Cheakamus River Benthic Invertebrate Recovery Monitoring Program 2005

Prepared for:

Canadian National Railway Company
CN Environment
13477 – 116 Avenue
Surrey, BC V3R 6W4

FINAL REPORT
April 2008

Prepared By:

TRITON ENVIRONMENTAL CONSULTANTS LTD.
8971 Beckwith Road, Richmond, BC V6X 1V4

This page has been intentionally left blank for two sided printing

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

This page has been intentionally left blank for two sided printing

Summary

On August 5, 2005, the Cheakamus River was affected by a spill of sodium hydroxide (NaOH) following a train derailment in the Cheakamus canyon approximately 15 km north of Squamish, British Columbia. Although the product did not persist in the system, it caused mortalities to invertebrates and fish in the river at the time of the spill. The spill represented a short-term “pulse disturbance” to aquatic assemblages, and benthic invertebrate recovery was studied during the summer and fall months following the derailment.

Post-spill community structure, biomass and abundance of individual benthic invertebrates was compared to pre-spill data, as well as upstream controls and reference sites in the Georgia Basin following procedures outlined by the Canadian Biomonitoring Network (CABIN). Kick net and colonization baskets were used to sample invertebrate assemblages over a three month period following the spill.

The Reference Condition Approach (RCA) was one method used to compare affected invertebrate sampling sites. Based on the assessment of site specific habitat conditions all sites were predicted to be in the same sub-group (“Group 1”) of Georgia Basin Reference Sites within the Basin-wide reference database. Invertebrate community structure from a total of seven post-spill kick net sampling sites, including one control, showed sites furthest downstream were most similar to the regional Reference Condition. The density of post-spill invertebrate samples was also found to be greater at sites further downstream from the spill location within the three month sampling period. While the mechanisms of recovery are unclear it appears downstream drift from tributary streams contributed to recovery. Data from kick net and colonization basket studies indicates rapid recovery was occurring within weeks of the spill.

This page has been intentionally left blank for two sided printing

1.0 Introduction

1.1 Background

The Cheakamus River is one of the largest tributaries of the Squamish watershed, draining a 1,070 km^2 area of the Coastal Mountain range in southwestern BC (NHC, 2000) (Figure 1-1). The flow regime of the Lower Cheakamus River is regulated by the Daisy Lake Dam and reservoir, which diverts a portion of the annual discharge to the Cheakamus Powerhouse in the Squamish valley. Diversion volumes and power production vary with both climate and regulation (Marmorek and Parnell, 2002). The traditional territory of the Squamish Nation encompasses the entire Cheakamus watershed, and Squamish Nation members have traditionally relied on the river and the watershed for cultural activities, food gathering as well as transportation (Marmorek and Parnell, 2002).

Downstream of Daisy Lake Dam, the Cheakamus River flows 24 km through a high-gradient canyon, dropping 450 m before entering the valley floor and joining the Squamish River at Baynes Island near the community of Brackendale. The Cheakamus River reaches below the Daisy Lake Dam are joined by several tributary streams. These tributaries including Rubble Creek, Chance Creek, Culliton Creek, Swift Creek and the Cheekye River contribute flows and invertebrate drift to the Cheakamus River (Figure 1-2). These tributaries and the Cheakamus River support a variety of anadromous and/or resident fish species.

1.2 Train Derailment

On August 5, 2005, a train derailment occurred at Mile 56.6 of the Squamish Subdivision of the Canadian National Railway Company (CN) mainline, resulting in the release of approximately 45,000 L of sodium hydroxide (NaOH) into the Cheakamus River (Teal Solutions, 2005). As the product was carried down the river it affected anadromous and resident fish in the river (McCubbing *et al.*, 2006). The spill also resulted in a 24-hour closure of the Cheakamus River for recreational purposes and a 48-hour closure of drinking water wells (Triton, 2007a). Although the product did not persist in the environment, it did cause mortalities to invertebrates and fish in the river at the time of the spill (McCubbing *et al.*, 2006).

NaOH is highly soluble in water and dissociates to sodium and hydroxyl ions. This has the effect of increasing water pH and alkalinity, particularly in waters of low buffering capacity such as the Cheakamus River. There is little or no specific data on the mode of toxicity to benthic invertebrates, however there have been fish studies on this topic. Environment Canada (EC) indicates the adverse effects resulting from fish exposure to sodium hydroxide are mostly a consequence of increased pH where pH levels above 9.0 cause burns to the external membranes of the gills, accompanied by slime formation. The fish die from suffocation as the blood/oxygen barrier is increased to a point where gas exchange cannot take place (EC, 1984).

Benthic macroinvertebrates are aquatic fauna retained by a net of mesh diameter >500 micrometres (µm) (Hauer and Resh, 1996), and reside amongst the stream substrate. These organisms can serve as an indicator of stress in the aquatic environment. Juvenile and adult fish are known to feed upon benthic invertebrates hence their density and biomass are useful

