Comparison of Two Benthic Invertebrate Sampling and Analysis Methods for Streams in Greater Vancouver

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SUMMARY

This study compares two approaches for collecting and analyzing benthic invertebrate community data that are used for stream assessment in Greater Vancouver: 1) a benthic index of biological integrity (B-IBI) method that uses multiple samples collected with a Surber sampler and multimetric data analysis; and, 2) a reference condition approach (RCA) advocated by the Canadian Aquatic Biomonitoring Network (CABIN) that uses a single sample collected with a kicknet sampler and multivariate data analysis. Data collected from eight streams in North Vancouver from September–October 2003 and from 11 streams located more broadly in Greater Vancouver from September 2004 were used for the comparison.

Generally, richness and composition of benthic invertebrate communities collected using Surber and kicknet sampling devices were similar in the 19 study streams. Total taxa richness was significantly higher in composite Surber samples than kicknet samples by almost two taxa (mean of 20.3 and 18.5 respectively; p=0.0105). However, more organisms were collected in a single kicknet sample than a sample composited from three Surber placements. The higher richness in Surber samples is likely due to the collection of taxa which occur deeper in the substrate.

While taxa richness was higher in composite Surber samples, B-IBI values from kicknet samples were significantly higher (mean difference 3.0; p=0.0076). The mean B-IBI was 24.4 (SD 10.1) in kicknet samples and 21.4 (SD 8.8) in Surber samples. This suggests that small increases in total taxa richness in Surber samples do not necessarily have a corresponding increase in B-IBI values. More organic debris and possibly more sensitive taxa associated with this microhabitat were collected with the kicknet.

Ordination showed that Surber and kicknet samples from the same stream were generally more similar to one another than to samples from other streams.

The B-IBI and RCA-CABIN assessment approaches were in agreement in identifying that the benthic invertebrate communities showed some degree of anthropogenic stress. Where the degree of stress differed between methods, they differed by only one condition category. Both techniques were able to detect departures from acceptable conditions, however, the degree of stress indicated by each approach varied.

The results of this study showed that the Surber and kicknet sampling and lab processing methods provide similar estimates of benthic invertebrate community structure, and that B-IBI and RCA-CABIN analysis methods provide similar assessments of biological condition for streams in Greater Vancouver. While this study has shown general similarity of invertebrate assemblages collected using Surber and kicknet sampling methods from the same stream, differences in the richness and abundance of taxa groups and resulting differences in metric scores for B-IBI values indicate they should not be used interchangeably for stream assessment in Greater Vancouver without calibration. Consistency of sample collection (e.g., equipment, number of samples, seasonality, etc) and laboratory processing (e.g., subsampling and taxonomy) is more important for effective monitoring programs than specific sampling or analysis methods.

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INTRODUCTION

This study compares two approaches for collecting and analyzing benthic invertebrate community data that are used for stream assessment in Greater Vancouver: 1) a B-IBI (benthic index of biological integrity) method that uses multiple samples collected using a Surber sampler and multimetric data analysis; and, 2) a reference condition approach (RCA) advocated by the national Canadian Aquatic Biomonitoring Network (CABIN) program, which uses a single integrated sample collected using a kicknet sampler and multivariate data analysis. Data from eight streams in North Vancouver from September–October 2003 and from 11 streams located more broadly in Greater Vancouver from September 2004 were used for the comparison.

Throughout this study we separate the comparison of field sampling and lab processing results from the comparison of data analysis results. For clarity, we refer to the comparisons of benthic invertebrate richness and abundance from the two field sampling and lab processing methods as *Surber* versus *kicknet*, and to the comparison of the data analysis results as *B-IBI* versus *RCA-CABIN.*

Two research questions were asked:

1. *Are the estimates of richness and abundance of benthic invertebrate taxa collected by the Surber and kicknet methods statistically equal?* The question focuses on the effect of field sampling and lab processing on attributes of the benthic invertebrate community.

2. *Do the analysis methods differ in their assessment of biological condition?* This question addresses the similarities in the assessment of biological condition provided by the B-IBI and RCA-CABIN analysis methods.

Biomonitoring Approaches

A variety of sampling and data analysis methods exist for using benthic invertebrates to assess or monitor the biological condition of streams and rivers (Carter *et al.* 2006). Differences in field sampling, lab processing, and data analysis affect both the measurement of benthic invertebrate community structure, and the amount and usefulness of the information on biological condition provided by the sample (Rosenberg and Resh 1993). Different field sampling methods can provide different estimates of invertebrate diversity and abundance through variation in sampling intensity (e.g., amount or area of substrate sampled), sampling period, type of habitat sampled (e.g., riffle versus pool habitat), and the mesh size of the sampling equipment. Laboratory procedures such as the number of organisms identified, and the level of taxonomic resolution (e.g., family or lowest practical taxonomic level) also influence the estimates of taxa richness and abundance that are provided by the sample. Finally, data analysis methods strongly influence how data are evaluated and interpreted since different aspects (e.g., number of taxa versus the types of taxa) are emphasized or data are summarized in different ways (e.g., multivariate vs. multimetric).

Two common methods for collecting and analyzing benthic invertebrate data in Greater Vancouver are the focus of this study. It is important to stress that while the data analysis procedures of these two methods are fundamentally different, the purpose of each method is the same. Both methods attempt to assess the biological condition of streams or small rivers based on the benthic invertebrate community. An introduction to each method is described below and more details on field sampling, laboratory analysis, and data analysis are provided in the Methods section.

B-IBI Approach

The benthic index of biological integrity (B-IBI) is used by numerous organizations in the Pacific Northwest to assess and monitor the biological condition of streams and rivers (Karr 1998; Karr and Chu 1999; Fore *et al.* 2001). The B-IBI uses predictable changes in the benthic invertebrate community along a defined gradient of anthropogenic stress, such as urbanization, to assess biological condition. The index is composed of a series of scoring metrics that summarize information on the benthic invertebrate community. Expectations for metrics are derived from values observed for stream invertebrate communities in undisturbed streams (Karr and Chu 1999).

Several different scoring systems have been proposed for the Puget Sound region of Washington State depending on the level of taxonomic resolution of the benthic invertebrate data. For this study, we used a 10-metric scoring system with lowest practical taxonomic level (LPTL) data (Fore *et al.* 2001). Metrics for each system are scored as a 5, 3, or 1 according to whether they are similar to values observed at reference sites (score = 5), deviate somewhat from expected values (3), or deviate substantially (1). Scores are added to obtain a B-IBI value that can range from 50 (indicating best condition) to 10 (indicating poorest condition). B-IBI values can be compared to numerically defined classes to summarize the stream's biological condition: *Excellent* (46–50); *Good* 38–44; *Fair* (28–36); *Poor* (18–26); *Very Poor* (10–16).

