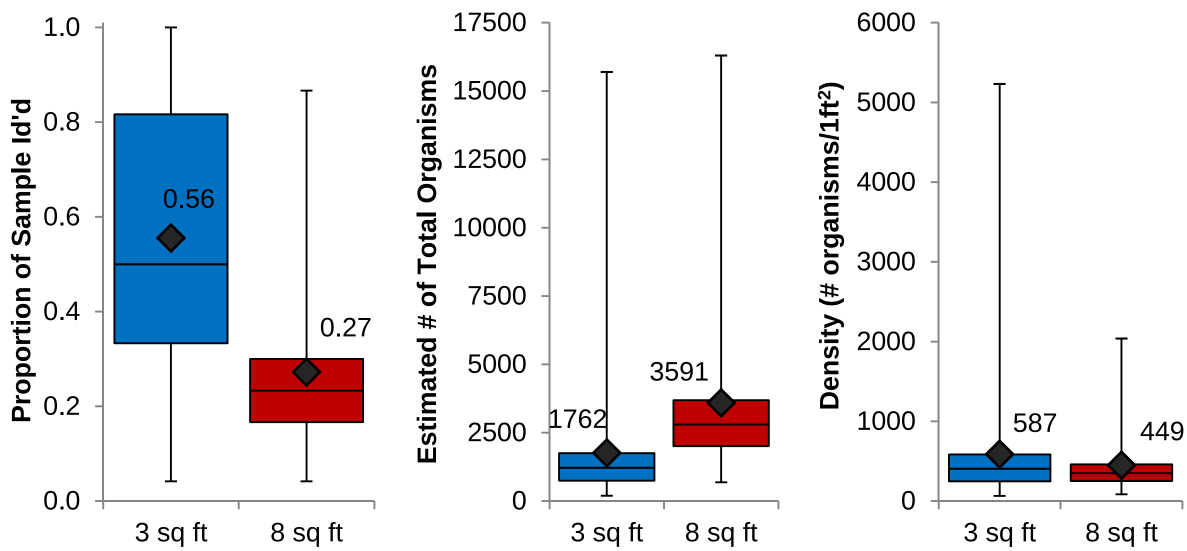


Figure 6. Box plots comparing proportion of sample identified, abundance, and density of of organisms.
Box plots range from the first to third quartile with the median designated by a black line. Error bars extend to the minimum and maximum values. Average is shown by the diamonds and data labels. N =55.

3.3 B-IBI total scores: 3 ft² vs. 8 ft²

When B-IBI scores were regressed against percent urbanization, the resulting regression lines for 3 ft² and 8 ft² were nearly identical to the line of perfect agreement and were highly correlated with one another (B-IBI$_{0-100}$ r^2=0.78, B-IBI$_{10-50}$ r^2=0.61), which indicates that B-IBI scores collected from 3 ft² and 8 ft² are comparable (Figure 7). Additionally, mean B-IBI scores for samples collected from both 3 ft² and 8 ft² were not statistically different for both B-IBI scoring systems based on t-test results (B-IBI$_{0-100}$ $p>0.80$, B-IBI$_{10-50}$ $p>0.50$, Table 3).

[9] Three 3-ft² samples had fewer than 500 organisms: "Boulder" on the Boulder River (196), "PIMA" on Pipers Creek (231), and "SwanCk" on Swan Creek (253).

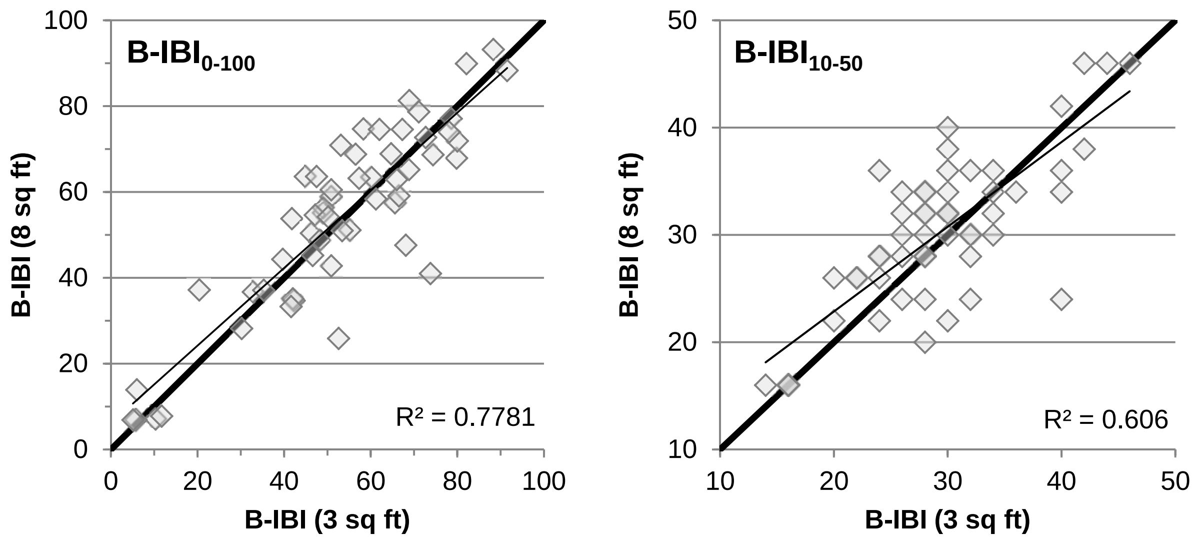


Figure 7. Comparison of B-IBI scores for macroinvertebrate samples collected from 3 ft^2 and 8 ft^2 surface areas; N=55.
The wide line is the 1 to 1 line representing an r^2 of 1; the thin line is the best fit line for the regression.

Table 3. Summary statistics for 3 ft^2 and 8 ft^2 B-IBI results; N=55.

Metric	Mean (3 ft^2)	Mean (8 ft^2)	t-test p-value	95% CI Residuals	Range (3 ft^2)	Range (8 ft^2)
B-IBI$_{0-100}$	53.1	54.2	0.7987	-6.9 to 9.0	5.1 to 91.5	6.9 to 93.2
B-IBI$_{10-50}$	29.1	30.0	0.5017	-1.8 to 3.7	14.0 to 46.0	16.0 to 46.0

CI = Confidence Interval

Residuals were calculated by subtracting the 3 ft^2 B-IBI score from the 8 ft^2 B-IBI score for both B-IBI$_{0-100}$ and B-IBI$_{10-50}$ (Figure 10). The mean residual was 1.0 for B-IBI$_{0-100}$ and ranged from a minimum of -32.8 to a maximum of 18.7. The mean residual was 0.9 for B-IBI$_{10-50}$ with a minimum of -16 and a maximum of 12. Having a mean residual close to zero with a distribution of points on both sides of zero support the conclusion that 3 ft^2 and 8 ft^2 results are comparable.

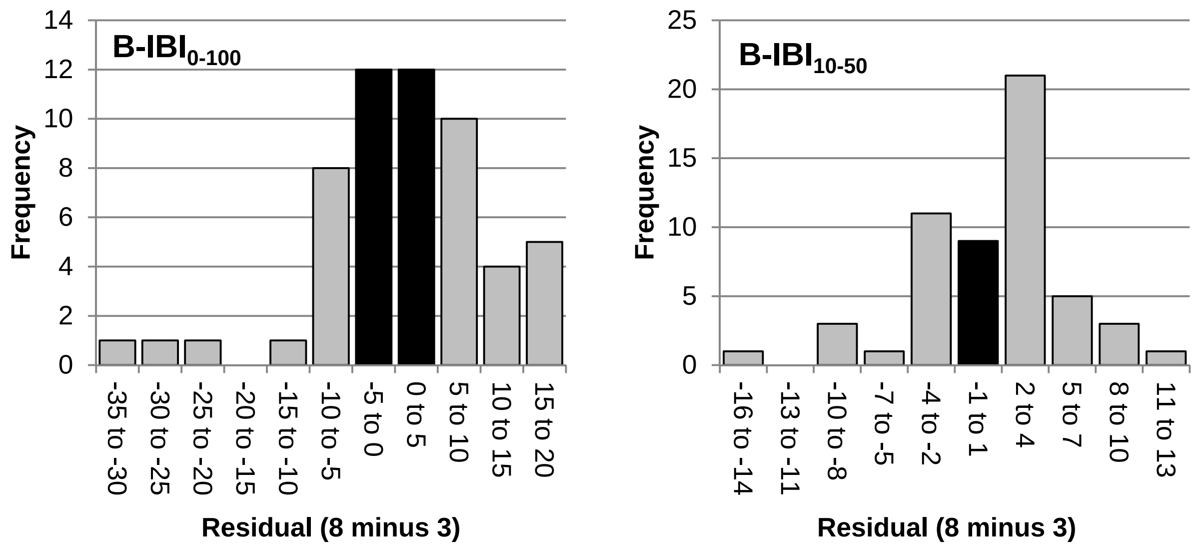
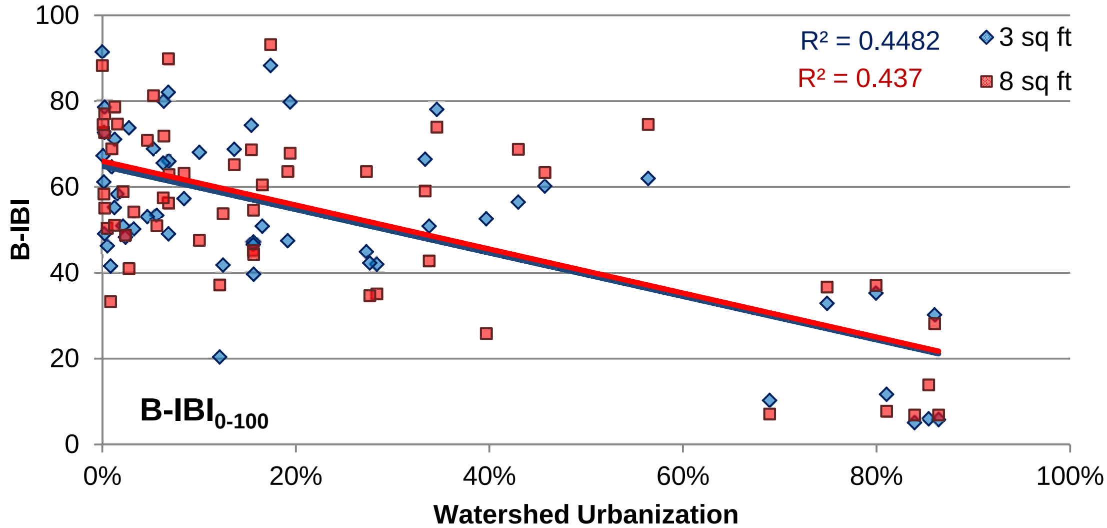


Figure 8. Histograms of B-IBI residuals (8 ft^2 minus 3 ft^2); N=55. Dark bars represent the bins that include or bracket a residual of zero.

B-IBI scores decreased with increasing percent watershed urbanization for samples collected from both 3 ft^2 and 8 ft^2 surface areas and both B-IBI$_{0-100}$ and B-IBI$_{10-50}$ (Figure 11), although the correlation with urbanization is stronger for B-IBI$_{0-100}$ (r^2=0.44-0.45) than B-IBI$_{10-50}$ (r^2=0.28-0.32). The best fit lines for samples collected from 3 ft^2 and 8 ft^2 were nearly identical to each other indicating B-IBI scores are comparable for both surface areas.



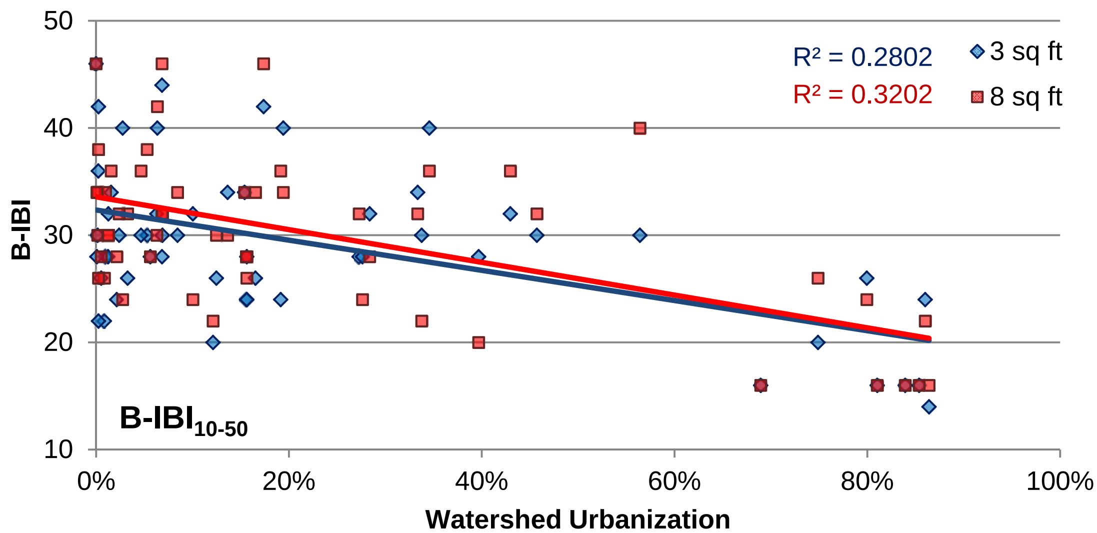


Figure 9. Percent watershed urbanization versus B-IBI scores (3 ft^2 and 8 ft^2) for both B-BIBI scoring types.
Lines represent the best fit line for either 3 ft^2 (blue) or 8 ft^2 (red) collection areas.