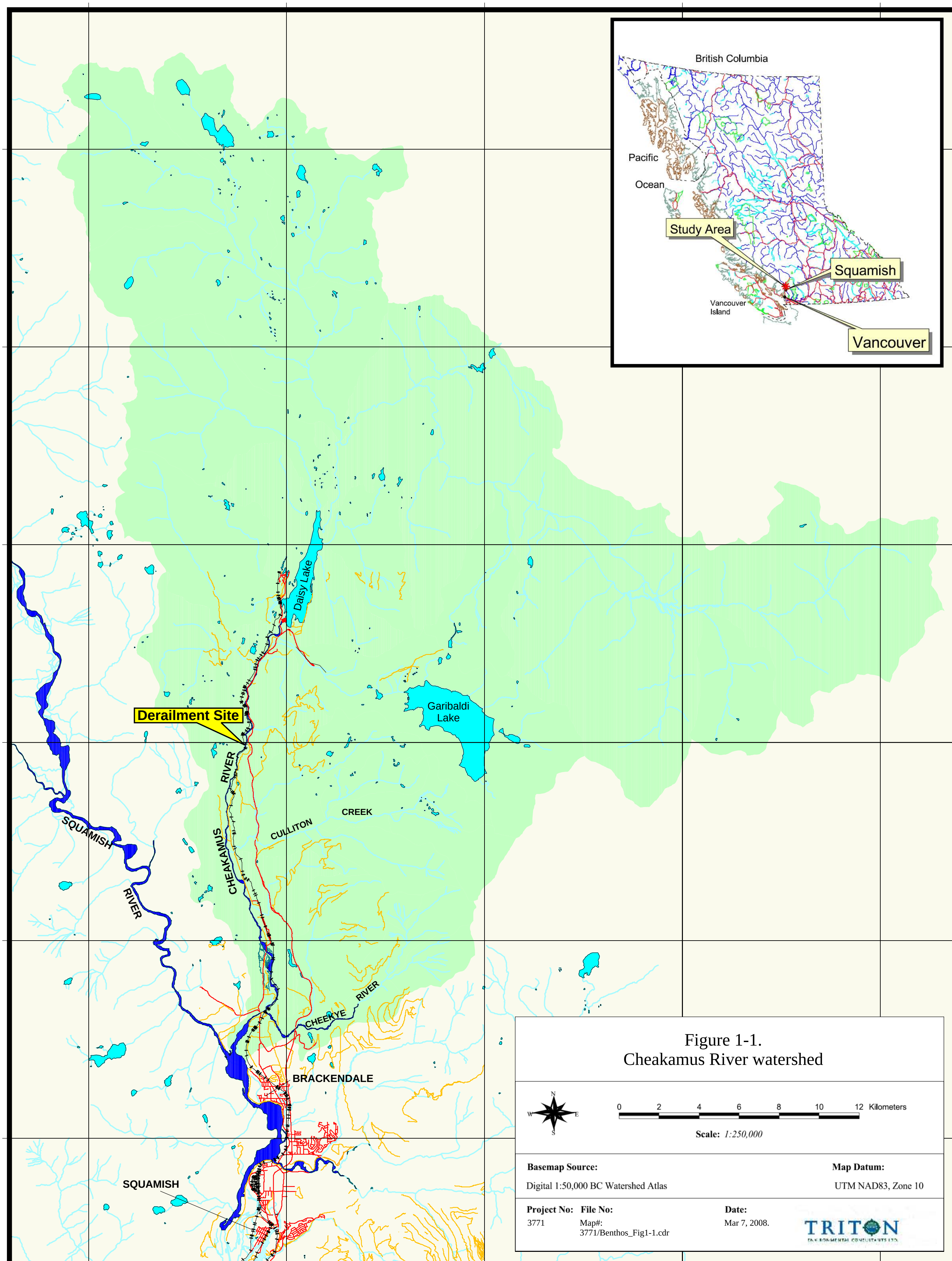


Figure 1-1.
Cheakamus River watershed

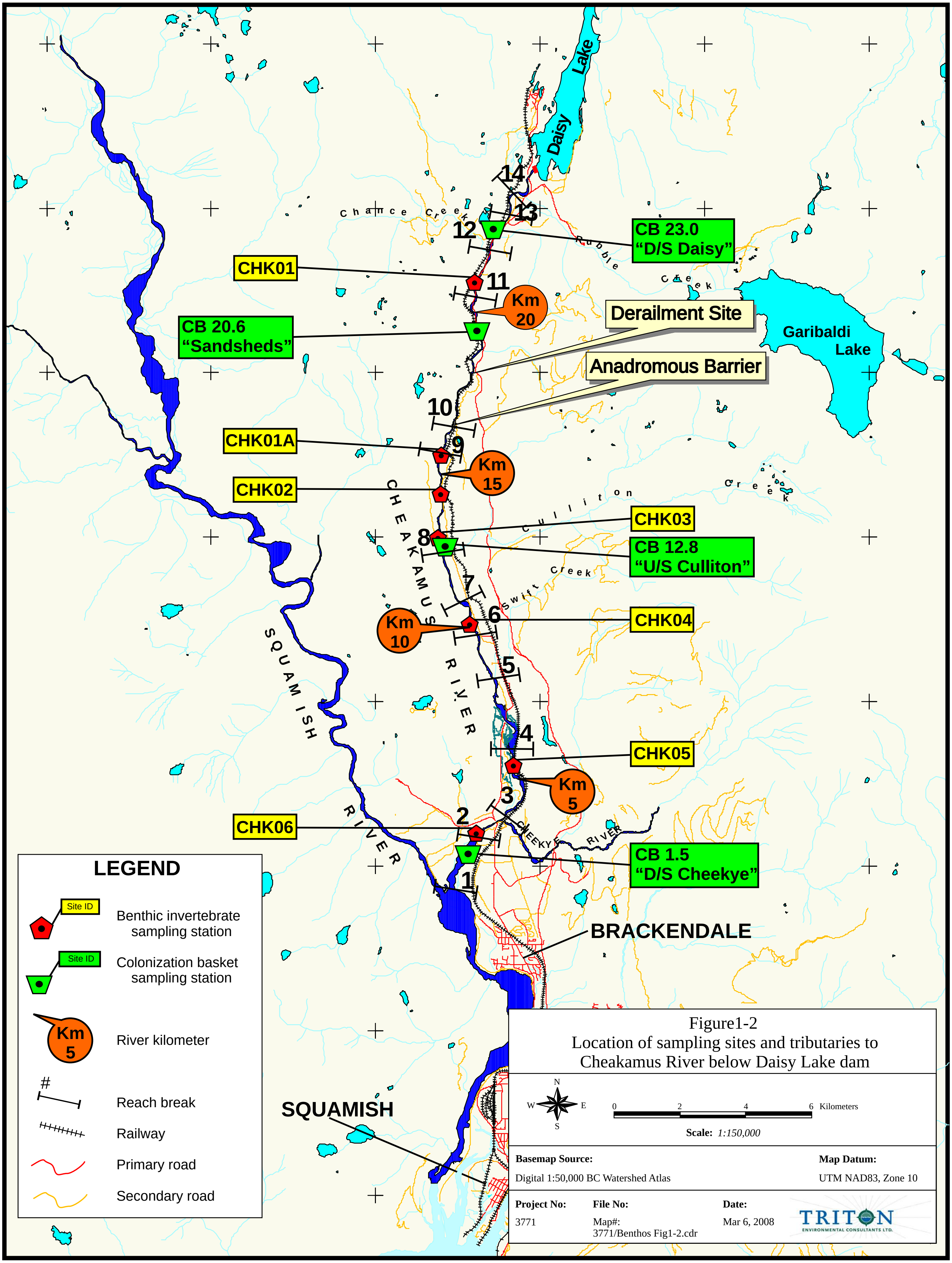


Figure1-2
Location of sampling sites and tributaries to Cheakamus River below Daisy Lake dam

indicators of available food resources for fish. Evaluation of benthic community abundance and diversity is also used as an indicator of stream health.

The benthic invertebrate community in the Cheakamus River was sampled after the NaOH spill to provide an estimate of the:

- Measurable impacts, if any, resulting from exposure to NaOH; and,
- Recovery in the weeks and months after the spill.

Recovery in this context is defined as returning to pre-spill condition. In the absence of benthos data from the hours immediately before the spill, this study has used the following comparisons for evaluating recovery:

- A Before-After-Control Impact (BACI) study design using Cheakamus pre-spill benthos data (Before) (colonization baskets, Perrin, 2001), re-sampling of these sites after the spill (After) and including sites upstream (Control) and downstream of the spill;
- Regional data [*i.e.*, Reference Condition Approach (RCA) using Canadian Biomonitoring Network (CABIN) Group 1 Reference Sites for Georgia Basin, Sylvestre *et al*., 2005];
- A Cheakamus River control site (CHK01), upstream of the spill and sampled on the same dates as downstream sampling sites; and,
- Intra-river sample comparisons over five kick net sampling periods after the spill (included Analysis of Similarities, Similarity Percentages, Analysis of Variance).

Benthic invertebrates have often been used to detect and evaluate the significance of pollution in the water column (Lenat, 1998) and benthic community structure is considered to be responsive to changes in water quality. Particular insect taxa are considered to be more sensitive to pollution than others and therefore these taxa have been used in various bioassessment studies to develop biotic indices (Woodiwiss, 1964; Hilsenhoff, 1988; Kerans and Karr, 1994). These pollution-sensitive taxa include **E**phemeroptera (mayflies), **P**lectoptera (stoneflies) and **T**richoptera (caddisflies), collectively referred to as EPT taxa. Streams with higher relative abundance of pollution-sensitive taxa typically receive a higher “score” in these biotic indices (*e.g.*, B-IBI, Hilsenhoff, 1988) than streams with lower levels of these taxa. Biological indices have been applied to invertebrate taxa which have been exposed to pollution exposure over months and years, at varying levels of toxicity.

In the Cheakamus River, the acute NaOH exposure resulted in dead invertebrates (insect larvae) being found in the nets used to collect fish in the hours after the spill (Wilson, 2006, pers. comm.), but the magnitude and distribution (amongst taxa groups and geographically) of invertebrate mortalities were unknown. The principal objective of this study was to measure the status and recovery of the benthic invertebrates in the Cheakamus River following the spill.

1.3 Post-Spill Benthos

The following sets of invertebrate data were used for comparison with the data collected in this study:

1. Studies conducted in 1996 and 1999-2000 as part of BC Hydro Water Use Planning (WUP) studies (Perrin, 2001) – "pre-spill".
2. Sites on the Cheakamus River (upstream of Daisy Lake Dam) and across the Georgia Basin which were previously sampled as part of the CABIN program for establishing a set of "reference sites" (Sylvestre *et al*., 2005).
3. Post-spill samples collected by EC from Cheakamus River at CHK01 (below the dam and above the spill), CHK03 (below the spill) and CHK05 (below the spill) in November 2005 (see Section 2.1 for site descriptions). During this sampling session, EC also re-sampled one of their sites upstream of the Daisy Lake Dam (sampled in past CABIN studies (Sylvestre *et al*., 2005).
4. Sites upstream and downstream of the spill sampled the week of the spill (August 2005) by BC Ministry of Environment (MoE) and Stamford Environmental (Stamford *et al*., 2007).
5. Sites upstream and downstream of the spill sampled summer/fall 2005 by Triton Environmental. The results and comparison to previous years' studies are found within this report.

Estimating the spill-related changes in the benthic invertebrate community presented several challenges including:

- Distinguishing between natural variation and the ecological stress of the spill on the benthic community; and,
- Inherent natural variability among and between sites based on site-specific factors.