RCA-CABIN Approach

Environment Canada has developed a national benthic invertebrate monitoring program called the Canadian Aquatic Biomonitoring Network (CABIN). The CABIN uses the Reference Condition Approach (RCA; Bailey *et al.* 2004) to compare the observed benthic invertebrate community structure to a regional reference condition defined by reference sites. The Great Lakes (Reynoldson *et al.* 1995) and the Fraser River Basin (Rosenberg *et al.* 1999) have been used as regional case studies in Canada.

Environment Canada uses CABIN protocols to build a database of reference conditions to assess the status of Canada's freshwater, as well as to provide a biological monitoring component for the national Water Quality Index at routine water quality monitoring stations across the country. RCA-CABIN has been used to assess small streams in the Fraser River Basin, the Lower Fraser River Valley and on Vancouver Island (Sylvestre *et al.* 2005).

RCA-CABIN uses predictive models derived from the relationship of environmental and biological data to predict what the benthic invertebrate community should look like at a site. This uses natural variation of unimpacted sites as the benchmark for site assessment. It is assumed that if a site does not have the invertebrate community it is expected to have based on its environmental attributes, then there must be some anthropogenic stress exerted on it. The distance the test site falls from the group of reference sites in multivariate space gives an indication of the severity of stress. Four bands of biological condition have been established by Rosenberg *et al.* (1999) based on confidence ellipses around the range of reference sites. Any site that falls within the 90% confidence ellipse is *not stressed*, between the 90% and the 99% ellipses is *possibly stressed*, between the 99% and 99.9% ellipses is *stressed* and outside of the 99.9% ellipse is *severely stressed*.

METHODS

Site Selection

Benthic invertebrates were collected from eight[1] streams in North Vancouver during September–October 2003 and 11 streams located more broadly within Greater Vancouver in September 2004. All are permanently flowing streams representative of the range of physiographic and land use conditions in the Greater Vancouver area. The North Vancouver streams are moderately urbanized while the streams from Greater Vancouver range from undisturbed (e.g., Clear and Beaver creeks) to heavily urbanized (e.g., Como, Still, and Wagg creeks). General characteristics of the study area streams are presented in Table 1 and their locations are shown in Figure 1.

Sampling Methods

Separate benthic invertebrate samples were collected from each stream using both Surber and kicknet sampling methods defined in the B-IBI protocols for GVRD streams (EVS Consultants 2003) and CABIN protocols (Reynoldson *et al.* 2003). Key differences in field sampling, lab processing, and data analysis are summarized in Table 2. Using the Surber sampling method for B-IBI assessment, four composite samples were collected in a 500 m long reach in each stream following a protocol developed by EVS Consultants (2003) for Greater Vancouver. Each composite sample consisted of three individual Surber placements from one riffle. Using the kicknet sampling method for RCA-CABIN assessment, one or two samples were collected from each stream using a three-minute traveling kicknet transect following the protocol described by Reynoldson *et al.* (2003). The kicknet sampling site was generally adjacent to the most downstream Surber sampling site. In 16 streams, only one kicknet sample was collected, however, two kicknet samples were collected from Clear Creek, Stoney Creek, and Serpentine River in 2004 to evaluate sample variability.

The interval between Surber and kicknet sample collection differed in 2003 and 2004. In 2003, between 14 and 42 (average of 30) days elapsed between Surber and kicknet sample collection. This interval reflected the recommended sampling period for each protocol (e.g., August 1–September 30 for Surber sampling for B-IBI and between September 15–October 25 for kicknet sampling for RCA-CABIN). In 2004, we collected Surber and kicknet samples concurrently (e.g., <12 hrs difference) to minimize any potential differences in the benthic invertebrate community related to hydrologic, water chemistry, or other changes. Despite the differences in the sample timing between the two years, we analysed both datasets together. We assumed that very few life history changes would be observed in the benthic invertebrate community over 14–42 days and that only large pollution or hydrological events could cause significant changes in benthic community composition.

[1] Lynn Creek, Capilano River, and Seymour River were also sampled in September 2003. Capilano and Seymour rivers were not resampled with the kicknet in October 2003 because of high flows. Lynn Creek was resampled with a kicknet but subsequent sampling identified water quality issues. Based on this information and anomalous benthic community differences between September–October 2003, we excluded Lynn Creek from all analyses.

Table 1. Summary of physical characteristics of study streams (from GVS&DD 1999).

Stream	Drainage Area (km^2)	Elevation Min (m)	Elevation Max (m)	% Impervious Area	% Riparian Forest
2003 Study Streams					
MacKay Creek	8.0	0	1100	29	54
Mosquito Creek	6.9	0	900	26	77
Wagg Creek	8.0	2	310	39	71
Keith Creek	4.0	10	260	39	47
Hastings Creek	8.2	40	1000	21	54
McCartney Creek	7.2	0	560	13	98
Taylor Creek	2.2	0	560	17	83
Parkside Creek	0.4	0	120	37	59
2004 Study Streams					
Still Creek	28.2	14	95	69	40
Como Creek	8.9	0	170	54	38
Wagg Creek	3.6	0	300	44	72
Serpentine River	19.2	12	73	39	74
Stoney Creek	7.3	14	360	33	71
Yorkson Creek	2.0	2	100	15	35
Bertrand Creek	7.2	45	125	5	50*
Coghlan Creek	13.8	25	75	5	74
Mossom Creek	3.9	0	890	4	93
Beaver Creek	1.1	146	720	<1	>98*
Clear Creek	0.9	180	1350	<1	>98*

* estimate based on recent air photos.