3.4 Component B-IBI metrics: 3 ft^2 vs. 8 ft^2

Mean values for component B-IBI metrics were very similar for samples collected from both 3 ft^2 and 8 ft^2 and were not statistically different based on t-test comparisons (Table 4, p-value between 0.18-0.89). See Appendix D for box plot figures displaying the distribution of B-IBI metric scores. B-IBI scores for samples collected from 3 ft^2 and 8 ft^2 were generally highly correlated for both scoring systems (B-IBI$_{0\text{-}100}$ and B-IBI$_{10\text{-}50}$ r^2 ranges from 0.45 to 0.79, see figures in Appendix E).

Table 4. Summary statistics and statistical significance for B-IBI metrics.

Metric		Mean (3 ft²)	Mean (8 ft²)	t-test p-value	95% CI Residuals	Range (3 ft²)	Range (8 ft²)	r^2 3 vs. 8
Taxa Richness		38.4	40.3	0.2121	-1.1 to 4.9	21 to 58	21 to 54	0.4935
EPT Richness		15.9	16.3	0.6684	-1.6 to 2.6	3 to 27	4 to 28	0.7679
Ephemeroptera Richness		5.4	5.1	0.5560	-1.3 to 0.7	0 to 12	1 to 11	0.6732
Plecoptera Richness		5.5	6.1	0.1773	-0.3 to 1.5	0 to 10	0 to 12	0.6937
Trichoptera Richness		5.0	5.1	0.7059	-0.6 to 0.9	2 to 11	1 to 10	0.4521
Dominance (%)		54.1	53.7	0.8712	-5.5 to 4.7	32 to 86.2	29 to 82.8	0.5251
Clinger Richness	$\text{B-IBI}_{0\text{-}100}$	16.6	17.2	0.5198	-1.3 to 2.6	5 to 29	3 to 26	0.6336
Clinger Richness	$\text{B-IBI}_{0\text{-}50}$	15.4	16.0	0.4761	-1.2 to 2.5	4 to 28	3 to 27	0.6537
Intolerant Richness	$\text{B-IBI}_{0\text{-}100}$	3.9	3.8	0.8918	-1.1 to 1.0	0 to 12	0 to 10	0.7908
Intolerant Richness	$\text{B-IBI}_{0\text{-}50}$	2.0	2.2	0.6882	-0.6 to 0.9	0 to 7	0 to 8	0.5273
Long-Lived Richness	$\text{B-IBI}_{0\text{-}100}$	5.9	6.1	0.7484	-0.8 to 1.0	2 to 16	2 to 12	0.5893
Long-Lived Richness	$\text{B-IBI}_{0\text{-}50}$	2.8	2.9	0.7450	-0.5 to 0.6	0 to 6	0 to 6	0.5970
Predator (%)	$\text{B-IBI}_{0\text{-}100}$	11.1	10.9	0.8903	-3.7 to 3.2	1.2 to 65.6	1.2 to 57.4	0.7863
Predator (%)	$\text{B-IBI}_{0\text{-}50}$	10.9	10.7	0.8868	-3.7 to 3.2	1.2 to 65.4	1.2 to 57	0.7866
Tolerant (%)	$\text{B-IBI}_{0\text{-}100}$	14.7	17.2	0.4948	-4.7 to 9.6	0 to 72.2	0 to 70.8	0.6634
Tolerant (%)	$\text{B-IBI}_{0\text{-}50}$	15.0	13.7	0.5277	-5.0 to 2.6	0.8 to 40.7	1.2 to 42.8	0.5988

CI = Confidence Interval

3.5 Taxonomic composition: 3 ft² vs. 8 ft²

The results of the Mantel test show significant concordance between the 3 ft² and 8 ft² sampling methods (r = 0.62; p = 0.001), confirming similarity of species composition at each site regardless of sampling area. Results of the MRPP show no significant difference was seen in variances between samples collected from 3 ft² and 8 ft² (within-group agreement A = -0.003, delta = 0.82, p >0.99). A nonmetric multidimensional scaling (NMDS) ordination was used to visualize spread of the taxa abundance between the two methods (Figure 10). The spread of the data show very little differences and quite a bit of overlap, indicating that taxa composition is similar for the two sampling methods.

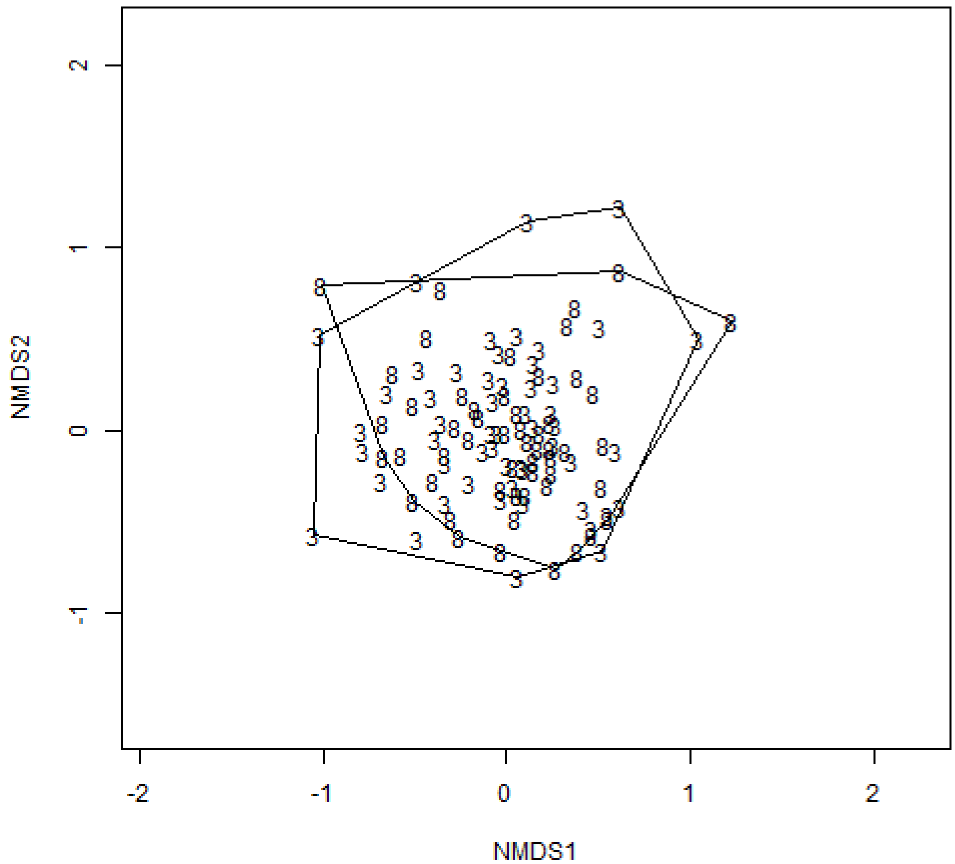


Figure 10. Nonmetric Multidimensional Scaling (NMDS) ordination plot of 3 ft^2 and 8 ft^2 sample data.
3 = 3 ft^2 samples; 8 = 8 ft^2 samples.

4.0. DISCUSSION

Comparability of 3 ft^2 and 8 ft^2 results, the applicability of these results, and the regional implications of this study are discussed here.

4.1 Are 3 ft^2 and 8 ft^2 results comparable?

Results indicate that B-IBI scores (Table 3, Figures 9 and 10) and component metrics (Table 4, Appendix E) were comparable regardless of surface collection area with no significant difference in means, data points scattered both above and below the 1:1 line of perfect agreement and residuals generally centered around zero. The response of B-IBI to watershed urbanization (Figure 9) and taxonomic composition (Figure 10) were also similar regardless of surface area (Figure 11). However, this project was undertaken with the assumption that B-IBI results from macroinvertebrate communities collected from different surface areas (3 ft^2 versus 8 ft^2) were not comparable and it would be necessary to develop some kind of scoring adjustment or ”cross-walk.” The working hypothesis was that samples from a larger surface area would likely sample more microhabitats and better represent the taxonomic diversity of the stream reach resulting in a greater number of macroinvertebrates from a greater diversity of taxa. Therefore, counts for richness metrics from 8 ft^2 would be greater and result in higher B-IBI scores compared with counts from 3 ft^2. The side by side data collected from both 3 ft^2 and 8 ft^2 samples at 55 sites across Puget Sound in the summer of 2011 do not support the pre-project hypothesis as long as a minimum subsampling target such as 500 organisms is employed. The correlation of 3 ft^2 versus 8 ft^2 B-IBI scores show the best fit line is nearly identical with the line of perfect agreement (Figure 7) and residuals centered around zero instead of skewed in the positive direction.

Mean total estimated abundance was the only result that was significantly different between 3 ft^2 and 8 ft^2 samples indicating, not surprisingly, that more organisms are collected from larger surface areas (Figure 6). These results emphasize the importance of subsampling as an essential consideration. It is logical to assume that if all organisms from a sample were sorted and identified that a greater variety of taxa and an increase in B-IBI scores *might* be found for 8 ft^2 samples compared to 3 ft^2 samples as found by Cazier (1993) and Vinson and Hawkins (1996). Subsampling at 500 organisms is a commonly adopted practice throughout the Puget Sound region and is used by Ecology (Adams 2010b) and advocated by PNAMP and EPA (Hayslip 2007). In addition, PSSB users can designate a subsampling target when exploring or downloading data in order to standardize taxa counts across samples.

Endorsement by regional experts

The results of the side by side sampling for B-IBI_{10-50} total score and individual metrics in addition to the correlation of different surface area B-IBI_{10-50} scores with percent watershed urbanization were presented at a workshop in May 2012 attended by 37 staff from federal, state, and Puget Sound agencies and tribes active in biomonitoring programs (King County 2012). Attendees discussed the results and collectively agreed that no scoring adjustments were required to compare results from 3 ft^2 and 8 ft^2 samples and that no additional sampling was necessary to further explore this topic.

4.2 Regional implications

Several agencies with ongoing biomonitoring programs have modified their macroinvertebrate collection methodologies based at least in part on the results presented here. King County DNRP and Snohomish County have switched from collecting samples from 3 ft^2 to 8 ft^2 and Clallam County switched from 9 ft^2 to 8 ft^2. Prior to this study, Kitsap County switched their collection area from 3 ft^2 to 8 ft^2. These agencies can now have confidence that if changes in taxonomic effort are accounted for, historical data are comparable to current data even when surface area collection methods have changed.

However, even though samples collected from different surface areas have been shown to be comparable based on the results presented in this report, there still are a number of things to consider when determining what surface area to sample when collecting benthic macroinvertebrates (Table 5). For example, modifying collection area protocols to 8 ft^2 meets Ecology's minimum collection area requirement for results to be considered for their water quality assessment (Ecology 2012). Waterbodies classified as impaired based on B-IBI data are listed on Washington State's 303(d) list and may be considered for stressor identification or total maximum daily load (TMDL) studies to try to improve biotic condition. Because benthic macroinvertebrates integrate both water quality and habitat (including flow conditions), use of the B-IBI in the water quality assessment and the 303d listing process provides an important tool to better characterize environmental conditions and potential stressors.

An additional factor to consider is level of effort and cost. Sampling from 8 ft^2 is more time intensive and therefore more costly. Collection from a larger surface area disturbs additional substrate and likely results in the sacrifice of more organisms compared to collection from a 3 ft^2 area. While regional standardization is generally desired, this study suggests that standardization of surface area is not essential for data to be comparable and therefore it is up to the individual monitoring agencies to determine what is in their best interest.