Comparing samples from locations exposed to NaOH with samples from locations within the same river without exposure (*i.e.*, upstream control) is a common approach in lotic studies. Therefore the benthos sampling sites selected by the Ministry of Environment (MoE) and District of Squamish (DoS) in the week after the spill included a location upstream of the spill. This study also used this same control site for comparison to post-spill sampling results. Using this upstream data and the other data sets listed above, the following questions were asked with respect to comparing samples from spill-affected and unaffected sites:

- What are the differences in community composition, density, biomass and relative abundance?
- How much of this difference can be attributed to expected year to year variation?
- How do the inter-site similarities within the benthos communities of the affected reach compare with the similarities between the control site and affected communities across the 2005 sampling dates?

This report provides insight into benthic macro invertebrate recovery using data analysis from multiple sampling events and two sampling methods.

2.0 Benthic Invertebrate Surveys

Beginning August 20, 2005 benthic invertebrate (*i.e.*, aquatic insect larvae) kick net, Hess and Surber samples were collected every two weeks from seven sites on the Cheakamus River (Figure 1-2, Table 2-1). All but one site (control) were located downstream of the spill. Colonization baskets were installed on November 2 and 3, 2005 at four sites (Figure 1-2) and were subsequently retrieved on December 13 and 14, 2005 to replicate the incubation period and timing of basket studies conducted in fall 1996 and 1999/2000 (Perrin, 2001). Of the four colonization basket sites in this study, two were located upstream of the spill and two downstream (Table 2-1). Colonization basket sites were located in similar locations to earlier studies with the exception of the site downstream of the Cheekye River which was sampled in 1999/2000 but not in 1996 (Perrin, 2001). Protocols for installation and collection of colonization baskets are provided in Perrin (2001), and the colonization basket approach is further discussed in Section 2.3 of this report.

Table 2-1. Benthic invertebrate sampling sites and dates, 2005

Site name (ID)	Location relative to spill	Sampling method	Number of samples	Sample collection dates
CHK01	Upstream	Kick net, Surber and Hess	9 (3 per method)	26-Aug, 13-Sept, 25-Sept, 12-Oct, 26 Oct
CHK01A	Downstream	Kick net, Surber and Hess	9 (3 per method)	31-Aug, 13-Sept, 25-Sept, 11-Oct, 25 Oct
CHK02	Downstream	Kick net, Surber and Hess	9 (3 per method)	30-Aug, 13-Sept, 25-Sept, 11-Oct, 25 Oct
CHK03	Downstream	Kick net, Surber and Hess	9 (3 per method)	30-Aug, 14-Sept, 26-Sept, 11-Oct, 26 Oct
CHK04	Downstream	Kick net, Surber and Hess	9 (3 per method)	30-Aug, 14-Sept, 26-Sept, 12-Oct, 25 Oct
CHK05	Downstream	Kick net, Surber and Hess	9 (3 per method)	31-Aug, 14-Sept, 26-Sept, 12-Oct, 26 Oct
CHK06	Downstream	Kick net, Surber and Hess	9 (3 per method)	26-Aug, 14-Sept, 26-Sept, 12-Oct, 26 Oct
d/s Daisy Lake Dam: Site 1	Upstream	Colonization Basket	4	2-Nov to 14-Dec
Sandsheds: Site 2	Upstream	Colonization Basket	4	2-Nov to 14-Dec
u/s Culliton Creek: Site 3	Downstream	Colonization Basket	4	3-Nov to 13-Dec
d/s of Cheekye: Site 3	Downstream	Colonization Basket	4	3-Nov to 13-Dec

Note: Kick net, Surber, and Hess samples listed in this table were collected by Triton Environmental Consultants Ltd. Colonization basket samples were collected by Limnotek Research and Development Inc.

2.1 Site Descriptions

The Cheakamus River joins the Squamish River at Brackendale before entering Howe Sound (Figure 1-1), and both the Squamish and Cheakamus River watersheds fall within the Georgia

Basin. All sampling sites were located on the mainstem of the Cheakamus River (Figure 1-2). Wetted widths at individual sampling sites ranged from 23 to 44 m (Table 2-2). During the first 10 days of August 2005, the average discharge was estimated to be 40 m^3/s, with a monthly average discharge of 33 m^3/s based on the Water Survey of Canada (WSC) gauge at Brackendale (Stn. 08G0A43). Site-specific discharges were estimated using the Brackendale gauge and Daisy Lake Dam release data provided by BC Hydro, along with estimated watershed areas (Appendix 1). Discharges were calculated by comparing the relative proportion of the catchment area downstream of Daisy Lake Dam but upstream of the sampling site, to the difference in discharge between the two gauging stations (Figure 2-1). This calculation assumes uniform rainfall and runoff across each watershed draining into the lower Cheakamus River.

Table 2-2. Location and description of kick net sampling locations, 2005

SITE	UTM Zone	Easting	Northing	River km	Reach #	Bankfull width (m)	Wetted Width (m)	Comment
CHK01	10U	487978	5532811	21.9	11	47	44	Upstream (u/s) control site
CHK01A	10U	486923	5527387	15.6	8	35	24	3 km downstream (d/s) of derailment
CHK02	10U	486985	5526452	14.75	8	47	23	End of gravel road
CHK03	10U	486937	5525325	13.6	8	43	36	Misty Lane Suspension bridge
CHK04	10U	487880	5522266	10.2	6	40	31	2.4 km d/s of Culliton Creek
CHK05	10U	489176	5518238	5.3	3	57	34	1.4 km downstream of Bailey Bridge
CHK06	10U	487866	5515750	1.7	1	72	44	1.2 km downstream of Cheekye River

2.2 Sample Collection

Kick net samples were collected from the left bank except at sites CHK03 and CHK05 which were collected from the right bank. Sampling was limited to sections of the channel ranging from 0.2 to 0.8 m deep with moderate velocities, which could be safely accessed by wading. A description of the individual kick net sampling sites is provided in Table 2-2.

Samples of benthic invertebrates were collected every second week between August 26 and October 10, 2005 (four sampling sessions). Sampling sites were chosen to match locations used by BC MoE and DoS staff for benthic invertebrate sampling conducted the week after the spill (Stamford *et al*., 2007). The one exception was CHK01A, which was added to this study in order to gain a downstream sampling site in closer proximity to the derailment location. Stamford *et al*. (2007) collected three kick net samples per location (*i.e.*, one per riffle), and this methodology was repeated at each location in this study.