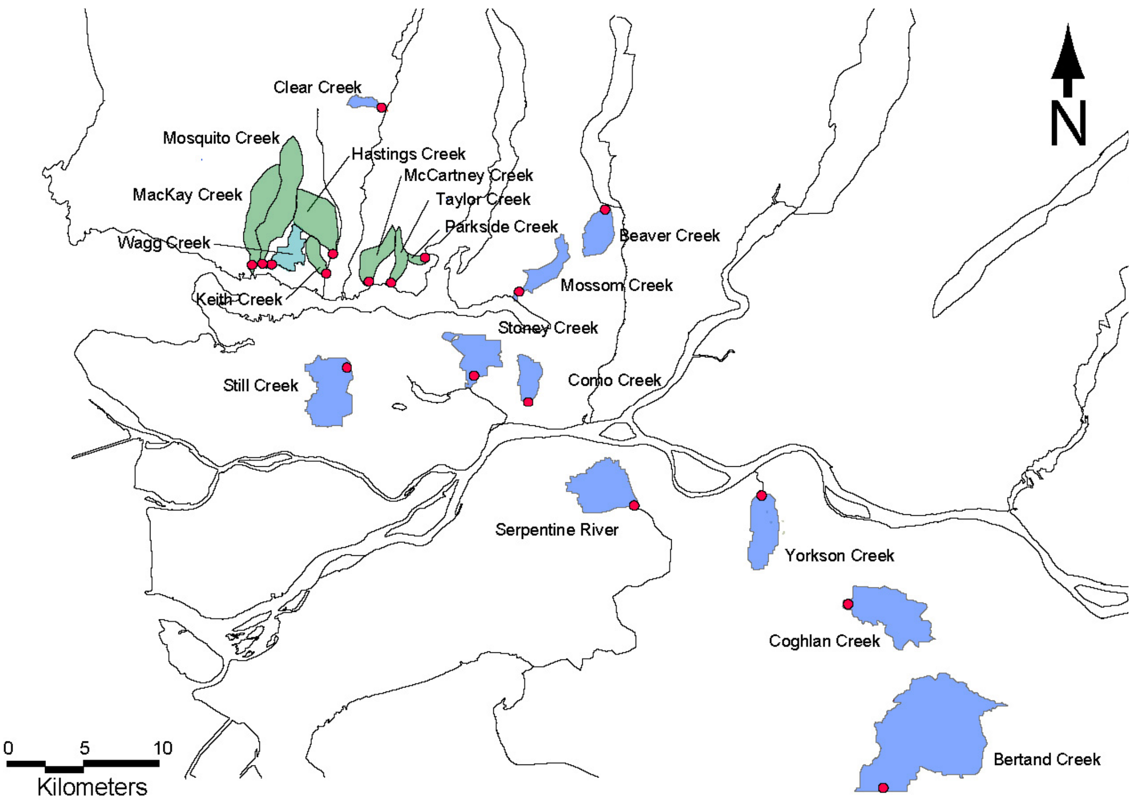

Figure 1. Location of 2003 (green) and 2004 (blue) study streams in Greater Vancouver. Note Wagg Creek was sampled in both years.

Table 2. Summary of differences in field sampling, lab processing, and data analysis between the B-IBI and RCA-CABIN protocols.

Component	B-IBI (Surber)	RCA-CABIN (Kicknet)
Field Sampling		
Recommended sampling period:	August 1 – September 30	September 15[3] – October 25
Sampling device:	Surber	Kicknet
Mesh size:	500 micron	400 micron
Habitat sampled:	Riffle less than 40 cm deep with coarse substrate (sand, gravel, cobble)	Multiple microhabitats within a site; fine to coarse substrate; depth limited by wadability (<1.0 m)
Samples standardized by:	Area: 0.09 m^2 Surber sampler frame and time: 2 minutes of substrate disturbance.	Sampling effort (time): 3 minute travelling kick
# samples collected to represent site:	3 Surber samples at 1 riffle in each 125 m stream segment; 3 Surber samples amalgamated to form 1 composite sample	1 sample per stream
# samples analysed per stream:	12 Surber samples integrated into 4 composite samples (4 x 125 m segments with 1 composite sample each).	1 sample per stream
Lab Processing		
Subsampling[1]	400 organisms	300 organisms
Taxonomic resolution[2]	Lowest practical taxonomic (genus- or family-level)	Family-level
Data Analysis		
Biological data:	10 or 5 metric summary of key taxa groups depending on taxonomy	Multivariate summary of all taxa
Environment variables used:	No – not integrated into biological condition assessment, but used for interpretation	Yes – required for model
Biological condition assessment:	Scaled on defined gradient of stress related to urbanization – 5 defined categories of biological condition Based on 1 integrated sample.	Predictive models – comparison of observed vs. expected; 4 defined categories based on probability ellipses Based on average of 4 composited samples.
Output:	Numerical score indicating relative biological condition; 10 metric values that can be interpreted.	Probabilities of membership with reference groups; visual and statistical assessment of biologic condition using reference sites and probability ellipses; expected and observed taxa.

[1] A Marchant subsampling box was used for both Surber and kicknet samples (see Marchant, 1989).
[2] Lowest practical taxonomic level defined for the Pacific Northwest by Plotnikoff and White (1996).
[3] Note, 2004 kicknet samples were collected between August 18 and September 27, 2004.

Comparison of Surber vs. Kicknet Samples

We used several univariate and multivariate methods to compare the composition of the benthic invertebrate community collected using Surber and kicknet sampling methods. First, paired t-tests were used to test for differences between the average composite Surber sample (n=4) and the single kicknet sample from each stream for 14 composition and richness variables. The attributes tested were:

1) total number of invertebrate taxa;
2) total number of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa;
3) percentage of EPT individuals;
4) percentage dominance (3 taxa);
5) Shannon's diversity index (H') (-sum (Pi*ln(Pi));
6) sample evenness (H' / ln (richness);
7) number of intolerant taxa;
8) percentage of individuals in tolerant taxa;
9) number of Ephemeroptera taxa;
10) number of Plecoptera taxa;
11) number of Trichoptera taxa;
12) number of long-lived taxa;
13) number of clinger taxa; and,
14) percentage of predator organisms.

Prior to analysis, normality of the data was examined using a Shapiro-Wilk W-test and two variables were $\log_{10}$ transformed to improve normality. We calculated mean values for composite Surber samples from each stream.

Second, similarity of total taxa richness and EPT taxa richness in Surber and kicknet samples was evaluated using correlation analysis. Correlation provided a readily interpreted visual depiction of relationships between biological attributes of Surber and kicknet samples.

Third, we used non-metric multidimensional scaling (NMDS) ordination to show relationships between composite Surber and kicknet samples based on the entire composition of the benthic invertebrate community. NMDS ordination provided a graphical depiction of sample similarity that was used to depict and interpret relationships between samples. The NMDS ordination was performed using PC-ORD, an ordination program designed for ecological data. We used an initial test to assess the target dimensionality (e.g., two or three dimensions). Based on this initial test, a three-dimensional solution was selected and a final analysis was rerun using the Sorenson distance measure (based on presence-absence data) with 250 iterations to assess solution stability.

As a separate analysis, we assessed the effect of organism abundance on sample similarity. The methods and results of this analysis are presented in Appendix 6.

Comparison of Biological Condition

We used multiple analyses to compare differences in the assessment of biological condition. First, we compared B-IBI values from kicknet samples and mean B-IBI values from composite Surber samples using a paired sample t-test. Second, correlation between B-IBI calculated from composite Surber and kicknet samples was tested. Third, we qualitatively compared the assessments of biological condition provided by the B-IBI and RCA-CABIN methods.

A 10-metric scoring with organisms identified to lowest practical taxonomic level (LPTL) was used to calculate B-IBI values for each sample (Fore *et al.* 2001; Salmonweb 2004). The average B-IBI value for each stream was calculated from four composite Surber samples. Each stream was assigned to a biological condition class (Table 3).

Table 3. Lowest practical taxonomic level B-IBI biological condition classes.