Table 5. Summary of considerations regarding selection of sample collection area.

	3 ft^2	8 ft^2
Pros	• Fewer organisms are sacrificed • Easier to find sufficient riffle habitat • Sample collection takes less time, and therefore saves money • Less habitat disruption	• More streams meet minimum organism counts • In line with federal and state protocols[10] • Meets Ecology's surface area requirements for the state water quality assessment • Compatible with Ecology's O/E model collection protocols
Cons	• Does not meet Ecology's surface area requirement for the State water quality assessment • May not be appropriate to apply Ecology's O/E model • In some situations it can be difficult to meet minimum organism counts	• More organisms are sacrificed • Some sites do not have sufficient riffle habitat for the larger surface area and therefore are dropped from sampling • Sample collection takes more time and therefore costs more money • More habitat disruption

B-IBI is now a PSP vital sign indicator and has two ecosystem recovery targets (PSP 2012). Progress towards these targets is evaluated approximately biannually in the State of the Sound Reports (PSP 2013). The results of this project confirm the comparability of regional data from different surface areas. These results combined with the results of Gerth and Herlihy (2006) and Rehn et al. (2007) demonstrating that riffle-based sampling is comparable to reach-wide transect sampling greatly increases the regional data available to evaluate progress towards the ecosystem recovery targets.

4.3 Conclusions

In conclusion, this work establishes the comparability of B-IBI and macroinvertebrate community composition from 3 ft^2 and 8 ft^2 collection areas. Results reported from each collection method can be compared with confidence and reported interchangeably enabling the use of existing long-term and future data to evaluate trends in biological integrity over time. This has allowed jurisdictions within the Puget Sound region to transition to collection of 8 ft^2 samples without losing the ability to track long-term trends based on historical data collected from 3 ft^2 areas. In addition, results of this work enable direct comparison of a larger pool of regional data and in doing so promote data integration to evaluate ecosystem conditions across jurisdictional boundaries.

[10] See Hayslip 2007, Adams 2010a, Cusimano et al. 2006, Peck et al. 2006, and Klemm et al. 2006.

5.0. REFERENCES

Adams, K. 2010a. Standard operating procedures and minimum requirements for the collection of freshwater benthic macroinvertebrate data in wadeable streams and rivers. Prepared by Environmental Assessment Program, Washington Department of Ecology, Olympia, Washington.

Adams, K. 2010b. Quality Assurance Monitoring Plan: Ambient Biological Monitoring in Rivers and Streams: Benthic Macroinvertebrates and Periphyton. Publication No. 10-03-109. Prepared by Environmental Assessment Program, Washington Department of Ecology, Olympia, Washington.

Anderson, M. J., T. O. Crist, J. M. Chase, M. Vellend, B. D. Inouye, A. L. Freestone, N. J. Sanders, H. V. Cornell, L. S. Comita, K. F. Davies, S. P. Harrison, N. J. Kraft, J. C. Stegen, and N. G. Swenson. 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecology Letters 14: 19-28.

Anderson, M. J. and D. C. Walsh. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecological Monographs 83:557–574.

Caton, L. W. 1991. Improving subsampling methods for the EPA's "Rapid Bioassessment" benthic protocols. Bulletin of the North American Benthological Society 8:317-319.

Cazier, L. D. 1993. Bioassessment of small agricultural streams: the Palouse-Camas Prairie Region of Northern Idaho. University of Idaho, Moscow, Idaho.

Cusimano, R., G. Merritt, R. Plotnikoff, C. Wiseman, C. Smith & Washington State Department of Fish and Wildlife. 2006. Status and Trends Monitoring for Watershed Health and Salmon Recovery: Quality Assurance Monitoring Plan. 62 pages. Environmental Assessment Program, Washington State Department of Ecology, Ecology Publication No. 06-03-203, Olympia, Washington.

[Ecology] Washington State Department of Ecology. 2012. Water Quality Program Policy 1-11. Chapter 1: Assessment of water quality for the Clean Water Act sections 303(d) and 305(b) integrated report. July 2012 revisions. Lacey, Washington.

[EPA] U.S. Environmental Protection Agency. 2013. Level III ecoregions of the continental United States. Map. (1:7,500,000). U.S. EPA - National Health and Environmental Effects Research Laboratory, Corvallis, Oregon.

Fore, L. 2011. Memorandum to Deb Lester and Jo Wilhelm, King County: Effect of natural features on B-IBI and recommendations for the 2011 sampling design. Statistical Design, Seattle, Washington.

Fore, L. S., J. R. Karr and R. W. Wisseman. 1996. Assessing invertebrate responses to human activities: evaluating alternative approaches. Journal of the North American Benthological Society 212-231.

Fore, L. S., K. Paulsen and K. O'Laughlin 2001. Assessing the performance of volunteers in monitoring streams. Freshwater Biology 46(1): 109-123.

Gerth, W. J., and A. T. Herlihy. 2006. Effect of sampling different habitat types in regional macroinvertebrate bioassessment surveys. Journal of the North American Benthological Society 25:501-512.

Gesch, D. B. 2007. The National Elevation Dataset. Pages 99-118 in D. Maune, editor. Digital Elevation Model Technologies and Applications: The DEM User's Manual, 2nd Edition. American Society for Photogrammetry and Remote Sensing, Bethesda, Maryland.

Gesch, D., M. Oimoen, S. Greenlee, C. Nelson, M. Steuck, M., and D. Tyler. 2002. The National Elevation Dataset. Photogrammetric Engineering and Remote Sensing 68:5-11.

Hayslip, G., editor. 2007. Methods for the collection and analysis of benthic macroinvertebrate assemblages in wadeable streams of the Pacific Northwest. Pacific Northwest Aquatic Monitoring Partnership (PNAMP), Cook, Washington.

Karr, J. R. 1998. Rivers as sentinels: using the biology of rivers to guide landscape management. Pages 502-528 in R. J. Naiman and R. E. Bilby (editors), River Ecology and Management: Lessons from the Pacific Coastal Ecosystems. Springer, New York.

Karr, J. R. and E. W. Chu. 1999. Restoring life in running waters: better biological monitoring. Island Press, Washington, D.C.

Kerans, B. L. and J. R. Karr. 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. Ecological Applications 4:768-785.

King County. 2009. Puget Sound Stream Benthos: Monitoring Status and Data Management. Prepared by Jenée Colton, Doug Henderson, Deb Lester, and Jim Simmonds, Water and Land Resources Division, Seattle, Washington.

King County. 2011a. Puget Sound Region Benthic Macroinvertebrate Field Collection and Laboratory Sorting Methods for a Side by Side Comparison of 3 ft^2 vs 8 ft^2. Prepared by Jo Opdyke Wilhelm, Deb Lester (Water and Land Resources Division), and Leska Fore (Statistical Design). Seattle, Washington.

King County. 2011b. Enhancement and Standardization of Benthic Macroinvertebrate Monitoring and Analysis Tools for the Puget Sound Region: Quality Assurance Project Plan. Prepared by Deb Lester (Water and Land Resources Division), Jo Opdyke Wilhelm (Water and Land Resources Division), and Leska Fore (Statistical Design). Seattle, Washington.

King County. 2012. Results of 2011 side by side sampling efforts: 3 vs. 8 sq ft. A PowerPoint presentation prepared by Jo Opdyke Wilhelm, Deb Lester (King County), Leska Fore (Statistical Design), Karen Adams (Washington State Department of Ecology), and Gretchen Hayslip (Environmental Protection Agency) for a May 8, 2012 advisory board meeting. Seattle, Washington.

King County. 2013a. Watershed delineation and land cover calculations for Puget Sound stream basins. Prepared by Jo Opdyke Wilhelm (King County Water and Land Resources Division), Peter Leinenbach (US Environmental Protection Agency), Leska Fore (Statistical Design), Deb Lester (King County Water and Land Resources Division), Karen Adams (Washington State Department of Ecology), and Gretchen Hayslip (US Environmental Protection Agency). Seattle, Washington.

King County. 2013b. Using natural history attributes of stream invertebrates to measure stream health Prepared by Leska Fore (Statistical Design), Bob Wisseman (Aquatic Biology Associates), Jo Opdyke Wilhelm (King County Water and Land Resources Division), Deb Lester (King County Water and Land Resources Division), Karen Adams (Washington State Department of Ecology), Gretchen Hayslip (U.S. Environmental Protection Agency), and Peter Leinenbach (U.S. Environmental Protection Agency). Seattle, Washington.

King County. 2014a. Examining the influence of natural site features on B-IBI response. Prepared by Elene Dorfmeier, King County Department of Natural Resources, Water and Land Resources Division, Seattle, Washington.

King County. 2014b. Recalibration of the Puget Lowland Benthic Index of Biotic Integrity (B-IBI). Prepared by Leska Fore (Statistical Design), Jo Opdyke Wilhelm, Deb Lester, and Elene Dorfmeier. Water and Land Resources Division, Seattle, Washington.

Kleindl, W. J. 1995. A Benthic Index of Biotic Integrity for Puget Sound Lowland Streams, Washington, USA. M.S. Thesis, University of Washington, Seattle, Washington.

Klemm, D. J., J. M. Lazorchak, and D. V. Peck. 2006. Section 10 Benthic Macroinvertebrates.in D. V. Peck, A. T. Herlihy, B. H. Hill, R. M. Hughes, P. R. Kaufmann, D. J. Klemm, J. M. Lazorchak, F. H. McCormick, S. A. Peterson, P. L. Ringold, T. Magee, and M. R. Cappaert, editors. Western Pilot Study: Field operations manual for wadeable streams. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

Leinenbach, P. 2011a. Automated watershed digitization using NHDPlus datasets and the ArcHydro extension. United States Environmental Protection Agency, Seattle, Washington.

Leinenbach, P. 2011b. Landscape sampling of the 1027 sampling sites in the Puget Sound Basin. United States Environmental Protection Agency, Seattle, Washington.

Marconi, D. R. 2010. Comparing paired scores from the overall Benthic Index of Biotic Integrity of 20 stream sites located in the Lake Washington/Cedar/Sammamish Watershed. Senior Project Capstone, School of Forest Resources, University of Washington. Advisor S. Bolton, Seattle, Washington.

May, C. W., R. R. Horner, J. R. Karr, B. W. Mar, and E. B. Welch. 2000. Effects of urbanization on small streams in the Puget Sound lowland ecoregion. Watershed Protection Techniques 2(4):483-494.

Morley, S. A., and J. R. Karr. 2002. Assessing and restoring the health of urban streams in the Puget Sound Basin. Conservation Biology 16(6):1498-1509.

Omernik, J. M. 1987. Ecoregions of the conterminous United States. Map Supplement (scale 1:7,500,000). Annals of the Association of American Geographers 77:118-125.

Peck, D. V., A. T. Herlihy, B. H. Hill, R. M. Hughes, P. R. Kaufmann, D. J. Klemm, J. M. Lazorchak, F. H. McCormick, S. A. Peterson, P. L. Ringold, T. Magee, and M. R. Cappaert, editors. 2006. Environmental Monitoring and Assessment Program - Surface Waters: Western Pilot Study Field Operations Manual for Wadeable Streams. U.S. Environmental Protection Agency, Washington, D.C.

Plotnikoff, R. W. and C. Wiseman. 2001. Benthic Macroinvertebrate Biological Monitoring Protocols for Rivers and Streams. Washington Publication No. 01-03-028, Environmental Assessment Program, Department of Ecology, Washington Publication No. 01-03-028, Olympia, Washington.

[PSP] Puget Sound Partnership. 2012. The 2012/2013 Action Agenda for Puget Sound. 499 pages. Tacoma, Washington.

[PSP] Puget Sound Partnership. 2013. 2013 State of the Sound: A biennial report on the recovery of Puget Sound. 177 pages. Tacoma, Washington.

Rehn, A. C., P. R. Ode, and C. P. Hawkins. 2007. Comparisons of targeted-riffle and reach-wide benthic macroinvertebrate samples: implications for data sharing in stream-condition assessments. Journal of the North American Benthological Society 26:332-348.

Vinson, M. R. and C. P. Hawkins. 1996. Effects of Sampling Area and Subsampling Procedure on Comparisons of Taxa Richness among Streams. Journal of the North American Benthological Society 15:392-399.

Appendices

Appendix A: Data Sheet

Appendix B: Site Specific Watershed Data and B-IBI Scores

Appendix C: Site Specific Habitat Data

Appendix D: Box plots of mean B-IBI scores and individual B-IBI metric values

Appendix E: Correlation graphs and residual histograms for B-IBI metrics

APPENDIX A: DATA SHEET

Page one of a two-page data sheet is shown on the following page. The second page of the data sheet was blank and was used to hand draw the approximate location of sampled collected and the location of riffles.