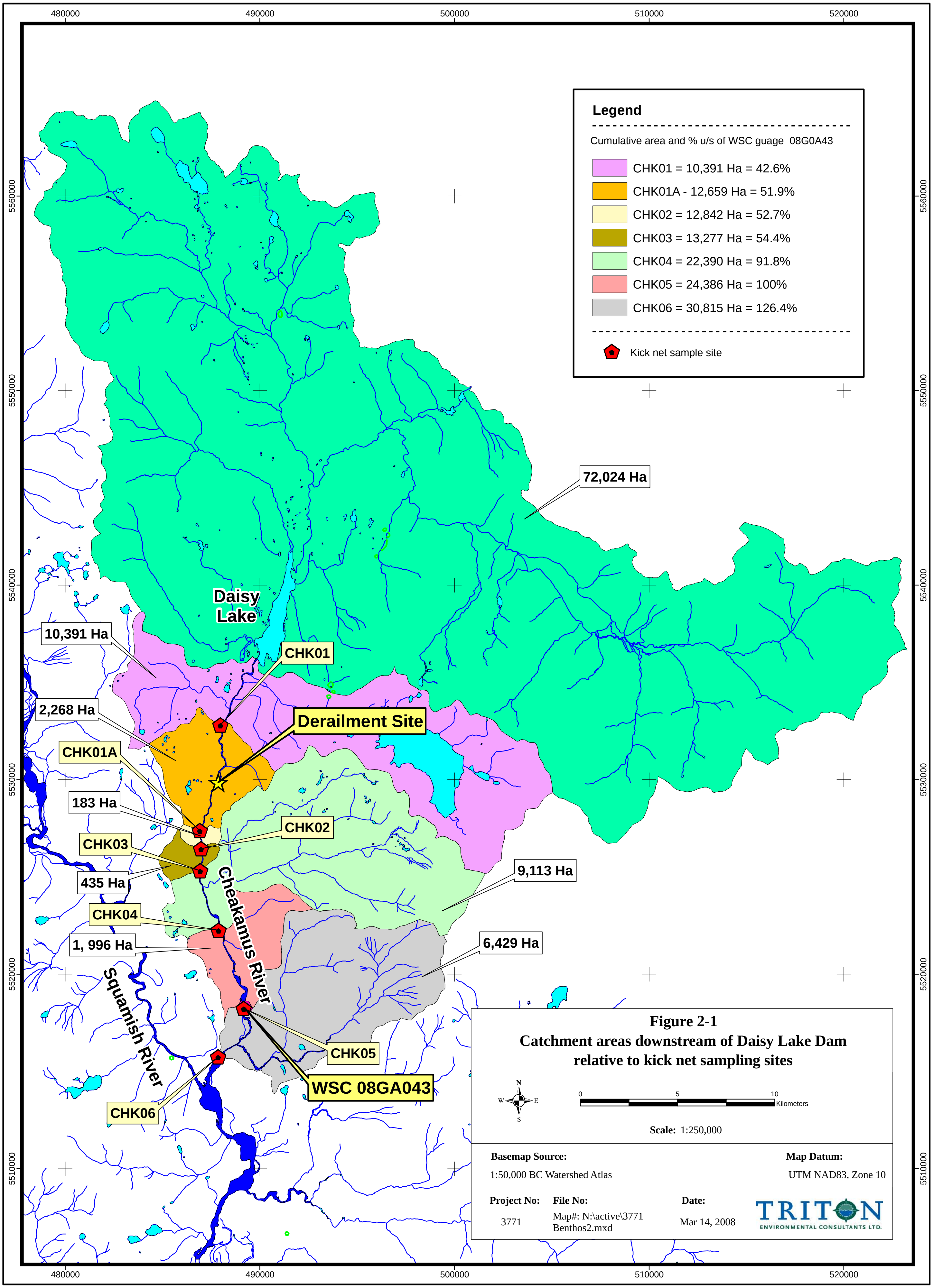


Figure 2-1
Catchment areas downstream of Daisy Lake Dam relative to kick net sampling sites

2.3 Kick Net Sampling

Kick net samples were collected following field protocols published by EC as part of the CABIN program (Reynoldson *et al.*, 2001b). This included a three (3) minute “travelling kick” period to collect each sample using a net with a 400 µm mesh size. This method involves capturing drifting invertebrates by loosening substrate with a kicking motion while walking away from the net, and moving upstream in a zigzag pattern. The process is repeated for a set time period (*i.e.*, three minutes) to standardize effort. Invertebrates collected in kick nets were fixed in the field using 10% buffered formalin, and later transferred to 70% ethanol for storage.

Daily *in situ* water quality measurements including water temperature, pH, conductivity and dissolved oxygen (% and mg/L) were collected at each kick net sampling station. Conductivity, dissolved oxygen and temperature measurements were collected with a Yellow Springs Instruments (YSI) Model 85D handheld meter, while pH was measured with a pH Testr 2 manufactured by Oakton Instruments. Meters were calibrated daily according to manufacturers’ instructions, and recorded similar conditions in each of the five sampling areas during each sampling period (Appendix 2).

One of three samples at each location was processed (*i.e.*, invertebrates were identified to the lowest practical taxonomic level) and the other two samples were archived. This is consistent with the CABIN protocols where a single sample per site is required for comparisons using the Reference Condition Approach (RCA) (Rosenberg *et al*., 1999). The most upstream samples from each site were chosen for processing from sites CHK01, 02, 04, 05, and 06 (over all dates) while the most downstream samples were selected for sites CHK01A and 03. These samples were selected because they all came from riffle habitat with similar site characteristics (*e.g.*, depth, velocity and substrate) thereby reducing the potential for variation caused by site characteristics.

Samples were processed in the laboratory by an experienced invertebrate taxonomist[1], certified under the CABIN program standards, to identify and enumerate individual invertebrates. Invertebrates were identified to the lowest practical taxonomic level using a modified version of the CABIN protocol (Reynoldson *et al.*, 2001b), whereby sub-sampling was done with a plankton splitter device rather than a Marchant box. The use of a plankton splitter instead of the CABIN-recommended Marchant Box to separate and sub-sample the invertebrate community within each sample is not considered to have resulted in any bias (Perrin, 2006, pers. comm.). It should be noted this is the same taxonomist who provided taxonomic identification (ID) for 1996, 1999/2000 and 2005 colonization basket sampling in the Cheakamus River (Perrin, 2001).

The CABIN program has constructed a database of Georgia Strait and Fraser River basin sites and their corresponding benthic invertebrate communities. Site data used to establish the basin-wide database of biological and physical conditions are based on kick net samples and habitat measurements collected by EC staff and other professionals experienced in biomonitoring sampling techniques (Rosenberg *et al*., 1999).

[1] D. Dolecki, Limnotek Reseach and Development

In addition to the RCA approach, an upstream control site (CHK01) was sampled to compare invertebrate communities affected by the NaOH spill to another unaffected site within the same system (Figure 1-2). The taxa group representation and abundance of the control and the post-spill invertebrate community were compared for the nine week period following the spill (*i.e.*, BACI study design).

2.4 Surber and Hess Sampling

In addition to kick net samples, benthic invertebrates were collected during a 3 minute time period with Surber and Hess samplers. These samplers are designed for a more precise estimate of density by providing a defined sampling area within which benthic invertebrates can be collected. The Surber sampler encloses a 0.097 m^2 area, and uses a 253 µm mesh, while the Hess sampler encloses a 0.086 m^2 area with a 243 µm mesh. Invertebrates are collected by disturbing or washing rocks within the defined area encompassed by the sampling device, and collecting invertebrates in the downstream collection net. After collection, Surber and Hess samples were field-sieved using a 425 µm screen to reduce the sample volume by removing smaller particles or organisms. Samples were then fixed in 10% buffered formalin and subsequently transferred to 70% ethanol for longer-term storage, within 72 hours of collection. These samples can provide a quantitative view of invertebrate densities as well as community composition. Samples remain archived for possible future analysis.

2.5 Colonization Baskets

Colonization baskets consisted of wire baskets filled with natural substrate, which were placed in the river during November 2005. In December, after a 41-day incubation period, the baskets were removed to examine colonization by benthic invertebrates (Appendix 4). The colonization basket study was undertaken by Limnotek Research and Development Inc. Site locations, sampling approach and sample processing associated with colonization baskets repeated the methods and locations used previously by Limnotek (Perrin, 2001). This allowed comparison to pre-spill benthic invertebrate density data published as part of the Water Use Planning process, when two seasons of data (fall 1996, and 1999/2000) were collected from the Cheakamus River using the colonization basket approach. In 2005 the average depth of water above the top of the baskets was 0.026 m (N = 146). As in the previous studies, five replicate baskets were installed at each colonization basket sampling site to account for potential patchy distribution of invertebrates, and to ensure the area covered at each sample site was the same. Of all the samples processed in 2005, those collected with the colonization basket method represented the most reliable method of estimating and comparing the relative quantity of invertebrates in the river. The greater reliability is due to the precision of the basket method (using a more defined sampling area and volume within each basket) and because it provided a comparison with pre-spill basket data [*e.g.,* Before-After-Control-Impact (BACI) study design].

The number of individuals and biomass (dry weight) per unit area were estimated based on the known area of the wire baskets. As with the 1996 and 2000 samples, the microbenthos (<1mm maximum dimension) fraction of the samples was also measured for biomass and abundance. Samples were collected at the same locations (two upstream and two downstream of the spill site) and time periods as used in the 1996 and 1999/2000 studies (Perrin, 2001). *In situ* parameters including mean daily water temperature, along with weekly measurements of pH, conductivity, total dissolved solids (TDS), turbidity and dissolved oxygen were recorded at each of the four basket locations used in 2005 (Appendix 5).