Condition Class	LPTL B-IBI score
Excellent	46 – 50
Good	38 – 44
Fair	28 – 36
Poor	18 – 26
Very Poor	10 – 16

The RCA-CABIN analysis requires that environmental data are used to predict to which reference group each of the Surber and kicknet samples should be compared. In 2003, environmental measurements were taken during the kicknet sampling period but not during the Surber sampling period, thus the same environmental measurements were used to predict the Surber samples to an appropriate reference group. In the current Fraser River/Georgia Basin assessment model, five reference groups exist for family level data (Sylvestre *et al.* 2005). Worms and mites were not identified to family level for the GVRD study streams, therefore a modified reference dataset from the Fraser River/Georgia Basin database was used that used class level data for worms and mites rather than family level data. The reference and test communities in RCA-CABIN were plotted in ordination space using hybrid-multidimensional scaling (HMDS) using the PATN software (Belbin 1993). The assessment of biological condition is based on the distance the test sample falls from the cloud of reference sites using confidence ellipses (Table 4) from Rosenberg *et al.* (1999). Three axes are necessary to describe the community in ordination space in order to reduce the “stress” of the ordination to <0.2 (a low stress indicates that the ordination plot adequately represents the actual relationships of all the points to each other). The overall assessment is based on the axes where the site is furthest away from the reference community.

Table 4. RCA-CABIN biological condition classes.

Condition Class	Distance from reference sites
Not stressed	Within 90% confidence ellipse
Possibly stressed	90-99% confidence ellipse
Stressed	99-99.9% confidence ellipse
Severely stressed	Outside 99.9% confidence ellipse

RESULTS

Comparison of Surber and Kicknet samples

Sample Abundance

A total of 44,201 organisms from 119 invertebrate taxa were identified and enumerated from 76 composite Surber samples (1 composite sample = 3 Surber placements) and 22 single integrated kicknet samples collected in 2003 and 2004. Organism abundance ranged from as low as 249 individuals/sample to 13,800 individuals/sample (Table 5). Taxa richness ranged from six in Como Creek to 36 in Mossom Creek. Raw data are provided in Appendix 1.

The average abundance of organisms collected by composite Surber samples was similar in 2003 and 2004 (Table 5). The average abundance of organisms in kicknet samples was four times higher in 2004 than in 2003 due to the timing of the sampling. In 2003, the kicknet samples were taken at the end of the sampling window for the RCA-CABIN protocol while in 2004 they were taken at the very beginning of the RCA-CABIN protocol sampling window. Sampling in 2003 corresponded with the onset of the rainy season in the GVRD and storms may have affected sample comparisons by reducing the number of organisms in the streams through washout or by expanding the sampling area into banks of the streams from increased water levels.

Table 5. Abundance of organisms collected in composite Surber samples and kicknet samples from GVRD streams in 2003 and 2004.

2003 Study Streams	**Composite Surber Samples** (n=4)	**Kicknet sample**	**2004 Study Streams**	**Composite Surber Samples** (n=4)	**Kicknet sample(s)**
Keith	931	311	Bertrand	2757	13800
Hastings	1050	1003	Still	376	1937*
MacKay	1341	2247	Stoney	1915	2371
McCartney	1300	1024	Como	2942	7160
Mosquito	1239	1123	Beaver	549	1244*
Wagg	1459	825	Wagg	373	981
Parkside	1081*	1805	Mossom	710	2777
Taylor	249	393	Coghlan	3714	7850
			Serpentine	2172	2515*
			Clear	576	2913
			Yorkson	639	1665
Average	**1081.2**	**1016.5**	**Average**	**1520.4**	**4110.4**

*n = 2

In 2003, when kicknet samples were collected ~30 days later than Surber samples, the average abundance of organisms in composite Surber samples was higher at six of nine streams than in a single kicknet sample (Table 5). However, in 2004, when Surber and kicknet samples were collected at the same time, the kicknet consistently collected more organisms than a composite Surber sample.

Based on the abundance data presented in Table 5, one kicknet sample had approximately the same number of organisms as one composite sample of three Surber placements in 2003 when samples were collected at different times. However, in 2004 when samples were collected at the same time, a single kick-net sample had the same number of organisms as eight Surber placements which is the equivalent of nearly three composite Surber samples. This difference in abundance has implications to the RCA-CABIN analysis because relative abundances are the basis for the assessment.

Biological Attributes

We found that the richness and composition attributes of benthic invertebrates in samples collected using Surber and kicknet devices were similar in the 19 study streams (Table 3[2]; Appendix 3). The coefficient of variation (%CV) values (a standardized measure of variability) also indicated that kicknet and Surber samples were equally variable in measuring many of the community attributes (Table 6).

Total taxa richness was significantly higher in Surber samples than kicknet samples (mean of 20.3 and 18.5 respectively; p=0.0105). This result may be due to the greater depth to which the substrate is disturbed collecting a Surber sample versus the kicknet sample, thereby collecting different invertebrate taxa which reside deeper in the substrate or due to the greater number of invertebrates subsampled from the Surber samples (i.e., 400 vs. 300). We found that six taxonomic families (Ancylidae, Astacidae, Dytiscidae, Ephydridae, Sialidae and Sphaeriidae)

[2] Appendix 3 includes paired t-test results from 2003 and 2004 separately, as well as amalgamated data from both years.

were captured in the Surber samples that were not found in the kicknet samples from the 19 study streams, while only one taxon (Glossiphonidae) was found solely in the kicknet samples. As many of these families are not indicator taxa, nor were they common taxa, many of the community attributes were not significantly different (Table 6). Kicknet samples had significantly more mayfly (Ephemeroptera) taxa than the Surber samples (mean of 3.7 and 3.2; p=0.0345), but stonefly (Plecoptera) and caddisfly (Trichoptera) taxa richness were not significantly different between sampling methods. Despite the significant increase in mayfly taxa in kicknet samples, EPT taxa richness and % EPT individuals was also not significantly different between sampling methods.

Table 6. Summary of paired t-tests between 19 paired Surber and kicknet samples collected in 2003 and 2004. Variables with significant differences are indicated by p values <0.05 highlighted in bold.

Variable[1]	**Surber Samples**		**Kicknet Samples**		**Mean Difference**	**T- value** (paired)	***P*-value**
	Mean[1]	%CV	Mean	%CV			
Total Taxa	20.3	33.9	18.5	35.6	-1.8	-2.682	**0.0152**
EPT Taxa	9.8	63.7	10.1	58.1	0.3	0.799	0.4345
% EPT Organisms	48.6	45.1	47.7	43.9	-0.9	-0.205	0.8396
%Dominance (3 taxa)	70.1	21.1	67.8	23.2	-2.3	-0.864	0.3989
Diversity (Shannon)	1.906	23.8	1.910	23.1	0.004	0.057	0.9548
Evenness (Shannon)	0.638	13.4	0.661	12.7	0.023	1.038	0.3131
Intolerant taxa	1.2	137.9	1.2	121.8	0.01	1.663*	0.1136*
% tolerant organisms	25.3	66.1	29.3	53.0	-4.0	-1.220	0.2384
Ephemeroptera Taxa	3.2	61.2	3.7	61.7	0.5	2.287	**0.0345**
Plecoptera Taxa	2.9	81.9	2.9	78.0	-0.02	-0.2054	0.8395
Trichoptera Taxa	3.7	60.7	3.5	59.4	-0.2	-0.7751	0.4484
Long-lived Taxa	2.2	60.4	3.6	81.9	1.4	2.067	0.0534
Clinger Taxa	9.5	53.5	11.3	58.7	1.8	2.726	**0.0139**
% Predator Organisms	4.1	160.5	2.2	170.3	-1.9	-2.077*	0.0524*

[1] Mean values for Surber samples are derived from four composite samples per stream; mean values for kicknet samples are derived from single samples. N=19 for both years combined.
* Indicates significance or t-test values derived from log_{10} transformed means to meet or improve normality. Mean and %CV values reflect untransformed values.