Site Name_Number ______________________	Personnel: ______________________
Partner Organization ______________________	Date & Time: ______________________
Location Description ______________________	PSSB Data Entry: Check when complete ☐
______________________	Date: ____________ Initials: ____________

Weather & Water Conditions	Current Weather: ☐ rain ☐ mostly cloudy (>50%) ☐ partly cloudy (10-50%) ☐ sunny	> 0.5" rain in last 24 hours? **Y** **N** Air Temperature (°C): ____________ Water Temperature (°C): ____________ Water Clarity: ☐ Clear ☐ Turbid/Opaque

Sampling Metadata Sample collected? Y N	Reach length ____________ ft Wetted width ____________ ft Sampling Device: ☐ D-frame ☐ Surber # riffles sampled: **1** **2** **3** **4** Mussels in sample? Species & count: ______________________

Riffle Data

Sample Unit:
FT = Fast Turbulent (riffle, cascade, waterfall);
FN = Fast Non-Turbulent (sheet, run, glide)

Dominant Substrate:
(Snd) Fines/Sand (< 2mm; < ladybug)
(Grvl) Gravel (2-64mm; ladybug-tennis ball)
(Crs) Coarse (>64 mm; > tennis ball)
(Oth) Other

Sample #	3 or 5 sq ft	Sample Unit	Substrate	Riffle Depth (")	Riffle #
1	3 5	FT FN	Snd Grvl Crs Oth	________	________
2	3 5	FT FN	Snd Grvl Crs Oth	________	________
3	3 5	FT FN	Snd Grvl Crs Oth	________	________
4	3 5	FT FN	Snd Grvl Crs Oth	________	________
5	3 5	FT FN	Snd Grvl Crs Oth	________	________
6	3 5	FT FN	Snd Grvl Crs Oth	________	________
7	3 5	FT FN	Snd Grvl Crs Oth	________	________
8	3 5	FT FN	Snd Grvl Crs Oth	________	________

Notes (regarding riparian condition, landuse, buffer width, sampling details, etc.)	

APPENDIX B: SITE SPECIFIC WATERSHED DATA AND B-IBI SCORES

Table A-1 Watershed characteristics for the 55 project sampling sites.

Site Code	Agency	Ecoregion	WRIA	Stream	Latitude	Longitude	Net	Elevation (m)	WS Area (Hectares)	WS Urbanization	Date	B-IBI$_{0-100}$ (3ft^2)	B-IBI$_{0-100}$ (8 ft^2)	B-IBI$_{10-50}$ (3 ft^2)	B-IBI$_{10-50}$ (8 ft^2)
Chuckanut_Arroyo	Bellingham	Puget Lowland	1 - Nooksack	Chuckanut Ck	48.701664	-122.4807	Kicknet	29	1728	5.3%	9/28/2011	68.9	81.3	30	38
Squalicum_IronGate	Bellingham	Puget Lowland	1 - Nooksack	Squalicum Ck	48.777714	-122.454344	Kicknet	34	3146	12.1%	9/28/2011	20.4	37.2	20	22
JimmyComeLately0pt8	Clallam Co.	Puget Lowland	17 - Quilcene-Snow	Jimmycomelately Ck	48.011202	-123.002596	Surber	28	3805	0.2%	8/28/2011	72.7	72.7	36	34
Bagley_0pt7	Clallam Co.	Puget Lowland	18 - Elwha-Dungeness	Bagley Ck	48.105212	-123.338054	Surber	43	1866	5.6%	9/28/2011	53.4	51	28	28
Morse_1pt7	Clallam Co.	Puget Lowland	18 - Elwha-Dungeness	Morse Ck	48.096492	-123.357312	Surber	28	14512	1.0%	9/22/2011	64.7	68.9	28	34
Siebert_0pt6	Clallam Co.	Puget Lowland	18 - Elwha-Dungeness	Siebert Ck	48.111501	-123.282918	Surber	21	4939	3.3%	9/13/2011	50.2	54.2	26	32
Tumwater_01a	Clallam Co.	Puget Lowland	18 - Elwha-Dungeness	Tumwater Ck	48.122125	-123.446971	Surber	6	1522	15.6%	9/8/2011	47.2	54.6	28	28
WestTwin_1pt0	Clallam Co.	Coastal Range	20 - Sol Duc	West Twin Ck	48.162056	-123.953667	Surber	6	3264	0.2%	9/19/2011	61.2	58.4	30	30
08CED4192	King Co.	Puget Lowland	8 - Cedar-Sammamish	Rock Ck (L. Cedar)	47.3748	-122.0177	Surber	151	530	17.4%	9/19/2011	88.3	93.2	42	46
08CED5032	King Co.	Puget Lowland	8 - Cedar-Sammamish	Rock Ck (U. Cedar)	47.415086	-121.887131	Surber	328	514	0.0%	8/17/2011	91.5	88.3	46	46
08EAS2272	King Co.	Puget Lowland	8 - Cedar-Sammamish	Kelsey Ck	47.623286	-122.156843	Surber	52	1698	68.9%	9/12/2011	10.3	7.1	16	16
08ISS3877	King Co.	Puget Lowland	8 - Cedar-Sammamish	Issaquah Ck	47.551207	-122.046876	Surber	15	14753	12.5%	8/30/2011	41.8	53.8	26	30
08ISS4724	King Co.	Puget Lowland	8 - Cedar-Sammamish	Carey Ck	47.426952	-121.97338	Surber	148	1151	1.6%	9/8/2011	58.3	74.7	34	36
08ISS4748	King Co.	Cascades	8 - Cedar-Sammamish	Issaquah Ck - E Fork	47.531728	-121.983455	Surber	134	1654	6.4%	8/30/2011	80	71.9	40	42
08LAK3879	King Co.	Puget Lowland	8 - Cedar-Sammamish	Laughing Jacobs Ck	47.56535	-122.045569	Surber	46	1161	45.7%	8/30/2011	60.2	63.4	30	32
08LIT2585	King Co.	Puget Lowland	8 - Cedar-Sammamish	Little Bear Ck	47.758429	-122.160347	Surber	13	3970	33.8%	9/12/2011	50.9	42.8	30	22
08SAM2862	King Co.	Puget Lowland	8 - Cedar-Sammamish	Sammamish R trib (0090)	47.733381	-122.13722	Surber	49	221	34.6%	9/12/2011	78.1	74	40	36
08WES0622	King Co.	Puget Lowland	8 - Cedar-Sammamish	Ravenna Ck	47.673452	-122.310727	Surber	42	1593	86.0%	8/18/2011	30.2	28.2	24	22
08WES0629	King Co.	Puget Lowland	8 - Cedar-Sammamish	Maple Leaf Ck	47.700951	-122.30705	Surber	47	645	86.4%	8/18/2011	5.8	6.9	14	16
08WES0903	King Co.	Puget Lowland	8 - Cedar-Sammamish	McAleer Ck	47.752199	-122.281877	Surber	11	2027	74.9%	8/18/2011	32.9	36.7	20	26
09COV1756	King Co.	Puget Lowland	9 - Duwamish-Green	Covington Ck	47.32877	-122.022072	Surber	157	4882	6.8%	9/6/2011	82.1	89.9	44	46
09DUW0225	King Co.	Puget Lowland	9 - Duwamish-Green	Duwamish R trib (0003)	47.502054	-122.299381	Surber	14	303	81.0%	9/1/2011	11.7	7.8	16	16
09JEN1357	King Co.	Puget Lowland	9 - Duwamish-Green	Jenkins Ck	47.3689	-122.098899	Surber	115	2470	33.4%	9/8/2011	66.5	59.1	34	32
09LOW0751	King Co.	Puget Lowland	9 - Duwamish-Green	Olson Ck	47.343487	-122.20504	Surber	16	357	43.0%	9/15/2011	56.5	68.8	32	36
09MID1958	King Co.	Puget Lowland	9 - Duwamish-Green	Icy Ck	47.278886	-121.978571	Surber	119	103	0.5%	8/12/2011	46.3	50.4	26	28
09MID2426	King Co.	Cascades	9 - Duwamish-Green	Green R - Middle trib	47.315006	-121.867614	Surber	267	252	1.2%	9/6/2011	55.2	51.1	28	30
09NEW1657	King Co.	Puget Lowland	9 - Duwamish-Green	Newaukum Ck	47.250042	-122.037744	Surber	154	7020	15.4%	8/17/2011	74.4	68.7	34	34
09SOO1022	King Co.	Puget Lowland	9 - Duwamish-Green	Soosette Ck	47.3326	-122.1563	Surber	84	1135	56.4%	8/3/2011	62	74.6	30	40
09SOO1130	King Co.	Puget Lowland	9 - Duwamish-Green	Soos Ck	47.317784	-122.138384	Surber	45	19740	27.3%	9/15/2011	44.9	63.6	28	32
09SOO1283	King Co.	Puget Lowland	9 - Duwamish-Green	Little Soos Ck	47.3717	-122.1126	Surber	129	1745	13.6%	8/3/2011	68.8	65.2	34	30
KCSSWM003	Kitsap Co.	Puget Lowland	15 - Kitsap	Big Beef Ck	47.6495	-122.7824	Surber	4	3389	6.8%	8/24/2011	49.1	56.3	28	32
KCSSWM006	Kitsap Co.	Puget Lowland	15 - Kitsap	Big Anderson Ck	45.567	-122.9636	Surber	5	1542	0.9%	8/23/2011	41.6	33.3	22	26
KCSSWM007	Kitsap Co.	Puget Lowland	15 - Kitsap	Chico Ck	47.5922	-122.709	Surber	19	4069	6.9%	8/24/2011	66	62.9	30	32
KCSSWM009	Kitsap Co.	Puget Lowland	15 - Kitsap	Boyce Ck	47.6088	-122.9098	Surber	10	407	2.4%	8/23/2011	48.3	48.8	30	32
KCSSWM011	Kitsap Co.	Puget Lowland	15 - Kitsap	Little Anderson Ck	47.655733	-122.755017	Surber	30	921	16.5%	8/29/2011	50.9	60.5	26	34
KCSSWM030	Kitsap Co.	Puget Lowland	15 - Kitsap	Jump Off Ck	47.8068	-122.6692	Surber	6	336	28.4%	8/29/2011	42	35.1	32	28
KCSSWM034	Kitsap Co.	Puget Lowland	15 - Kitsap	Blackjack Ck	47.5349	-122.632	Surber	13	3323	19.1%	8/25/2011	47.5	63.6	24	36
KCSSWM038	Kitsap Co.	Puget Lowland	15 - Kitsap	Curley Ck	47.51505	-122.55135	Surber	7	3721	15.6%	8/25/2011	46.6	45.2	24	28
KCSSWM040	Kitsap Co.	Puget Lowland	15 - Kitsap	Gorst Ck	47.5305	-122.7136	Surber	17	1651	4.7%	8/29/2011	53.1	70.9	30	36
McAleer_187	Lake Forest Park	Puget Lowland	8 - Cedar-Sammamish	McAleer Ck	47.764128	-122.303422	Surber	52	1726	79.9%	9/17/2011	35.3	37.1	26	24
BIBI_008_Swan	Pierce Co.	Puget Lowland	10 - Puyallup-White	Swan Ck	47.2269	-122.3929	Surber	9	900	39.7%	8/19/2011	52.6	25.9	28	20
BIBI_006_Lacky	Pierce Co.	Puget Lowland	15 - Kitsap	Lacky Ck	47.34708	-122.73	Surber	7	708	15.6%	8/31/2011	39.7	44.3	24	26
BIBI_028_Purdy	Pierce Co.	Puget Lowland	15 - Kitsap	Purdy Ck (Burley Lagoon)	47.38931	-122.626	Surber	7	937	8.4%	8/26/2011	57.3	63.2	30	34
08BEA3474	Redmond/King	Puget Lowland	8 - Cedar-Sammamish	Bear Ck (Sammamish)	47.677597	-122.098301	Kicknet	15	12204	27.6%	9/7/2011	42.3	34.7	28	24
08BEA3650	Redmond/King	Puget Lowland	8 - Cedar-Sammamish	Bear Ck (Sammamish)	47.717871	-122.076997	Kicknet	37	3630	19.4%	9/7/2011	79.8	67.9	40	34
PIMA3714	Seattle	Puget Lowland	8 - Cedar-Sammamish	Pipers Ck	47.711	-122.3723	Surber	17	164	85.4%	8/22/2011	6	13.9	16	16
TNMA6462	Seattle	Puget Lowland	8 - Cedar-Sammamish	Thornton Ck	47.714962	-122.298236	Surber	49	1524	83.9%	8/22/2011	5.1	6.9	16	16
Benson	Snohomish Co.	North Cascades	5 - Stillaguamish	Benson Ck (Stillaguamish)	48.091067	-121.779031	Surber	294	568	0.2%	8/8/2011	78.6	77.1	42	38
Boulder	Snohomish Co.	North Cascades	5 - Stillaguamish	Boulder R	48.277869	-121.780953	Surber	103	6705	0.1%	8/15/2011	67.3	74.6	28	34
CCJensen	Snohomish Co.	Puget Lowland	5 - Stillaguamish	Church Ck	48.2481	-122.3135	Surber	39	2385	10.0%	8/16/2011	68.1	47.6	32	24
JIMWHITE	Snohomish Co.	Puget Lowland	5 - Stillaguamish	Jim Ck	48.177476	-122.050732	Surber	47	10739	1.3%	8/8/2011	71.1	78.7	32	30
PILC	Snohomish Co.	Puget Lowland	5 - Stillaguamish	Pilchuck Ck (Stillaguamish)	48.21024	-122.225622	Surber	8	19738	2.8%	8/16/2011	73.8	41	40	24
Squire	Snohomish Co.	North Cascades	5 - Stillaguamish	Squire Ck	48.270836	-121.671603	Surber	141	5141	0.2%	8/15/2011	49.1	55.1	22	26
Tiger	Snohomish Co.	Puget Lowland	5 - Stillaguamish	Tiger Ck	48.122247	-121.919867	Surber	158	17	2.1%	8/8/2011	50.9	58.9	24	28
TR30	Snohomish Co.	Puget Lowland	5 - Stillaguamish	Glade Bekken	48.204383	-122.290067	Surber	9	549	6.3%	8/16/2011	65.6	57.5	32	30