3.0 Data Compilation and Analyses

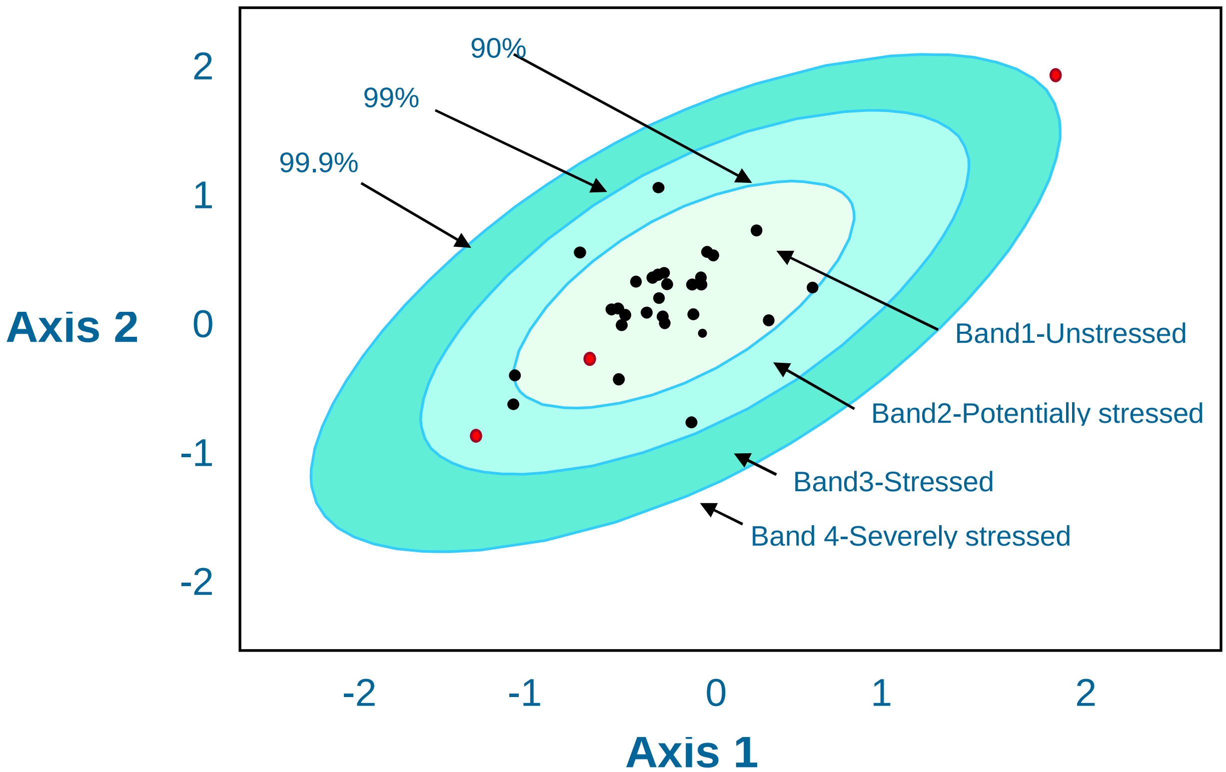
Kick net samples were assessed using the Reference Condition Approach (RCA) (Reynoldson *et al*., 1997; Rosenberg *et al*., 1999; Bailey *et al*., 2004). The RCA was successfully applied and tested in previous studies on the Georgia Basin (Sylvestre *et al*., 2005), (Reynoldson *et al*., 2001a) and provided a set of reference sites considered suitable for comparison to the Cheakamus River sites. A predictive model developed by EC was used to partition the invertebrate data from the reference sites into five subsets. The habitat variables EC found to best predict subset group membership in George Basin references sites were:

- Latitude
- Ecoregion
- Stream order
- Water Depth (avg.)
- Coniferous presence (riparian zone)
- Substrate size
- Substrate embeddedness
- Velocity (maximum)
- Channel width
- Slope
- pH

Given that the pH of water in the Cheakamus River had returned to baseline conditions prior to commencement of invertebrate sampling for this study (Triton, 2007a), it was determined the inclusion of pH in the model would not introduce additional error or bias to the classification of sites using the RCA.

The above listed habitat parameters were measured at each site using CABIN field protocols (Reynoldson *et al*., 2001b) and the data was then input into the EC predictive model. Using Discriminant Function Analysis (DFA), the CABIN analytical software tool assigned each of the Cheakamus River kick net sites (*i.e.*, “test” sites) to one of five subset groups based on site habitat data (Sylvestre *et al*., 2005). The DFA procedure provided a score of how well each test site matched its corresponding subset group from the regional database. The invertebrate community data from each sample was then compared to the invertebrate community of the predicted subset reference group. Since there are many invertebrate taxa groups (*i.e.*, variables) in each comparison, an ordination method called the **BE**nthic **A**ssessment of **S**edimen**T** (the BEAST, Reynoldson *et al*. 1995) is used to measure the proximity of test sample communities to the reference subset group communities. This ordination method reduces the number of variables to just three vectors which explain the majority of the variability, and the results are displayed on two-dimensional plots (Figure 3-1). Three plots are needed to show all the comparisons which result from the three vector scores. Within each plot, location of the test sample community is then measured from the centroid, or median of all the communities within the reference subset group. For this study, the largest distance from the centroid recorded in the three ordination plots was chosen as the overall score for each post spill test sample. To assess the meaning of a test sample's relative distance form the centroid, the BEAST has assigned confidence rating intervals which are plotted as ellipses around each ordination plot. The BEAST ratings for the 90, 99 and 99.9% confidence ellipses are: unstressed (inside 90% band), potentially stressed (inside 99% band), stressed (inside 99.9% band) and severely stressed (outside 99.9% band). An illustration based on data unrelated to the Cheakamus River study is included (Figure 3-1) to visually show these probability ellipses, which define the various levels of impairment (*e.g.*, stressed, severely stressed, etc.). The final result was a rating for each of the 2005 kick net samples collected over the nine (9) week sampling period.

Figure 3-1. Example of probability ellipses and site condition ratings of the RCA



Note: Figure is for demonstration purposes only. Source data is unrelated to Cheakamus study. Graphic source: Environment Canada, training material presentation, March 2006.

The similarity of the post-spill kick net samples was further explored with the RCA by plotting the distance of each group of samples, by site, to the subset reference group over the nine (9) week sampling period. This plot used the Bray-Curtis similarity measure (Bray and Curtis, 1957) to represent the relative distance of each post-spill sample of the invertebrate community with the median of its subset reference group community.

Assignment of kick net sample taxa into their respective functional feeding group types (*e.g.*, predators, collectors, gatherers etc.) was done using CABIN online analytical tools. With these CABIN tools family-level identifications are assigned their corresponding feeding group label, based on the known life history of that family. Since some invertebrate families fall into more than one feeding group, the software records them in both categories, and the result is the percent composition for samples will sum to >100%. However, this technique is not expected to introduce any directional bias since all samples are treated equally.

Quantitative comparison of the post-spill benthos communities was also completed without the use of the RCA or CABIN tools, using a multivariate analysis of the data to compare the relative similarity of post-spill benthos across sampling sites and dates (See Section 4.1.3). The quantitative comparison provided a complementary assessment of results in addition to the community structure assessment undertaken using the RCA approach.

The Analysis of Similarity (ANOSIM) and Similarity Percentages (SIMPER) procedures of the *PRIMER-E* 6 statistical software (Clark and Gorley, 2006) were used for multivariate comparisons. Taxa abundance data was pre-treated by converting it to the percent of total count for each respective sample, and then selecting the five most abundant Families (Baetidae, Ephemerellidae, Simuliidae, Heptageniidae, and Perlodidae). Pre-treatment was done to reduce dimensionality and facilitate comparisons between samples, since kick net sampling is only considered a semi-quantitative method of invertebrate collection and a poor indicator of total abundance. Given the semi-quantitative nature of the information, the percent composition also provided a comparative measure less prone to bias (*i.e.*, sampling error). Lastly, this pre-treatment standardized the data to facilitate comparisons.

A two-way ANOSIM was chosen for the comparison of kick net sample data using the ANOSIM and SIMPER procedures (Clark and Gorley, 2006). For the first factor, pre-treated data from kick net sites was grouped into one of three Site Blocks:

- Block #1 = CHK01 (N = 5) (upstream control)
- Block #2 = CHK01A, CHK02, CHK03 (N = 15) (all sites upstream of major tributaries such as Culliton Creek and Cheekye River)
- Block #3 = CHK04, CHK05, CHK06 (N = 15) (sites downstream of large tributaries)

To compare post-spill community similarities over time, a second factor was used by grouping the samples into two Week Blocks:

- Block #1 = first two sampling sessions
- Block #2 = last three sampling sessions

Prior to treatment, abundance data was plotted as a dendrogram using the resemblance matrix of Bray-Curtis similarities (Bray and Curtis, 1957) across samples in the *PRIMER* software version 6 (Clark and Gorley, 2006). The same Blocks of Sites and Weeks were used to group the samples in the dendrogram plot.

To compare the invertebrate community structure in samples from colonization baskets as it related to time (*i.e.*, sampling year) and location effects (*i.e.*, site), a plot was created using non metric multidimensional scaling (MDS) of the underlying Bray-Curtis similarity matrices in

PRIMER. The MDS allows for a two dimensional plot of multivariate taxa data. The plot does not have a scale nor axis, but instead the relative distances between samples is shown.