The proportion of the most abundant taxa (% dominance of three most abundant taxa) was similar between the sampling methods; the most abundant three taxa accounted for 70.1% of organisms in Surber samples and 67.8% of organisms in kicknet samples. Similarly, the analysis found that evenness, the distribution of abundance across taxa, and diversity, the combination of species richness and evenness, were similar for both sampling methods. This indicates that both sampling methods provided a similar estimate of the benthic invertebrate community. Taxa found in more than 65% of samples include (in decreasing commonness): the midge Chironomidae (present in all 98 samples); the mayfly *Baetis tricaudatus* (91); Oligochaeta worms (90); the stonefly *Zapada cinctipes* (78); *the mayfly Paraleptophlebia* (73); the blackfly *Simulium* (65); and the caddisfly Hydropsychidae (67). Both methods found Oligochaete worms were the most abundant taxon captured.

Kicknet samples had significantly more clinger taxa than the Surber samples (mean of 11.3 and 9.5 respectively; *p*=0.0139). Many mayflies are clingers which adhere to the substrates. This is likely a redundant result since the kicknet collected significantly more mayfly taxa than the Surber sampler.

Correlation of Sample Richness

The total number of taxa and the number of EPT taxa in Surber and kicknet samples were highly correlated indicating strong agreement between the sampling methods (Figure 2). The correlation between the total number of taxa in Surber and kicknet samples was 0.90 (Pearson's r, *p*<0.0001, N=19); for number of EPT taxa the correlation was 0.97 (Pearson's r, *p*<0.0001, N=19). In both cases, the regression slope is close to one indicating a nearly one to one relationship between samples.

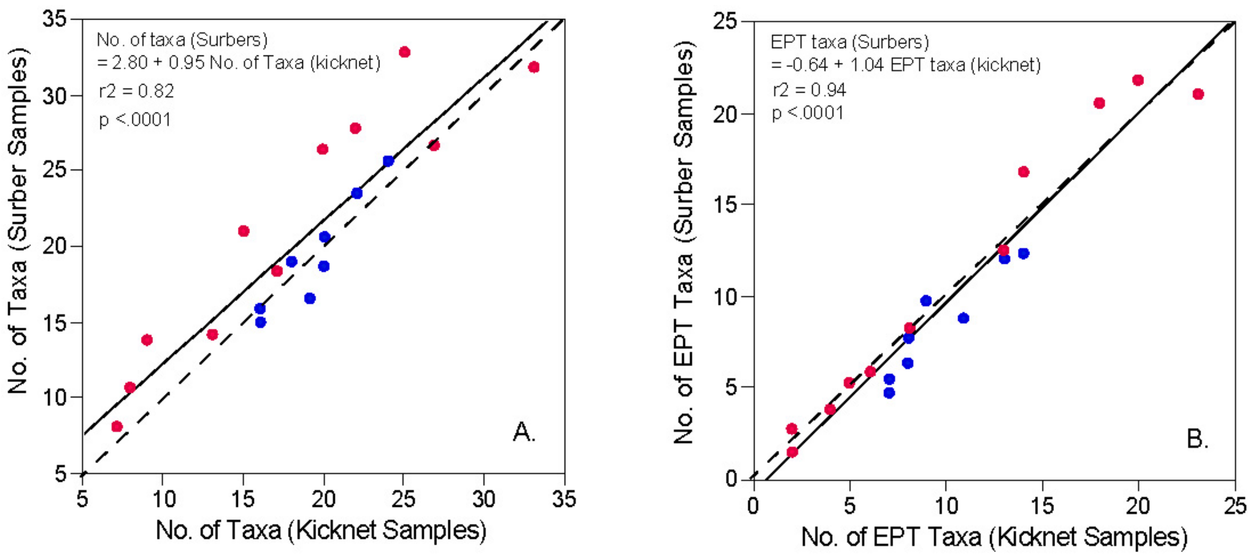

Figure 2. Correlation between taxa richness and EPT taxa richness in Surber and kicknet samples from 2003 and 2004. Samples from 2003 are shown in blue (N=8) and samples from 2004 are shown in red (N=11). The solid line shows the least-squares linear regression relationship and the dashed line shows the line of perfect agreement.

Multivariate Analysis of Similarity

Non-metric multidimensional scaling (NMDS) ordination showed that Surber and kicknet samples from the same stream were generally more similar to one another than to samples from other streams (Figure 3). More importantly, there was little difference between the kicknet sample(s) and the Surber samples from the same stream. In fact, kicknet samples were often more similar to Surber samples from the same stream than between the Surber samples from that stream themselves illustrating the variability of the Surber sampling method despite the composited samples. The NMDS ordination also showed the benthic community is less variable in the most urbanized streams (e.g., Still, Como, Wagg and Stoney creeks) than in less disturbed streams (e.g., Clear, Bertrand, and Beaver creeks) as indicated by the size of the polygons enclosing the samples.