APPENDIX C: SITE SPECIFIC HABITAT DATA

Table A-2 Habitat data for the 55 project sampling sites.

Site Code	Partner Organization	Location Description	Personnel	Date	Time	Weather	Air Temp (°C)	Water Temp (°C)	Reach Length (ft)	Wetted Width (ft)	Sampling Device	# Riffles Sampled
08BEA3474	Redmond + King Co.	Bear Ck, d/s of Microsoft footbridge, behind new Swedish Medical	J. Wilhelm, K. DuBose, T. MacFarlane, S. McQuincy	9/7/2011	9:15 AM	Sunny	16		461	24	Kick Net	3
08BEA3650	Redmond + King Co.	Bear Ck, u/s of 133rd @ 19660 133rd	J. Wilhelm, K. DuBose, T. MacFarlane, S. McQuincy	9/7/2011	12:10 PM	Sunny	21	15	302	20	Kick Net	4
08CED4192	King Co.	Roch Ck in Roch Ck Natural Area	J. Wilhelm, K. Foley, L. Llewellyn	9/19/2011	11:15 AM	P. Cloudy	13	11	203	16.5	Surber	4
08CED5032	King Co.	Rock Ck u/s of bridge	J. Wilhelm, K. DuBose, C. Gregersen	8/17/2011	9:00 AM	Sunny	9	8	233	13	Surber	4
08EAS2272	King Co.	Kelsey Ck @ Dentist #2066	J. Wilhelm, K. Foley, L. Llewellyn	9/12/2011	2:45 PM	M. Cloudy	19	15	92	12	Surber	4
08ISS3877	King Co.	Issaquah Ck u/s of 56th St. NW Pickering Farm	J. Wilhelm, K. Foley, L. Llewellyn	8/30/2011	11:50 AM	M. Cloudy	15	13	253	22	Surber	4
08ISS4724	King Co.	Carey Ck @ Dumovic Property	J. Wilhelm, L. Llewellyn, K. DuBose	9/8/2011	10:00 AM	Sunny	15	10.5	249	13	Surber	4
08ISS4748	King Co.	East Fork Issaquah Ck off High Point Way	J. Wilhelm, K. Foley, L. Llewellyn	8/30/2011	9:40 AM	M. Cloudy	15	13	237	10	Surber	4
08LAK3879	King Co.	Laughing Jacobs Ck @ Hans Jensen Camp	J. Wilhelm, K. Foley, L. Llewellyn	8/30/2011	2:20 PM	M. Cloudy	17	15	151	10	Surber	4
08LIT2585	King Co.	Little Bear Ck @ 134th Ave NE u/s of bridge	J. Wilhelm, K. Foley, L. Llewellyn	9/12/2011	9:45 AM	M. Cloudy	17	14	410	13	Surber	4
08SAM2862	King Co.	Tributary 0090 near 146th + 155th Trib of Sammamish River	J. Wilhelm, K. Foley, L. Llewellyn	9/12/2011	12:00 PM	M. Cloudy	15	13	113	7	Surber	4
08WES0622	King Co.	Ravenna Ck in Lowen Park d/s of 15th Ave Bridge	J. Wilhelm, L. Llewellyn	8/18/2011	11:00 AM	P. Cloudy	16	13	41	3	Surber	4
08WES0629	King Co.	Maple Leaf Ck @ Thornton Ck Park	J. Wilhelm, L. Llewellyn	8/18/2011	1:05 PM	P. Cloudy	17	15	255	12	Surber	4
08WES0903	King Co.	McAleer Ck @ Blue Heron Park Across from Shell Station	J. Wilhelm, L. Llewellyn	8/18/2011	8:50 AM	Sunny	15	13	366	13	Surber	4
09COV1756	King Co.	Covington Ck near Lake Sawyer in Black Diamond Park land	J. Wilhelm, K. Foley	9/6/2011	10:00 AM	Sunny	14	13	187	39	Surber	4
09DUW0225	King Co.	Unnamed Ck @ 116th	J. Wilhelm, L. Llewellyn, K. DuBose	9/1/2011	1:30 PM	P. Cloudy	18	14	89	6.25	Surber	4
09JEN1357	King Co.	Jenkins Ck near 256th + Hwy 18	J. Wilhelm, L. Llewellyn, K. DuBose	9/8/2011		Sunny	25	15	116	17	Surber	2
09LOW0751	King Co.	Olson Ck, just u/s of Green River @ Mary Olsen Farm	J. Wilhelm, L. Llewellyn, K. DuBose	9/15/2011	12:30 PM	M. Cloudy	15	13	89	5	Surber	4
09MID1958	King Co.	Key Ck, near hatchery rearing ponds just u/s of confluence w/ Green	J. Wilhelm, C. Gregersen, K. DuBose	8/17/2011	11:30 AM	Sunny	14	9	222	11	Surber	1
09MID2426	King Co.	Unnamed Ck just u/s of Green river @ old Parker residence (Hudson Rd).	J. Wilhelm, K. Foley	9/6/2011	1:30 PM	Sunny	23.5	15	113	5.5	Surber	4
09NEW1657	King Co.	Newaukum Ck at 212th	J. Wilhelm, K. DuBose, C. Gregersen	8/17/2011	2:14 PM	Sunny	16	12	359	25	Surber	3
09SOO1022	King Co.	Ravine below Hwy 18	J. Wilhelm, L. Llewellyn	8/3/2011	1:56PM	Sunny	19	13	280	228	Surber	4
09SOO1130	King Co.	Soos Ck along Auburn-Black Diamond Rd near 148th SE	J. Wilhelm, L. Llewellyn, K. DuBose	9/15/2011	10:00 AM	M. Cloudy	14	13.5	430	45	Surber	4
09SOO1283	King Co.	170th & 156th near Crystal Pk & Pump Station 14	J. Wilhelm, L. Llewellyn	8/3/2012	10:30AM	Sunny	18	16	97		Surber	3
Bagley_0pt7	S.K. of Clallam Co.	Bagley Creek at 0.7 mile	GCB, SBN, CVH	9/28/2011	10:00 AM	Sunny	10.6	10.1	80	7	Surber	4
Benson	Snohomish Co.	Mtn Loop Hwy @ Verlot Campground d/s of hwy, u/s of river	J. Oden, J. Wilhelm	8/8/2011	10:24 AM	M. Cloudy	11.5	10	271	11.5	Surber	4
BIBI_006_Lacky	Pierce Co. S.T.	Lackey Ck near mouth, across rd from YMCA camp Seymour	J. Wilhelm, I. Ragland, C. Towe, Z. & L. Potts	8/31/2011	9:30 AM	M. Cloudy	13.5	10.4	188	12.2	Surber	4
BIBI_008_Swan	Pierce Co. S.T.	Swan Ck @ Footbridge	J. Wilhelm, C. Towe, I. Ragland, A. Fewell	8/19/2011	9:15 AM	M. Cloudy	15	11	374	10.5	Surber	4
BIBI_028_Purdy	Pierce Co. S.T.	Purdy Ck @ Purdy P&R	J. Wilhelm, C. Towe, M. Buckingham	8/26/2011	9:45 AM	M. Cloudy	16	12.9	290	8.75	Surber	4
Boulder	Snohomish Co.	Boulder Ck @ Hwy 530	J. Wilhelm, J. Oden	8/15/2011	12:30 PM	P. Cloudy	17.5	11	682	48	Surber	4
CCJensen	Snohomish Co.	Church Ck @ Jensen Rd	J. Oden, J. Wilhelm	8/13/2011	1:30 PM	Sunny	18.5	13	188	8	Surber	4
Chuckanut_Arroyo	Bellingham	Chuckanut Ck @ Arroyo park	J. Wilhelm, S.B. Benjamin, R. LeCroix	9/28/2011	10:00 AM	Sunny	10	11	197	9.4	Kick Net	4
JimmyComeLately0pt8	S.K. of Clallam Co.	Jimmy Come Lately Creek at mile 0.8	GCB, JIB, JNM, RJB, ZDH, MTH	8/28/2011	9:00 AM	Sunny	17		140	7	Surber	3
JIMWHITE	Snohomish Co.	Jim Ck d/s and u/s of Whites Rd	J. Oden, J. Wilhelm	8/8/2011	3:43 PM	M. Cloudy	16.5	13	1555	74	Surber	4
KCSSWM003	Kitsap Co.	Big Beef Ck @ Research Station	J. Wilhelm, M. Heine, C. Knutson	8/24/2011		Sunny	20	14.7	338	26	Surber	4
KCSSWM006	S.K. of Clallam Co.	Big Anderson Ck	J. Wilhelm, K. DuBose, M. Heine	8/23/2011	10:30 AM	Sunny	17	13	182	4.2	Surber	4
KCSSWM007	Kitsap Co.	Chico Ck d/s of Golf Course Rd	J. Wilhelm, M. Heine, C. Knutson	8/24/2011		Sunny	19	15.4	397	25	Surber	4
KCSSWM009	Kitsap Co.	Boyce Ck @ Grillemont Cove	J. Wilhelm, K. DuBose, M. Heine	8/23/2011	1:50 PM	Sunny	19.5	13.7	235	7.2	Surber	4
KCSSWM011	Kitsap Co.	Lower Little Anderson Ck @ Rising Hill Lane & NW Anderson Hill Rd	J. Wilhelm, M. Heine, F. Stricklin	8/29/2011	10:50 AM	M. Cloudy	15.5	11	400	9.6	Surber	4
KCSSWM030	Kitsap Co.	Jump Off Joe Ck @ Edgewater Beach Community Club	J. Wilhelm, M. Heine	8/29/2011	2:00 OM	P. Cloudy	16.5	13	210	5	Surber	4
KCSSWM034	Kitsap Co.	Lower Blackjack Ck near end of Kendell	J. Wilhelm, M. Heine, K. Foley, L. Martin	8/25/2011		Sunny	20	13	568	23.9	Surber	4
KCSSWM038	Kitsap Co.	Lower Curley Ck, near 1570 Martin Lane	J. Wilhelm, M. Heine, K. Foley, L. Martin	8/25/2011		Sunny	19	16.2	423	25	Surber	3
KCSSWM040	Kitsap Co.	Gorst Ck @ Belfair Hwy	J. Wilhelm, M. Heine	8/29/2011	8:55 AM	M. Cloudy	15.5	11	274	12.3	Surber	4
McAleer_187	Lake Forest Park S.K.	McAleer Ck near end of 25th Ave NE	J. Wilhelm, M. Phillips, J. Holliday, D. Farkas + 7 other S.K.	9/17/2011	10:15 AM	M. Cloudy	13	15	259	12.4	Surber	3
Morse_1pt7	S.K. of Clallam Co.	Morse Creek at mile 1.7 u/s of restoration	MJB, RJB, GCB, SBN	9/22/2001	9:30 AM	M. Cloudy	16.5	12.5	200	45	Surber	2
PILC	Snohomish Co.	Pilchuck River u/s of Stilly Mouth @ Bridge 46 off 236th	J. Oden, J. Wilhelm	8/16/2011	9:30 AM	Sunny	15	15	1055	38	Surber	4
PIMA3714	Seattle	Piper's Ck d/s of Venema Ck in Carkeek Park	J. Wilhelm, K. Lynch, J. Starstead	8/22/2011	10:10 AM	M. Cloudy	18	14	132	10	Surber	3
Siebert_0pt6	S.K. of Clallam Co.	Siebert Creek at mile 0.6 near Lazy J Tree Farm	SBN, RJB, KDP, GCB	9/13/2011	9:00 AM	M. Cloudy	19.4	16			Surber	3
Squalicum_IronGate	Bellingham	Squalicum Ck @ Division along pipeline	J. Wilhelm, S.B. Benjamin	9/28/2011	1:30 PM	Sunny	16	13	233	5.4	Kick Net	4
Squire	Snohomish Co.	Squire Ck off Hwy 530	J. Oden, J. Wilhelm	8/15/2011	9:50 AM	P. Cloudy	17.5	11	1352	54	Surber	4
Tiger	Snohomish Co.	Tiger Ck @ One Lane Bridge	J. Oden, J. Wilhelm	8/8/2011	12:32 PM	M. Cloudy	12.5	11	395	15	Surber	4
TNMA6462	Seattle	N. branch Thornton Ck @ Homewood Park	J. Wilhelm, K. Lynch, J. Starstead	8/22/2011	12:55 PM	M. Cloudy	18	15	184	9	Surber	3
TR30	Snohomish Co.	Gladebekken Ck @ Sylvana Terrace Rd	J. Oden, J. Wilhelm	8/16/2011		Sunny	16.5	13	122	5.8	Surber	4
Tumwater_01a	S.K. of Clallam Co.	Tumwater Ck at mile 0.1	GCB, SBN, CMH, SLB, SMH, JNM	9/8/2011	12:00 PM	Sunny	17.4	14.8	100	10	Surber	
WestTwin_1pt0	S.K. of Clallam Co.	West Twin Ck at mile 1.0 near Telemetry Station	KDP, GCB, SNB	9/19/2011	10:30 AM	Sunny	12.5	14	220	30	Surber	3