The ANOSIM procedure was also used to compare the community structure of the colonization basket samples. Invertebrate abundance data was converted to its Bray-Curtis resemblance matrix after it was log (1+X) transformed. A one-way ANOSIM was run separately for the 2005 (N = 20), 2000 (N = 12) and 1996 (N = 12) samples with a factor denoting the position of the sites (*i.e.,* upstream or downstream of the spill). The intent of this multivariate test was to determine if the similarity between upstream and downstream communities was greater in 2005 than in past years.

Lastly, a comparison of the variation among and within sampling years for the colonization baskets was done using Analysis of Variance (ANOVA). A two-factor one-way ANOVA was used to compare microbenthos, mayflies, stoneflies and Diptera across basket samples from 2000 and 2005. This analysis examined the effect of year and location on samples from upstream and downstream of the spill location. A single-factor, two-way ANOVA was used to compare abundance within 2005 baskets samples prior to using Scheffe's pairwise comparisons among the four sites. ANOVA calculations were completed with SYSTAT 11 software, using log-transformed densities and log-transformed biomass results for the major groups of invertebrates collected in colonization baskets (Appendix 6). The log transformations were done to meet the ANOVA assumption that the effects of the factor levels must be additive and to convert a potentially skewed distribution to one more symmetrical around the mean.

4.0 Results

4.1 Kick Net Samples

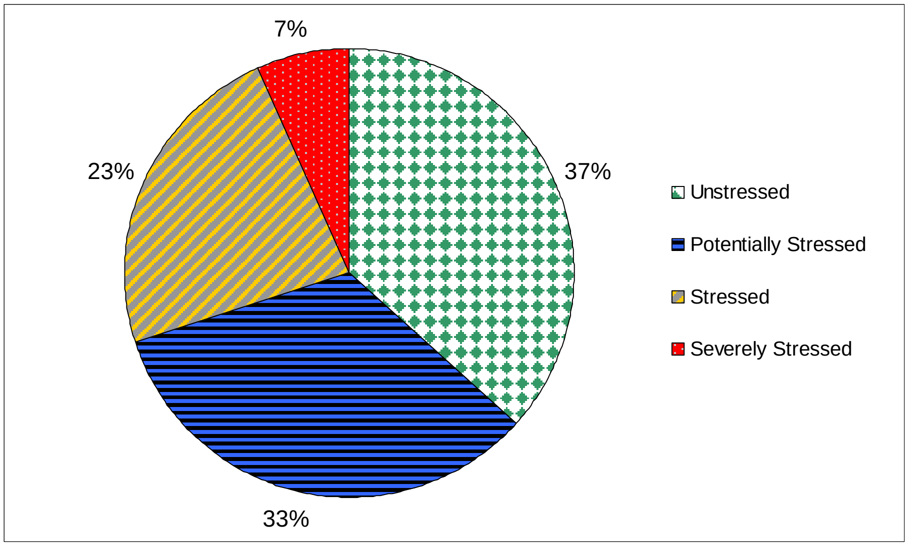
4.1.1 Comparison to Reference Condition

For the data analysis of kick net samples the RCA was used in the CABIN analytical tools such that each sample per location, per date was treated as a separate sample in all data analyses (*e.g.*, Site CHK01, Week 1 was a single site/data point). This allowed for comparisons over time and space (*e.g.*, upstream/downstream).

The CABIN analytical tools determined all of the 2005 kick net samples belonged in "Group 1" of the Reference Site database, with the median probability of membership being 83.1% (range = 63.2 to 99.5%). This is similar to results from Sept to Oct 2005 sampling (3 sites) by EC which reported a median probability of 85% (range = 76.8 to 96.9%) (Sylvestre 2005, unpublished data) and the range reported by Stamford *et al*. (2007) (range = 66.1 to 96.4%) for the same sites. The upstream control site (CHK01) sampled by Triton over a period of nine (9) weeks had an average Group 1 membership probability of 69% and was the lowest probability of all sites sampled. Since Group 1 membership probability of the control site was lower than other sites, this suggests differences between the control and affected sites may be related to factors other than the spill (*i.e.*, it may have site-specific environmental conditions which inherently separate it from average parameter values found in Group 1 streams).

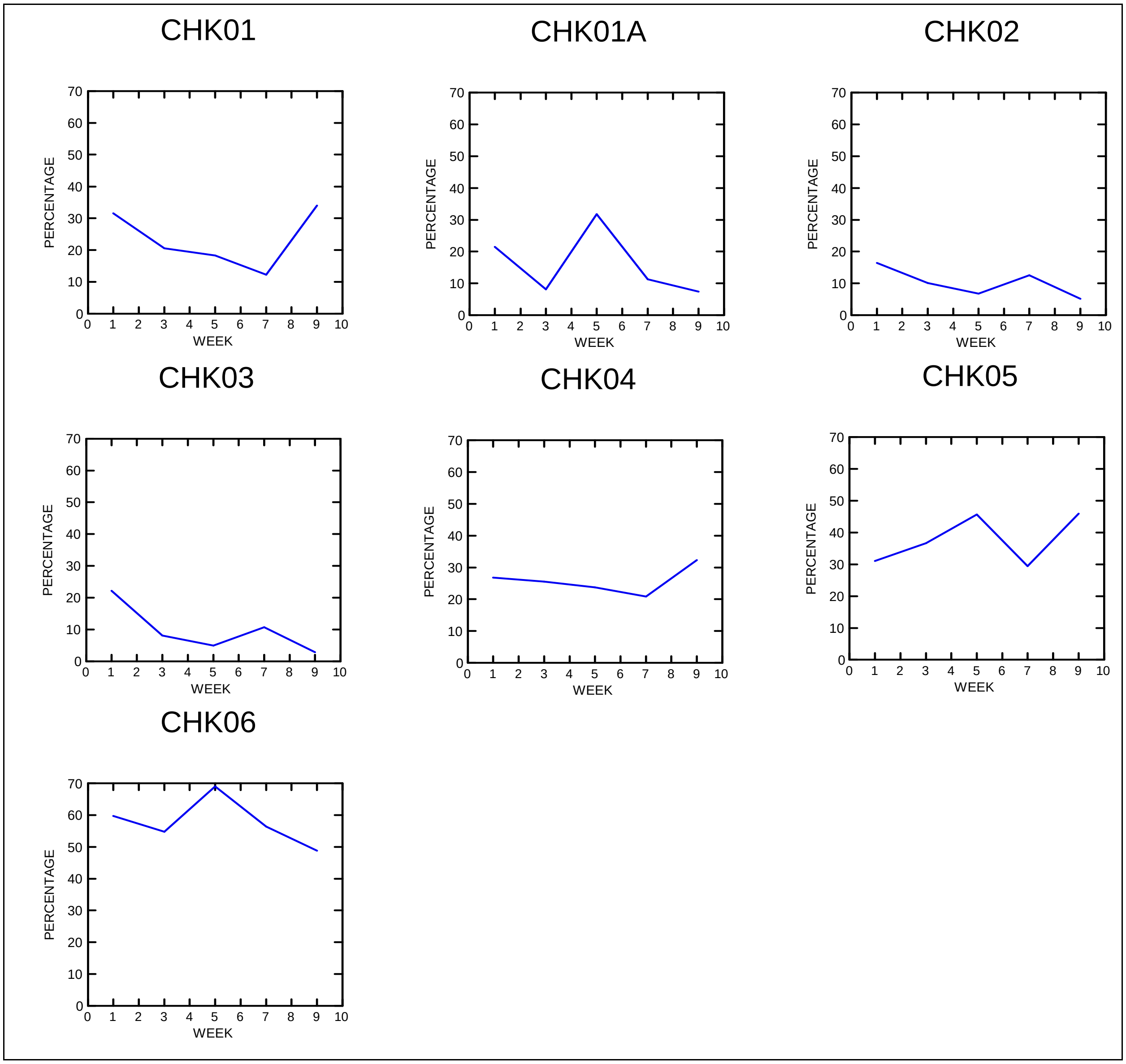
The majority of sites (70% of all samples) fell within the "unstressed" (37%) and "potentially stressed" (33%) categories (Figure 4-1). Two samples (7%), CHK02 Week 5 and 9, were considered "severely stressed" and 23% of samples were "stressed". The control site had a median rating of "potentially stressed".