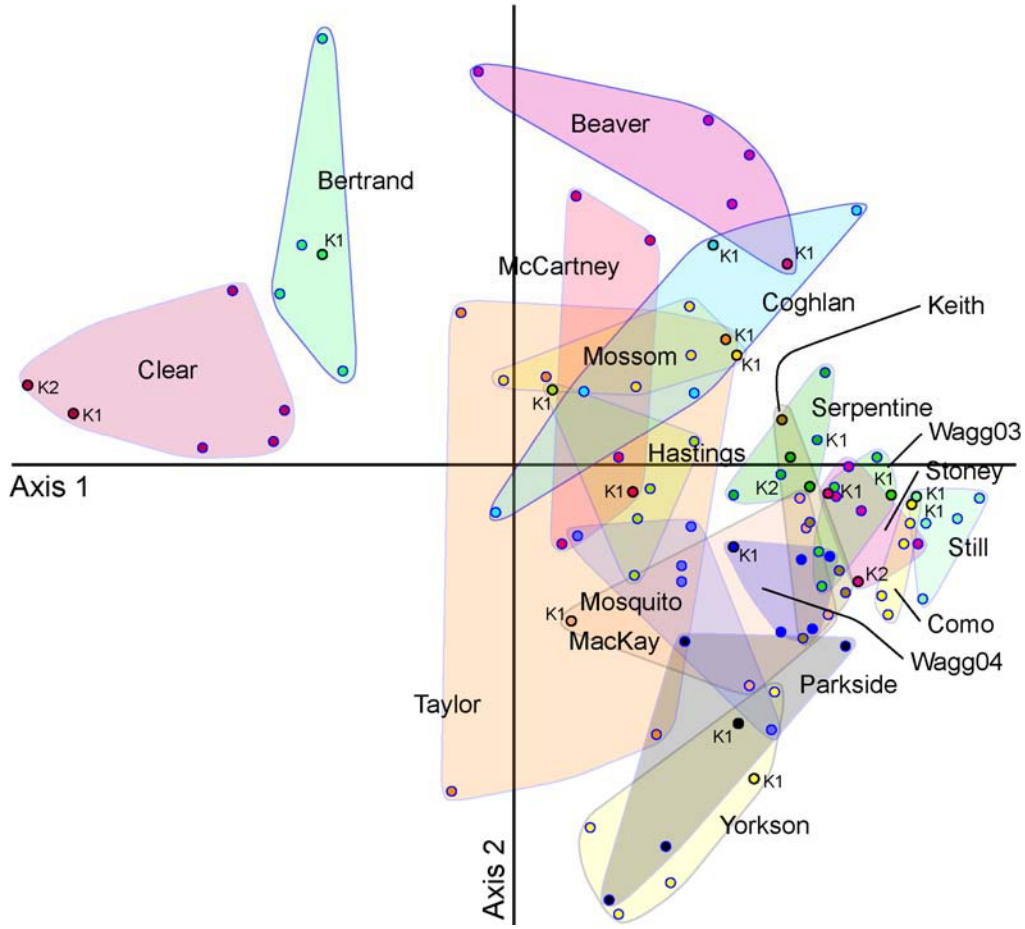

Figure 3. NMDS ordination results showing groupings of Surber and kicknet samples for 19 study streams. Kicknet samples are noted with a darker circle and the letter "K" ("K1" and "K2" refer to streams with multiple kicknet samples). The stress of the final 3-D solution was 15.7 and the final instability was 0.0047 after 47 iterations. Note that Wagg Creek was sampled in both 2003 and 2004.

B-IBI Values in Surber and Kicknet Samples

B-IBI values in kicknet samples were significantly higher than in Surber samples (mean difference 3.0; *p*=0.0076) (see Appendix 2 for B-IBI metric scores and values). The mean B-IBI was 24.4 (SD 10.1) in kicknet samples and 21.4 (SD 8.8) in Surber samples. Despite significantly higher taxa richness in Surber samples, B-IBI values were higher in kicknet samples. This indicates that small increases in total taxa richness in Surber samples, while statistically significant, have a minor effect on B-IBI values. However, significantly higher numbers of mayfly and clinger taxa in kicknet samples, although smaller in terms of mean abundance, resulted in an increase in B-IBI. This demonstrates the sensitivity of B-IBI scores to the presence of certain taxa regardless of their abundance.

B-IBI values in Surber and kicknet samples were highly correlated (Figure 4) with a Pearson's correlation coefficient of 0.91 ($p<0.0001$, N=19). The regression relationship in Figure 4 highlights the strong linear relationship of B-IBI values from the two sampling methods. The consistently higher B-IBI values from kicknet samples are evident by the number of points that fall below the dotted line which indicates perfect agreement. The 2003 samples (shown in blue) appeared to consistently fall below the line of perfect agreement and may be a result of the slight difference in sampling time.

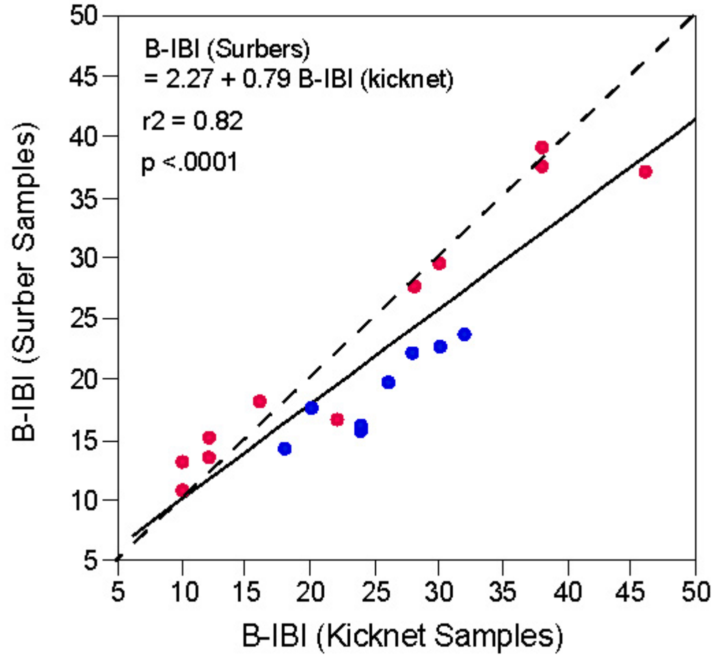

Figure 4. Correlation of B-IBI values from Surber and kicknet samples from 2003 and 2004. Samples from 2003 are shown in blue (N=8) and samples from 2004 are shown in red (N=11). The solid line shows the least-squares linear regression relationship and the dashed line shows the line of perfect agreement.

Although, we combined the 2003 and 2004 data for paired t-test analysis of community attributes, there appear to be consistent differences in B-IBI values between the sampling methods over the two years (Table 7). In 2003, B-IBI values from kicknet samples were an average of 6.5 (SD 2.3) points higher than Surber samples. All streams had higher B-IBI values in kicknet samples and ranged from +2.5 in Keith Creek to +9.0 in MacKay Creek. In 2004, B-IBI values from kicknet samples were more similar to Surber samples and were only 0.5 (SD 3.7) points higher. Six streams had higher B-IBI values in kicknet samples and five streams had lower values; maximum differences varied from -3.0 points in Como and Wagg creeks, to +9.0 in Mossom Creek. We suspect that this difference is due to the sample timing and the variance of conditions of the streams within the fall season. In 2003, when the B-IBI values differed by an average of 6.5 points, the kicknet samples were taken approximately 30 days after the Surber samples. Several storms and pollution effects may have occurred in that time. In 2004, the kicknet samples were taken within 12 hours of the Surber samples eliminating any potential temporal variation in stream condition resulting in a minor B-IBI difference of only 0.5 points on average.

Comparison of Biological Condition

B-IBI Biological Condition Assessment

Biological condition classes assessed using Surber and kicknet B-IBI values were equivalent for eleven of the 19 streams (Table 7). In seven streams, biological condition was one class higher in the kicknet samples, while in the other stream (Yorkson Creek), biological condition was one class lower. Kicknet samples tended to have higher B-IBI values compared to Surber samples as a result of the tendency for kicknets to collect more mayfly taxa. However, the overall effect was relatively small as differences tended to occur around the B-IBI category threshold.

Table 7. Mean B-IBI values and biological condition classes[1] for Surber and kicknet samples.