APPENDIX D: BOX PLOTS OF MEAN B-IBI SCORES AND INDIVIDUAL B-IBI METRIC VALUES

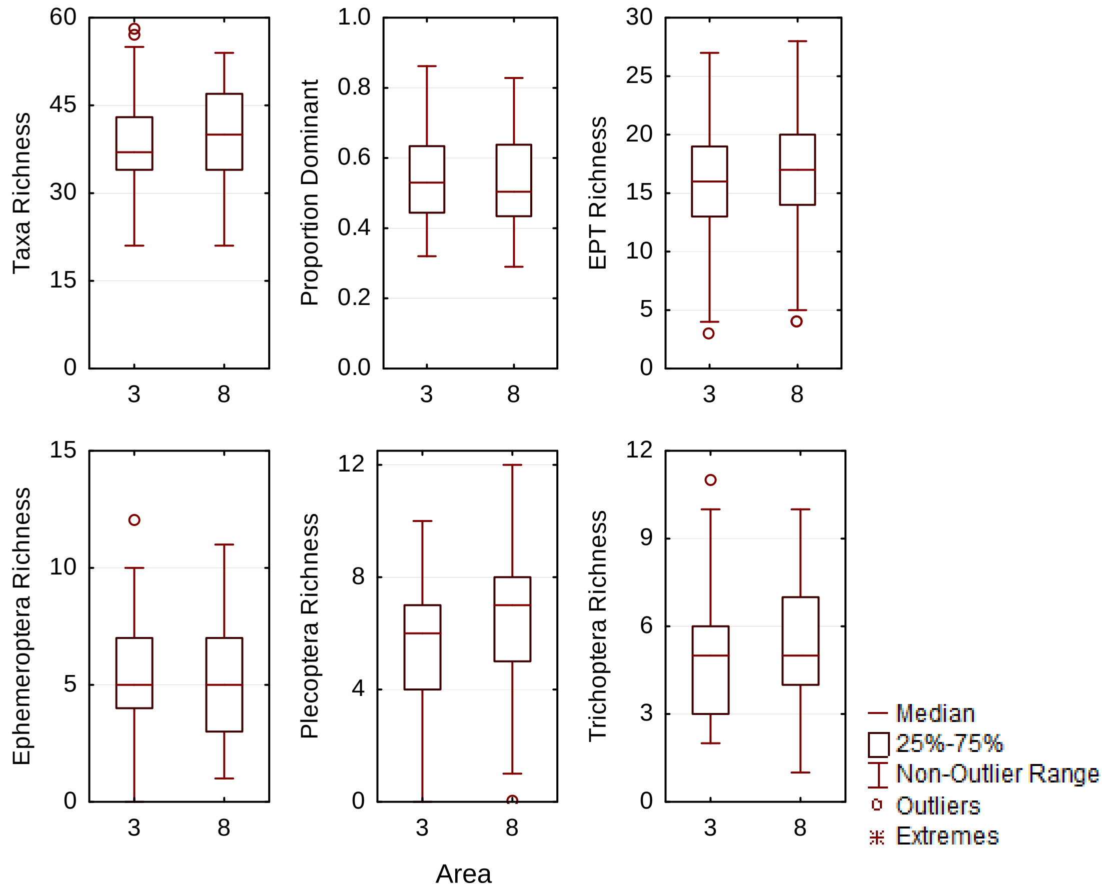


Figure A-1 **Box plots for the six B-IBI metrics that do not rely on attribute lists comparing 3 ft^2 and 8 ft^2 results. Box plots range from the first to third quartile with the median designated by a horizontal line. Error bars extend to the non-outlier range. Round circles designate outliers.**

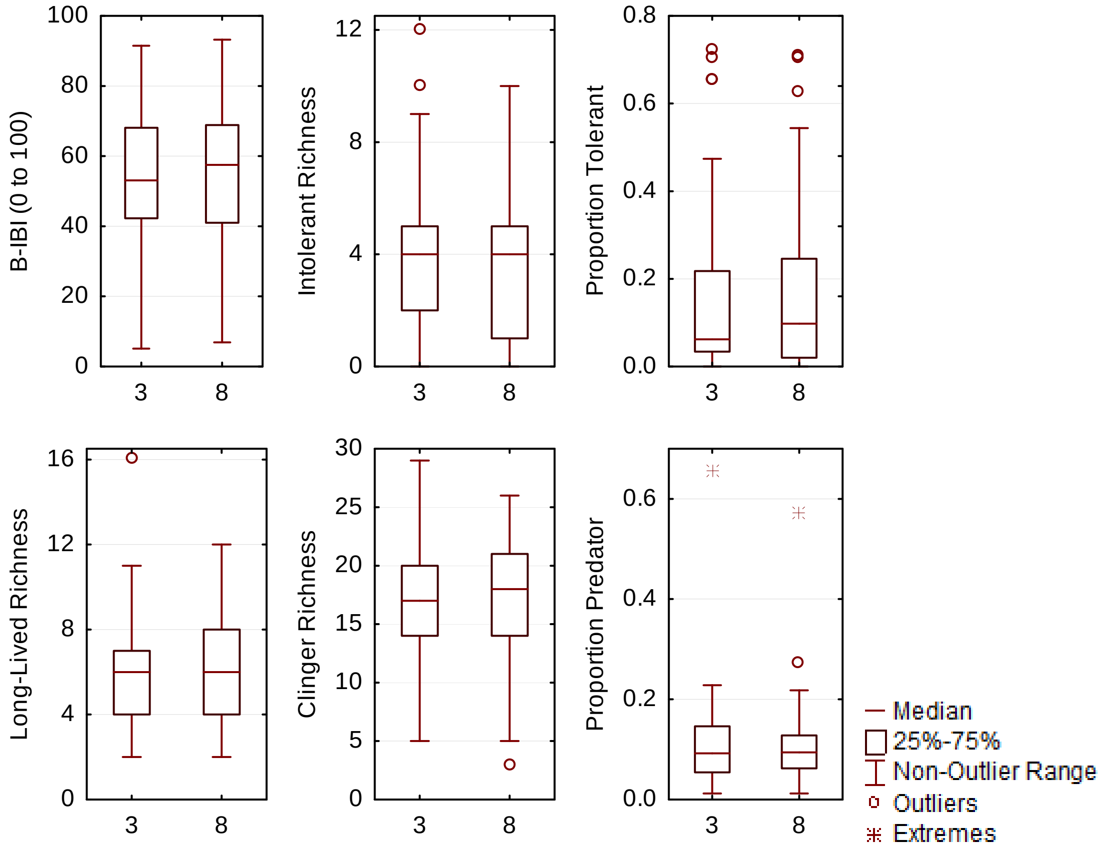


Figure A-2 **Box plots for B-IBI$_{0\text{-}100}$ and the five B-IBI$_{0\text{-}100}$ metrics that rely on attribute lists comparing 3 ft^2 and 8 ft^2 results. Box plots range from the first to third quartile with the median designated by a horizontal line. Error bars extend to the non-outlier range. Round circles designate outliers. Asterisks designate extreme values.**

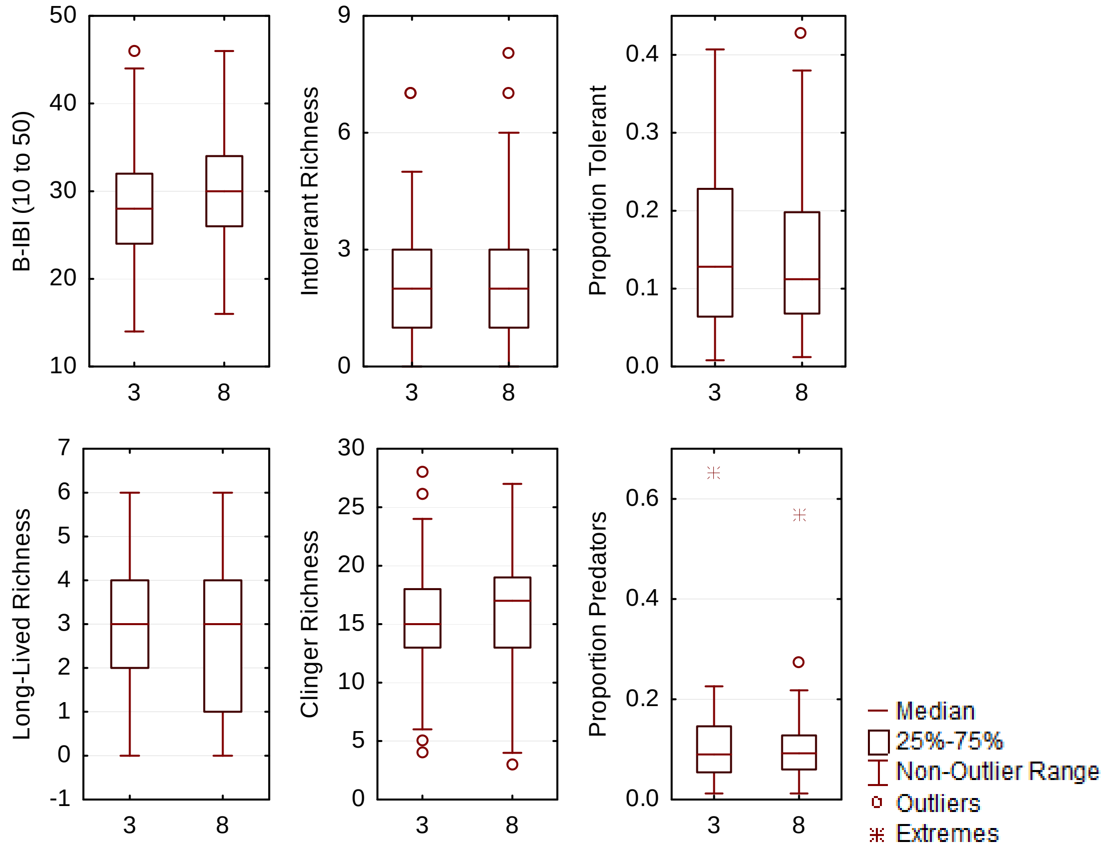


Figure A-3 **Box plots for B-IBI$_{10-50}$ and the five B-IBI$_{10-50}$ metrics that rely on attribute lists comparing 3 ft^2 and 8 ft^2 results. Box plots range from the first to third quartile with the median designated by a horizontal line. Error bars extend to the non-outlier range. Round circles designate outliers. Asterisks designate extreme values.**