Figure 4-1. Cheakamus benthic invertebrate community sample ratings compared to reference sites in the Georgia Basin (N = 35)



The similarity between the reference sites and the post-spill kick net samples was further assessed by plotting the percent similarity using the Bray-Curtis distance measure (Figure 4-2). The site downstream of the Cheekye River confluence was found to be most similar to the reference condition, although there was no consistent trend of downstream sites becoming more or less similar to the reference condition in the weeks following the spill (Figure 4-2). Compared to the upstream control over the same time period, it is evident some downstream sites were less similar (CHK02, 03) and some more similar to the reference condition (CHK05, 06).

Figure 4-2. Bray-Curtis (BC) similarity coefficient (% Similarity) between invertebrate assemblages in kick net sites and Environment Canada reference sites



Notes: 1) Samples with higher percent similarities are more similar to the Reference Condition than sites with lower percent similarities. B-C Similarity 100% = two samples are identical, 0% = two samples have no species in common (*e.g.*, samples with higher B-C % similarity are less stressed). Note CHK01 is Control site.

At the control site (CHK01), the invertebrate community displayed notable variation in relation to the Reference Condition. Results from the week of the spill (Stamford *et al*., 2007) and this study were also compared in terms of their Bray-Curtis distance to the Group 1 Reference Condition. This comparison showed week-to-week variation in the benthos community of >100% (<15% vs. >30% similarity). This variability at the control site suggests factors unrelated to the spill can also result in substantial difference in the community structure observed in collected samples.

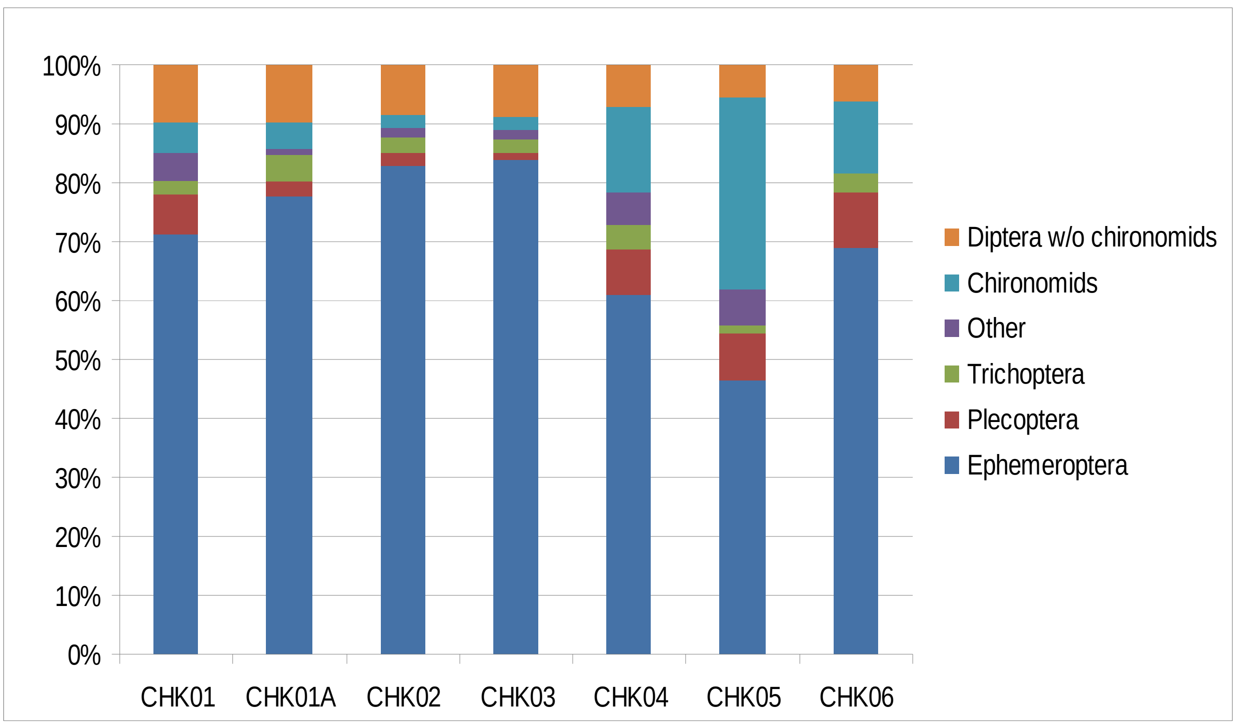
4.1.2 Community Structure and Reference Condition Approach

Mayflies (Ephemeroptera) constituted the majority of invertebrates collected at each site and in all cases except one they represented >60% of the total abundance (Figure 4-3).

When averaged over all sampling periods, some invertebrate groups were found in fewer numbers in downstream sites compared to the control site, including:

- Mayfly – Family: Heptageniidae; Genus: Rhithrogena; upstream control average per kick net sample = 76 (N = 5); downstream sites average = 6.5 (N = 25).
- Stonefly – Family: Chloroperlidae; Genus: Sweltsa; upstream control average per kick net sample = 152 (N = 5); downstream sites average = 11 (N = 25).

Figure 4-3. Percent composition of individuals from insect groups in kick net samples



Note: All samples were collected over nine weeks, beginning August 20th.

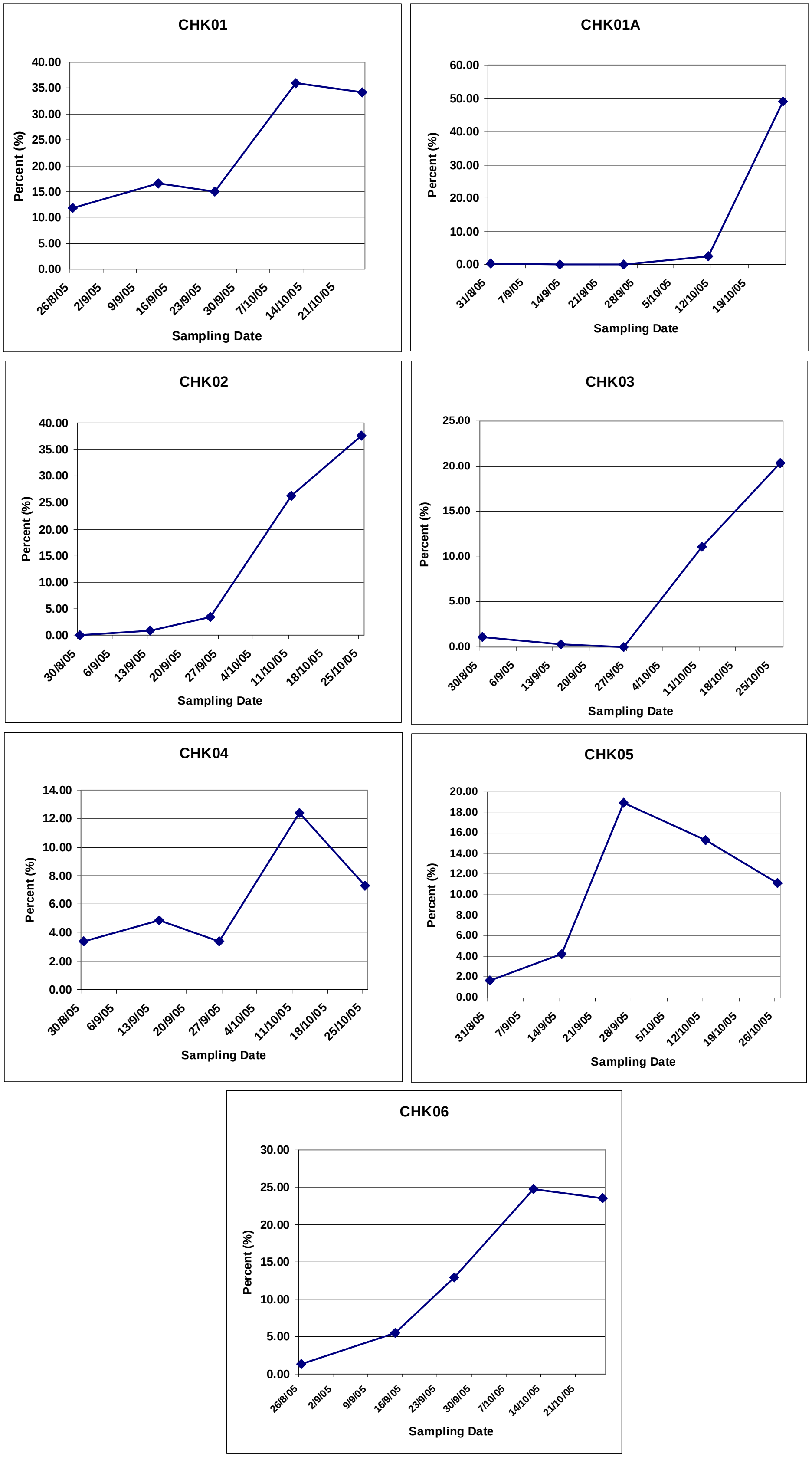
Further comparisons across taxa groups also revealed some differences in average abundance (*i.e.*, across all dates). At the Family level there were some abundance differences not observed at the Order level. The two taxa with the greatest upstream/downstream difference were a stonefly (Family: Chloroperlidae; Genus: *Sweltsa*) and a mayfly (Family: Heptageniidae; Genus: *Rhithrogena*) (Table 4-1). To provide further context for this variation, the Family and Order levels of these taxa were examined to see if this represented a systemic decline across all mayflies in the Family Heptageniidae or if it was limited to the Genus *Rhithrogena*. For the 2005 kick net samples, the median of the percent Mayflies which were Heptagenids at the CHK01 control site was 16.6% (range = 11.8 to 35.9%, N = 5) while the median for sites CHK02 to CHK05 was 4.5% (range = 0 to 37%, N = 20). An examination of how Heptagenid abundance changed over the sampling period revealed the relative abundance of Heptagenid in downstream samples increased consistently over the nine weeks of post-spill kick net sampling (Figure 4-4) whereby the median at week 9 was 15.6% for sites CHK02 to CHK05 (range = 7.3 to 37.6%, N = 4). Heptagenid relative abundance after nine (9) weeks was comparable to the unaffected control site (16.6% vs. 15.6%) and may be one of the clearest examples of recovery in the benthic invertebrate community. Results from the initial weeks of kick net sampling in this study agreed with the results reported by Stamford *et al.* (2007) where downstream sites had low Heptagenid abundance. These results supplement the single sampling event by Stamford *et al*. (2007), and suggest re-colonization of downstream sites was occurring shortly after the spill. It should be noted the control site also showed increased abundance with time, and part of the increasing abundance trend in downstream sites might be explained by natural increases in this mayfly family that take place from summer to fall.