Stream[1]	**Surber B-IBI**		**Kicknet B-IBI**		**B-IBI**
	Mean (SD)	Condition	Value	Condition	**Difference**
2003 Study Streams					
MacKay Creek	15.5 (3.4)	very poor	24	poor	+9.0
Mosquito Creek	22.5 (5.3)	poor	30	fair	+7.5
Wagg Creek	14.0 (1.6)	very poor	18	poor	+4.0
Keith Creek	17.5 (2.5)	poor	20	poor	+2.5
Hastings Creek	23.5 (1.9)	poor	32	fair	+8.5
McCartney Creek	22.0 (4.3)	poor	28	fair	+6.0
Taylor Creek	19.5 (4.1)	poor	26	poor	+6.5
Parkside Creek	16.0 (2.8)	very poor	24	poor	+8.0
2004 Study Streams					
Still Creek	10.5 (1.0)	very poor	10	very poor	-0.5
Como Creek	13.0 (1.2)	very poor	10	very poor	-3.0
Wagg Creek	15.0 (2.6)	very poor	12	very poor	-3.0
Serpentine River	16.5 (1.0)	vy. poor / poor*	22	poor	+5.5
Stoney Creek	13.5 (3.0)	very poor	12	very poor	-1.5
Yorkson Creek	18.0 (2.8)	poor	16	very poor	-2.0
Bertrand Creek	27.5 (1.9)	poor / fair*	28	fair	+0.5
Coghlan Creek	29.5 (1.9)	fair	30	fair	+0.5
Mossom Creek	37.0 (2.6)	fair / good*	46	excellent	+9.0
Beaver Creek	39.0 (5.0)	good	38	good	-1.0
Clear Creek	37.5 (4.4)	fair / good*	38	good	+0.5

[1] Biological condition classes: Excellent (46–50); Good (38–44); Fair (28–36); Poor (18–26); Very Poor (10–16).
* Indicates streams on the threshold between two biological condition classes.

RCA-CABIN Biological Condition Assessment

The RCA-CABIN assessment used a predictive model to select a group of reference sites that was appropriate for the each of the study streams based on the following habitat variables: ecoregion, average channel depth, presence of coniferous trees, dominant substrate category, embeddedness category, wetted channel width, latitude, stream order and pH. The model produced a probability of membership for each stream to one of the five reference groups defined in the Fraser River/Georgia Basin database (Sylvestre *et al.* 2005). Velocity and slope measurements were suggested predictor variables for the Fraser/Georgia Basin model based on Sylvestre *et al.* (2005) but were anomalously high for the North Vancouver streams in 2003 (likely due to poor instrument calibration) and therefore excluded from the model for assessment of the streams in this study. The exclusion of those variables increased the model error rate by only 1%.

Four Surber RCA-CABIN assessments were conducted at each stream and the average assessment was reported for each stream (Table 8). Ordination plots used to determine the biological condition classes are provided in Appendix 5. The RCA-CABIN assessments were identical among Surber samples for only six streams. At nine streams, only one Surber sample differed from the other Surber assessments. At least one of the Surber assessments was similar to the kicknet assessment at 13 of the 19 streams.

Table 8. RCA-CABIN assessments bands for Surber samples (A, B, C, D) and kicknet samples (K1, K2) at each site with the predicted reference group.

	Ref			**Surber**			**Kicknet**	
Stream	**Group**	**A**	**B**	**C**	**D**	**Avg**	**K1**	**K2**
2003 Study Streams								
Keith Ck	1*	3	2	3	2	**3**	2	
Hastings Ck	1*	2	2	2	2	**2**	2	
MacKay Ck	4*	2	3	2	2	**2**	2	
McCartney Ck	1*	2	1	2	2	**2**	2	
Mosquito Ck	3*	3	3	3	2	**3**	3	
Wagg Ck	4*	2	3	3	3	**3**	2	
Parkside Ck	1*	3	2			**3**	3	
Taylor Ck	1*	3	1	3	2	**2**	2	
2004 Study Streams								
Beaver Ck	1	1	1	1	1	**1**	1	
Wagg Ck	1	2	2	2	2	**2**	2	
Mossom Ck	1	1	1	1	1	**1**	2	
Serpentine R.	1	2	1	2	2	**2**	2	2
Stoney Ck	3	3	4	4	4	**4**	2	1
Still Ck	3	2	2	1	1	**2**	1	
Clear Ck	3	3	2	2	2	**2**	1	1
Yorkson Ck	4	2	2	2	2	**2**	3	
Bertrand Ck	5	3	4	3	3	**3**	2	
Como Ck	5	3	3	3	4	**3**	2	
Coghlan Ck	5	2	3	3	3	**3**	2	

Note: Band 1 = Not stressed, Band 2 = Possibly stressed, Band 3 = Stressed, Band 4 = Severely stressed; Similar assessments are shaded with the same colour.
*Environmental variables were not collected during the Surber sampling period in 2003 thus, the model prediction for the kicknet samples was applied to the Surber samples.

Generally, the average Surber assessment and the kicknet assessment were similar. There was 75% agreement between the average Surber assessment and the kicknet assessment in 2003 and only 27% agreement between the sampling methods in 2004. Assessments were identical for nine streams. In seven streams, the Surber samples indicated a worse condition class than the kicknet sample by only one category and in one case, Stoney Creek, Surber samples indicated a worse assessment by two categories. For Mossom and Yorkson creeks, the Surber sample indicated a better assessment than the kicknet sample.

B-IBI versus RCA-CABIN assessments

Generally, the interpretation of the B-IBI and RCA-CABIN assessments were in agreement in identifying that the invertebrate communities showed some form of anthropogenic stress with the exception of Still Creek and Stoney Creek (Table 9). While both techniques were able to detect departures from acceptable conditions, using the RCA-CABIN and B-IBI comparison on a site by site basis, the degree of stress indicated by either method varied. Table 9 summarizes the results from the RCA-CABIN and B-IBI assessments. Appendix 4 provides the predicted group membership results and Appendix 5 provides the ordination plots from the RCA-CABIN analysis from kicknet and surber samples.

Table 9. Summary of final biological condition assessment using RCA-CABIN and B-IBI values and assessment.

Stream	CABIN Assessment	Surber B-IBI	Surber B-IBI Assessment
2003 Study Streams			
MacKay Creek	Possibly stressed	15.5	very poor
Mosquito Creek	Stressed	22.5	poor
Wagg Creek	Possibly Stressed	14.0	very poor
Keith Creek	Possibly stressed	17.5	poor
Hastings Creek	Possibly stressed	23.5	poor
McCartney Creek	Possibly stressed	22.0	poor
Taylor Creek	Possibly stressed	19.5	poor
Parkside Creek	Stressed	16.0	very poor
2004 Study Streams			
Bertrand Creek	Possibly stressed	27.5	poor / fair*
Still Creek	Not stressed	10.5	very poor
Stoney Creek	Possibly stressed (K1) Not stressed (K2)	13.5	very poor
Como Creek	Possibly stressed	13.0	very poor
Beaver Creek	Not stressed	39.0	good
Wagg Creek	Possibly stressed	15.0	very poor
Mossom Creek	Not stressed	37.0	fair / good
Coghlan Creek	Possibly stressed	29.5	fair
Yorkson Creek	Stressed	18.0	poor
Serpentine River	Possibly stressed (K1+ K2)	16.5	vy. poor / poor*
Clear Creek	Not stressed (K1+K2)	37.5	fair / good*

* Indicates streams on the transition between two biological condition classes.