APPENDIX E: CORRELATION GRAPHS AND RESIDUAL HISTOGRAMS FOR B-IBI METRICS

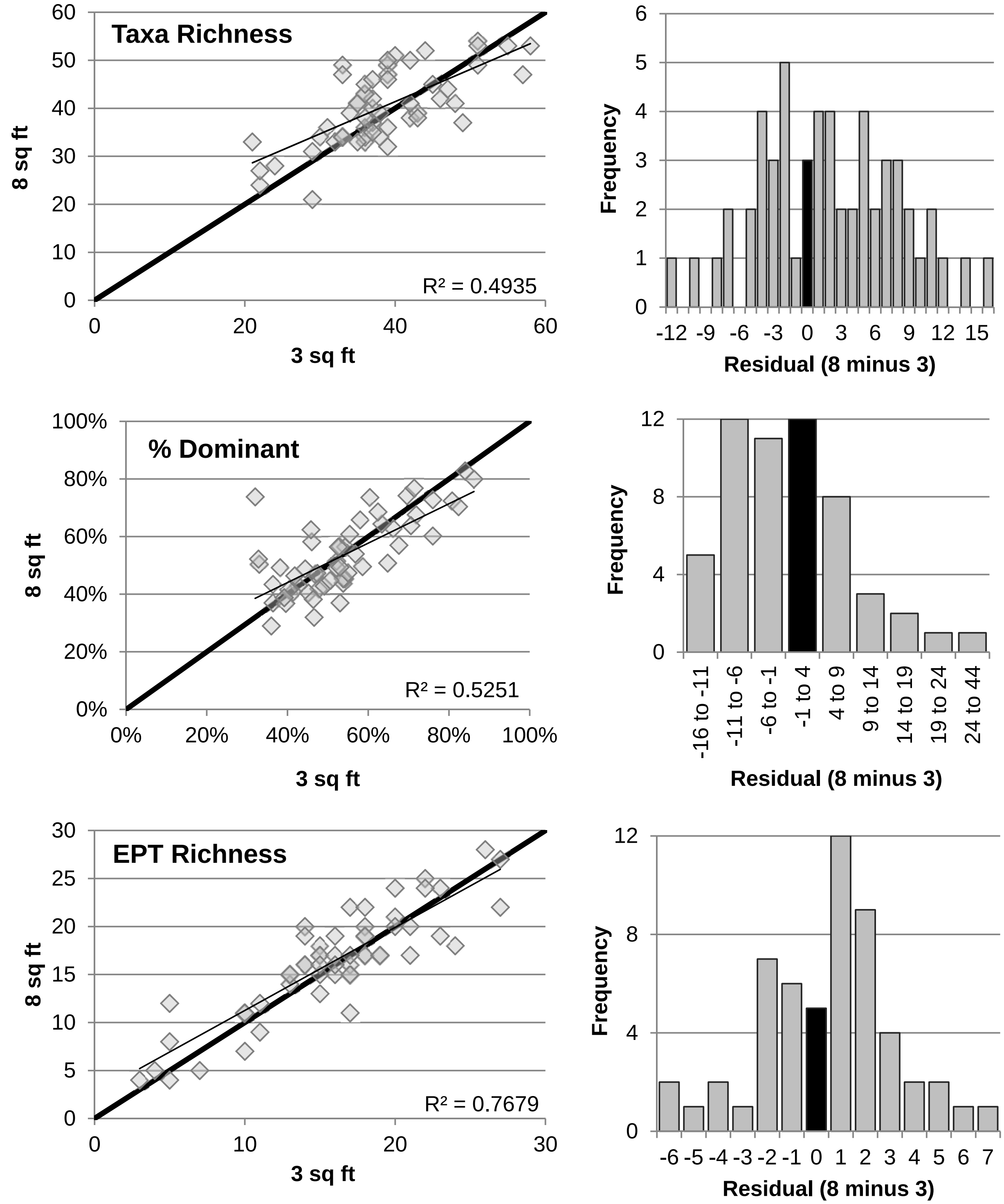


Figure A-4 **Comparison of individual B-IBI metric results for taxa richness (top), proportion dominant (middle) and EPT richness (bottom). These metrics are attribute-independent. Regressions are on the left and residual histograms on the right. The dark line is the 1 to 1 line; the light line is the best fit line for the regression. Dark bars represent the bins that include or bracket a residual of zero.**

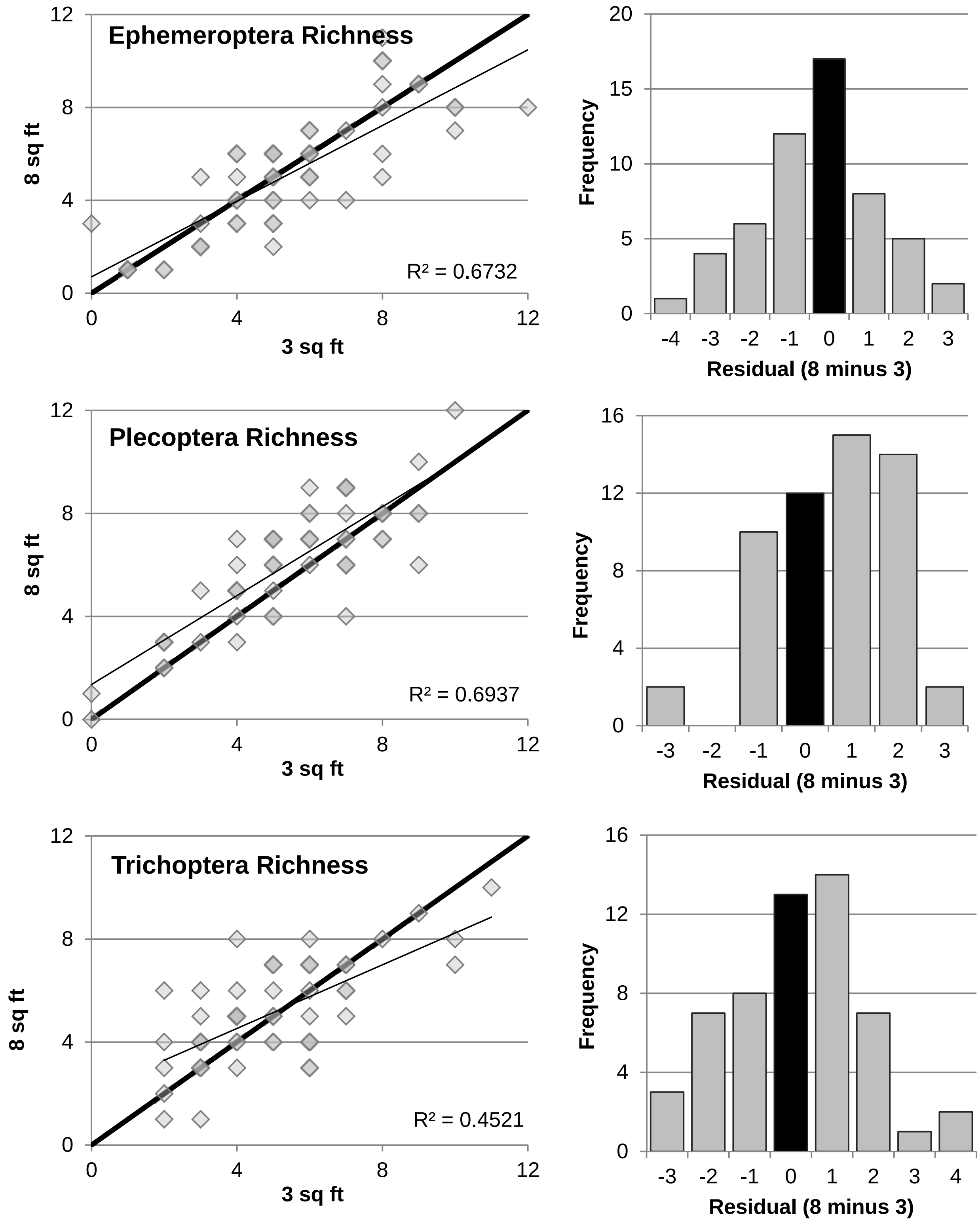


Figure A-5 **Comparison of individual B-IBI metric results for Ephemeroptera (top), Plecoptera (middle), and Trichoptera (bottom) richness. These metrics are attribute-independent. Regressions are on the left and residual histograms on the right. The dark line is the 1 to 1 line; the light line is the best fit line for the regression. Dark bars represent the bins that include or bracket a residual of zero.**

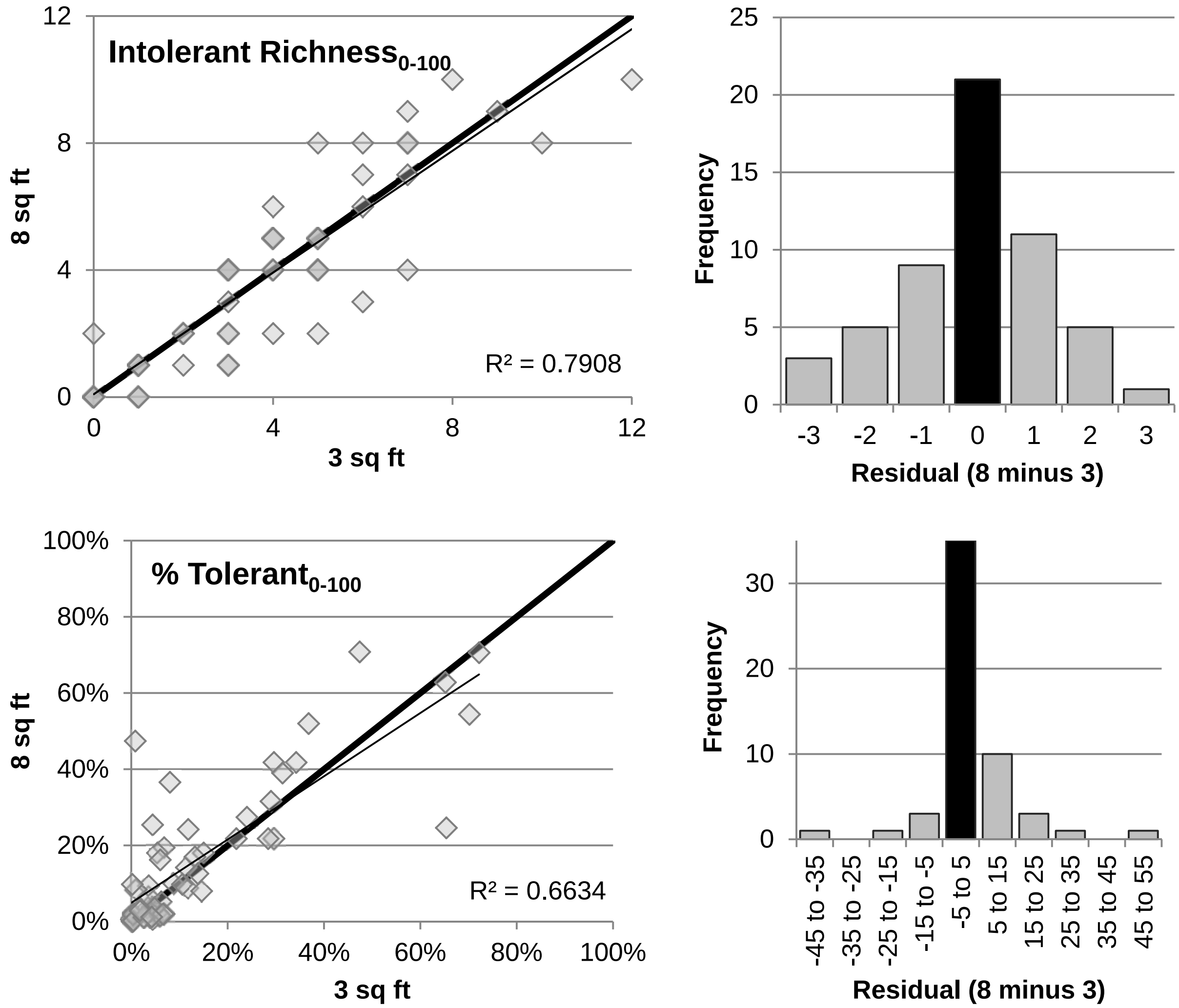


Figure A-6 **Comparison of two attribute-dependent B-IBI$_{0\text{-}100}$ metrics: intolerant richness (top) and percent tolerance (bottom). Regressions are on the left and residual histograms on the right. The dark line is the 1 to 1 line; the light line is the best fit line for the regression. Dark bars represent the bins that include or bracket a residual of zero.**