DISCUSSION

Comparison of Surber and Kicknet samples

The largest difference observed between the Surber and kicknet samples was the abundance of organisms collected in each sample. In 2003, similar numbers of organism were collected using the two sampling methods. In 2004, when the samples were collected at the same time, more organisms were captured in the kicknet samples than in a composite Surber sample. The kicknet sampling method appears to sample a larger area and collect more organic material than the Surber sampler. This may explain the larger number of organisms collected.

Despite this difference, many of the community attributes evaluated in this study were not significantly different between the two sampling methods. One exception is total richness which was significantly higher in Surber samples than kicknet samples. The subsampling protocol is a possible explanation for the increased taxa richness observed in the Surber samples. Only 300 organisms are subsampled from each kicknet sample compared with 400 organisms from each composite Surber sample. Previous studies have also found minor increases in taxa richness from subsamples greater than 300 organisms (Vinson and Hawkins 1996; Rosenberg *et al.* 1999). The increased richness may also be related to the depth of sample collection with the Surber sampling method. The substrate is disturbed to a much greater depth with the Surber method than with the kicknet method. The additional taxa found in Surber samples were generally rare and present at low abundance.

A recent study by Bennett (2004) in northwestern BC found Surber (3 placements or 1 placement) and kicknet sampling collected statistically similar numbers of invertebrate taxa. She also found that, similar to this study, many attributes were not significantly different between kicknet and Surber samples. In this study, kicknet sampling collected fewer organisms in 2003 than in 2004 probably due to the time of sampling. In 2003, kicknet sampling was conducted after a significant rainfall event, while in 2004 the kicknet samples were collected at baseflow.

There was a counterintuitive but significant increase in the number of mayfly (Ephemeroptera) taxa captured using the kicknet despite the higher total richness in Surber samples. Mayflies tend to cling to the surface of streambed substrates and to organic debris. An observation of the sample jars after collection was that Surber samples had a large proportion of inorganic material such as sand, while the kicknet samples had a large proportion of organic material such as leaves and twigs; this may partly explain the increase in mayfly taxa. The community richness values determined by the sampling methods were very highly correlated despite the significant difference in total richness and mayfly richness.

Neither sampling method produced more variable community attributes than the other suggesting that they are equally precise. Ordination plots, which take into account all the taxa present at a site, illustrated that the communities from the same stream were very similar regardless of the sampling method.

Comparison of Biological Condition

Overall, the community attributes measured with the Surber and kicknet devices were not substantially different. However, minor differences in these protocols such as subsampling counts, microhabitat sampled within the stream, and sampling period resulted in some statistically insignificant but biologically important differences between the methods. This was evident when the different data analysis approaches were applied to the two sampling methods.

A review of all community attributes that comprise the B-IBI and their associated metric scores found that despite small and statistically equal attribute values for Surber and kicknet samples, some metrics had a pronounced effect on overall B-IBI. Eight of ten B-IBI metric scores were higher for kicknet samples than for Surber samples, resulting in significantly higher B-IBI values for kicknet samples compared to Surber samples. For example, the mean richness of caddisfly taxa was 3.7 in Surber samples and 3.5 in kicknet samples, a difference that was not statically significant. When the richness values were translated into metric scores for the B-IBI, the mean metric score based on caddisfly taxa richness was 1.6 points in Surber samples and 1.9 points in kicknet samples.

Kicknet samples tended to have higher B-IBI values that Surber samples possibly as a result of collecting significantly more mayfly taxa or as a result of the translation of attribute values into metric scores. As noted above, the metric score thresholds may enhance minor differences in taxa richness. Regardless, kicknet and Surber B-IBI scores were highly correlated indicating that the methods agree on the relative differences in biological quality among GVRD streams. The corresponding biological condition classes were similar as well. Again, differences tended to occur around class thresholds.

Organism abundance was the primary factor for differing RCA-CABIN assessments between the sampling methods. The RCA-CABIN approach is reliant on both relative abundance and richness in measuring the similarity to the range of reference communities. Despite the different sampling periods in 2003, similar numbers of organisms were collected using both methods and the RCA-CABIN assessments were also found to be similar, regardless of the sampling method used. However, in 2004, organism abundance was higher in kicknet samples.

B-IBI assessments using Surber samples and the RCA-CABIN assessments using the kicknet samples equally identified streams with anthropogenic activities with the exception of Still Creek. Using the B-IBI assessment, both the Surber and kicknet sampling method produced an assessment of “very poor” due to the lack of stoneflies. These organisms tend to be predators and intolerant taxa which also contributed to the poor assessment. However, using the RCA-CABIN assessment, the Surber method produced an assessment of “not stressed” or “possibly stressed”. Similarly, the kicknet method also produced an assessment of “not stressed”. This is an example of where the two analytical approaches are fundamentally different despite the similarity of the benthic communities. Channelization of Still Creek may have also affected the assessment of biological condition using the RCA-CABIN approach because measurements of channel morphology affect model selection.

RECOMMENDATIONS

1. While this study has shown the similarity of Surber and kicknet samples from the same stream, differences in the richness and abundance of taxa groups and B-IBI values indicate they should not be used interchangeably for stream assessment in Greater Vancouver. If kicknet sampling is to be used for the collection of samples for B-IBI assessment, we recommend that a broader study to calibrate a B-IBI method for kicknet sampling be

undertaken in Greater Vancouver. This would be advantageous in that data collected using kicknet sampling methods could be analyzed using both RCA-CABIN and B-IBI approaches.

2. The results of this study showed that B-IBI and RCA-CABIN analytical approaches equally provide similar assessments of biological condition, with one exception, thus either method can be used to detect impacts to streams in Greater Vancouver. This study supports both methods as effective tools for assessing the biological condition of streams and small rivers in Greater Vancouver.
3. Both sampling methods are widely used for benthic community studies; Surber samplers are used in a broad range of stream studies in North America and the methodology is well established in monitoring programs in the Pacific Northwest. Similarly, kicknet sampling is widely used in Europe and Australia and has been adopted as the national method for biological monitoring in Canada. Every modification to a protocol provides additional uncertainty in the source of the variation of the data and therefore requires a pilot study to evaluate that modification. We stress that consistency of sample collection (e.g., equipment, number of samples, seasonality, etc) and laboratory processing (e.g., subsampling and taxonomic resolution and quality) are more important for effective monitoring programs than specific sampling or analysis methods.
4. Benthic community data that is collected using consistent and standardized field methods and with reliable taxonomic identifications can be analyzed using a variety of statistical methods.

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