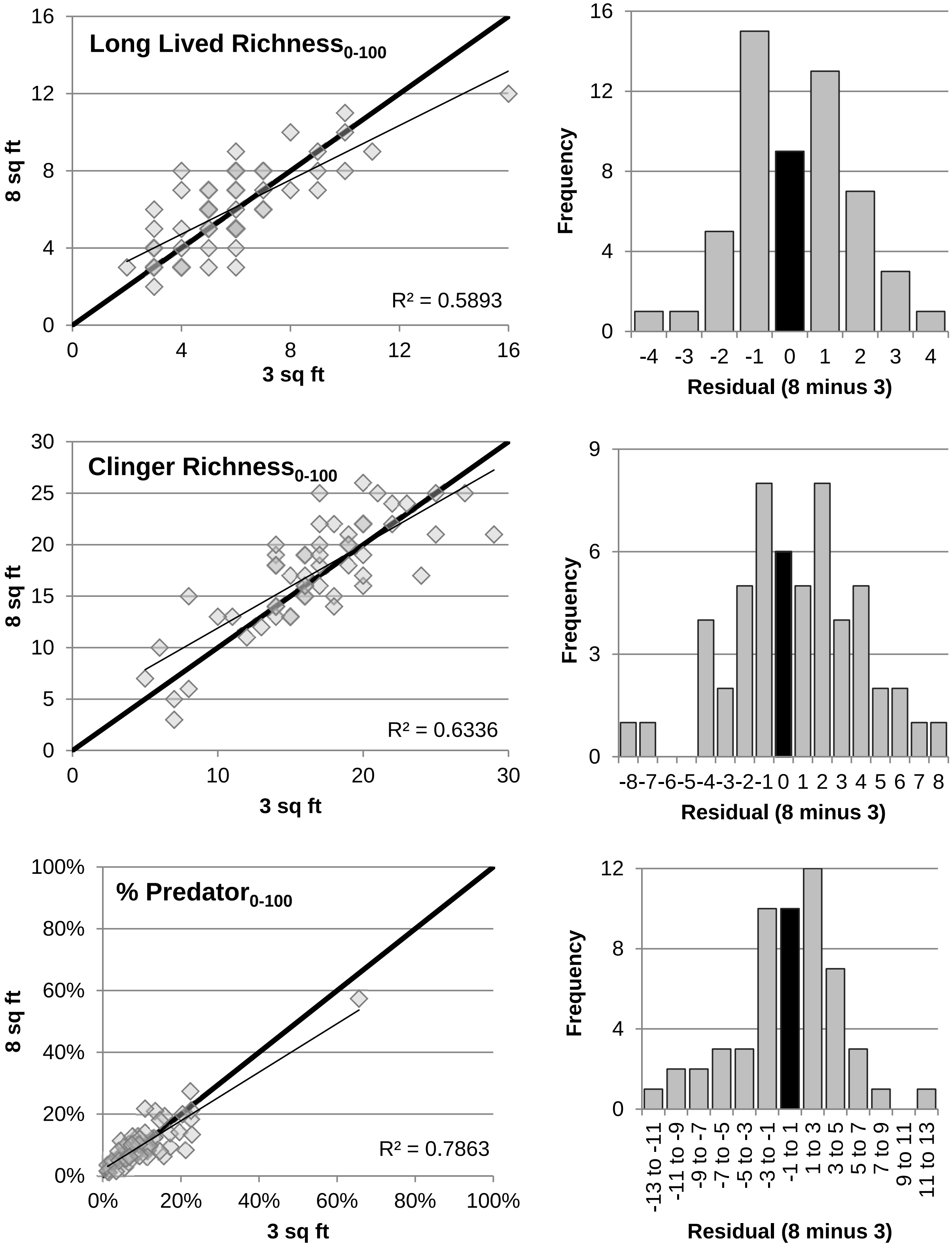


Figure A-7 **Comparison of three attribute-dependent B-IBI$_{0\text{-}100}$ metrics: long-lived richness (top), clinger richness (middle), and percent predators (bottom). Regressions are on the left and residual histograms on the right. The dark line is the 1 to 1 line; the light line is the best fit line for the regression. Dark bars represent the bins that include or bracket a residual of zero.**

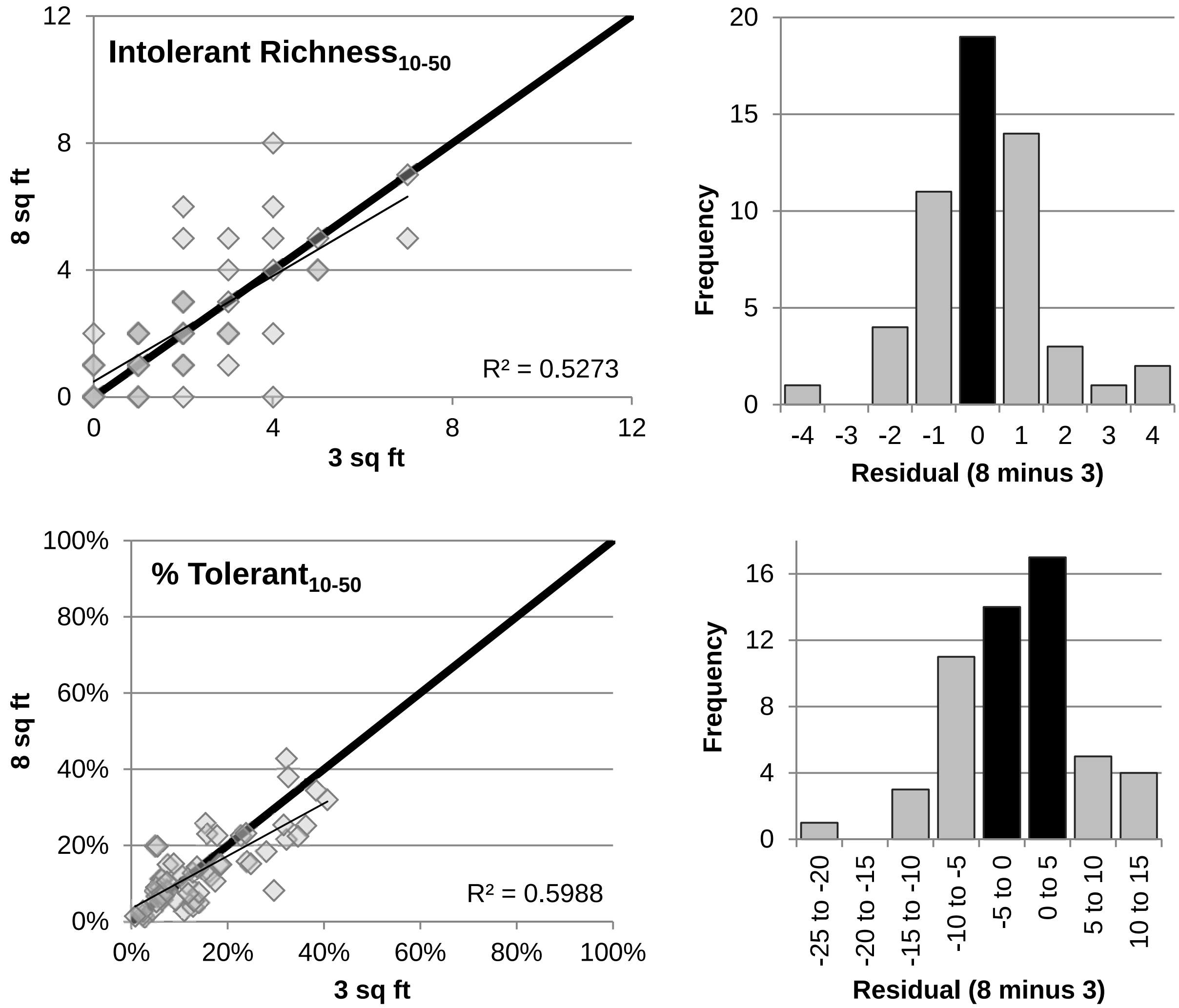


Figure A-8 **Comparison of two attribute-dependent B-IBI$_{10-50}$ metrics: intolerant richness (top) and percent tolerance (bottom). Regressions are on the left and residual histograms on the right. The dark line is the 1 to 1 line; the light line is the best fit line for the regression. Dark bars represent the bins that include or bracket a residual of zero.**

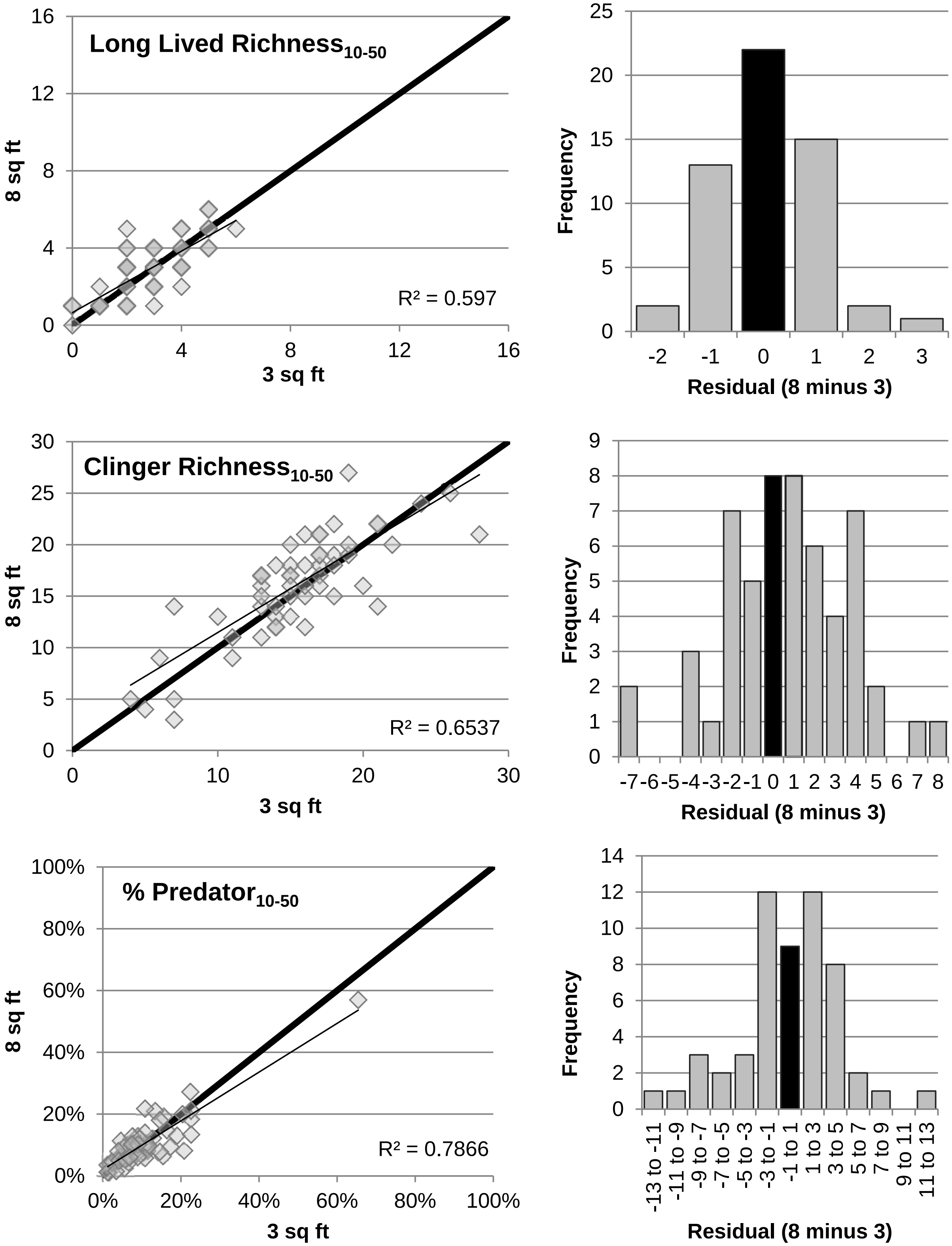


Figure A-9 **Comparison of three attribute-dependent B-IBI$_{10-50}$ metrics: long-lived richness (top), clinger richness (middle), and proportion predators (bottom). Regressions are on the left and residual histograms on the right. The dark line is the 1 to 1 line; the light line is the best fit line for the regression. Dark bars represent the bins that include or bracket a residual of zero.**