Table 4-1. Average number of mayflies and stoneflies in sites upstream and downstream of the spill

Taxanomic Level	Taxa Name	Average # +/- SD per kick net sample		Average # +/- SD per colonization basket sample	
		Upstream	Downstream	Upstream	Downstream
Common Name	Mayfly				
Order	Ephemeroptera	135+/-354 (N=80)	169+/-723 (N=480)	614+/-487 (N=20)	1295+/-467 (N=20)
Family	Heptageniidae	94+/-218 (N=30)	73+/-353 (N=353)	472+/-501 (N=20)	460+/-278 (N=20)
Genus	*Rhithrogena*	76+/-67(N=5)	6+/-24 (N=30)	54+/-75 (N=20)	13+/-11 (N=20)
Common Name	Stonefly				
Order	Plecoptera	16+/-44 (N=65)	8+/-27 (N=390)	66+/-52 (N=20)	297+/108 (N=20)
Family	Chloroperlidae	51+/-83 (N=15)	5+/-12 (N=90)	46+/-34 (N=20)	11+/-10 (N=20)
Genus	*Sweltsa*	152+/-59 (N=5)	11+/-18 (N=30)	n/a	n/a

Note: Average # of individuals from kick net samples across all sample dates. Average # of individuals in colonization baskets is based upon two upstream sites, each with five replicate baskets, and two downstream sites each with five replicates. Colonization baskets represent a single collection date.

Figure 4-4. Percent of total mayflies which were Heptageniidae in kick net samples.



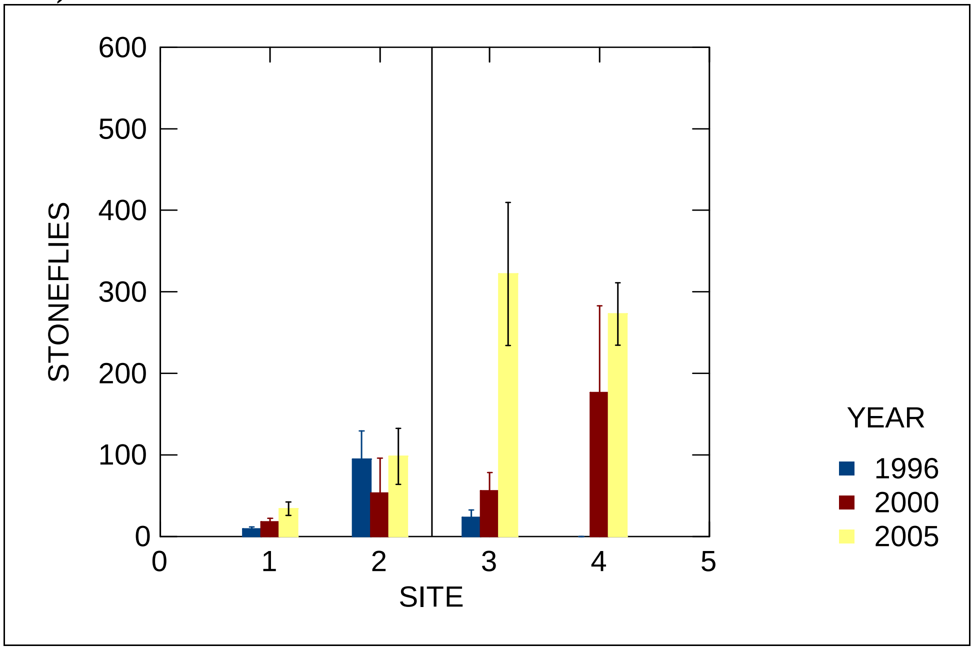
The colonization basket abundance of Heptagenids had less relative variability around the mean (*i.e.,* more reliable measure) and showed greater similarity between the upstream and downstream Heptagenids (472 vs. 460 per sample). At the Order level, mayflies were in greater average abundance in downstream samples (kick net and basket) compared with those upstream of the spill.

For stonefly taxa, Genus: *Sweltsa*, downstream kick net samples had fewer individuals compared with the upstream control. This trend was consistent at the Family and Order levels, and of all taxa measured it represented the greatest difference in upstream vs. downstream abundance. The Order-level trend (downstream sites had less abundance of individuals in Plecoptera Order than upstream site) is also supported by analysis of variance results from the basket biomass data (Section 4.2.2; Appendix 6) for stoneflies. The biomass variation across sites was not significantly affected ($P > 0.05$) by interaction of year and location, and both the year and location effects were found to be significant ($P < 0.05$).

Similar upstream vs. downstream spill comparisons were done for the samples collected from colonization baskets. These comparisons are considered a more accurate representation of invertebrate density because the sampling technique is a more standardized effort compared with kick net sampling. By using a defined area for each basket and completely removing the basket for processing, the basket approach provides more precise quantification than other methods. Furthermore, replication of baskets at each site ($N = 5$) accounted for the possibility of patchy species distribution. A similar trend observed in kick net samples for *Rhithrogena* mayflies was observed in the baskets, whereby the Genus-level had more individuals of *Rhithrogena* in upstream samples, and the Family level had comparable abundance. This trend was reversed at the Order level and downstream basket samples had the greatest abundance (Table 4-1).

Individuals of the stonefly, Genus: *Sweltsa*, could not be practically distinguished from another stonefly group, Genus: *Suwallia*, in the colonization basket samples. These two Genera are the only identified taxa groups within the Chloroperlidae Family of stoneflies sampled, and have similar physical features. With Chloroperlid stoneflies, the observed upstream/downstream trend of the kick net sample (*i.e.,* more abundant upstream) was further corroborated (Table 4-1). However, at the Order level, stoneflies were in greater average abundance in downstream basket samples (66 vs. 297, Figure 4-5). This was a result of the high number of individuals (*i.e.*, hundreds per basket) from the stonefly Family Capniidae at both downstream sampling sites. The Capniidae family of stoneflies is made up of detrivores and shredders, whereas the other stonefly Families found in Cheakamus River samples are predators. It is clear *Rhithrogena* and *Sweltsa* were less abundant in downstream samples; however this trend varied at higher taxonomic levels (*e.g.,* Family and Order) (Table 4-1) and among sampling methods.

Figure 4-5. Density (individuals/sample) +/- SE of stoneflies collected in colonization baskets (N = 5)

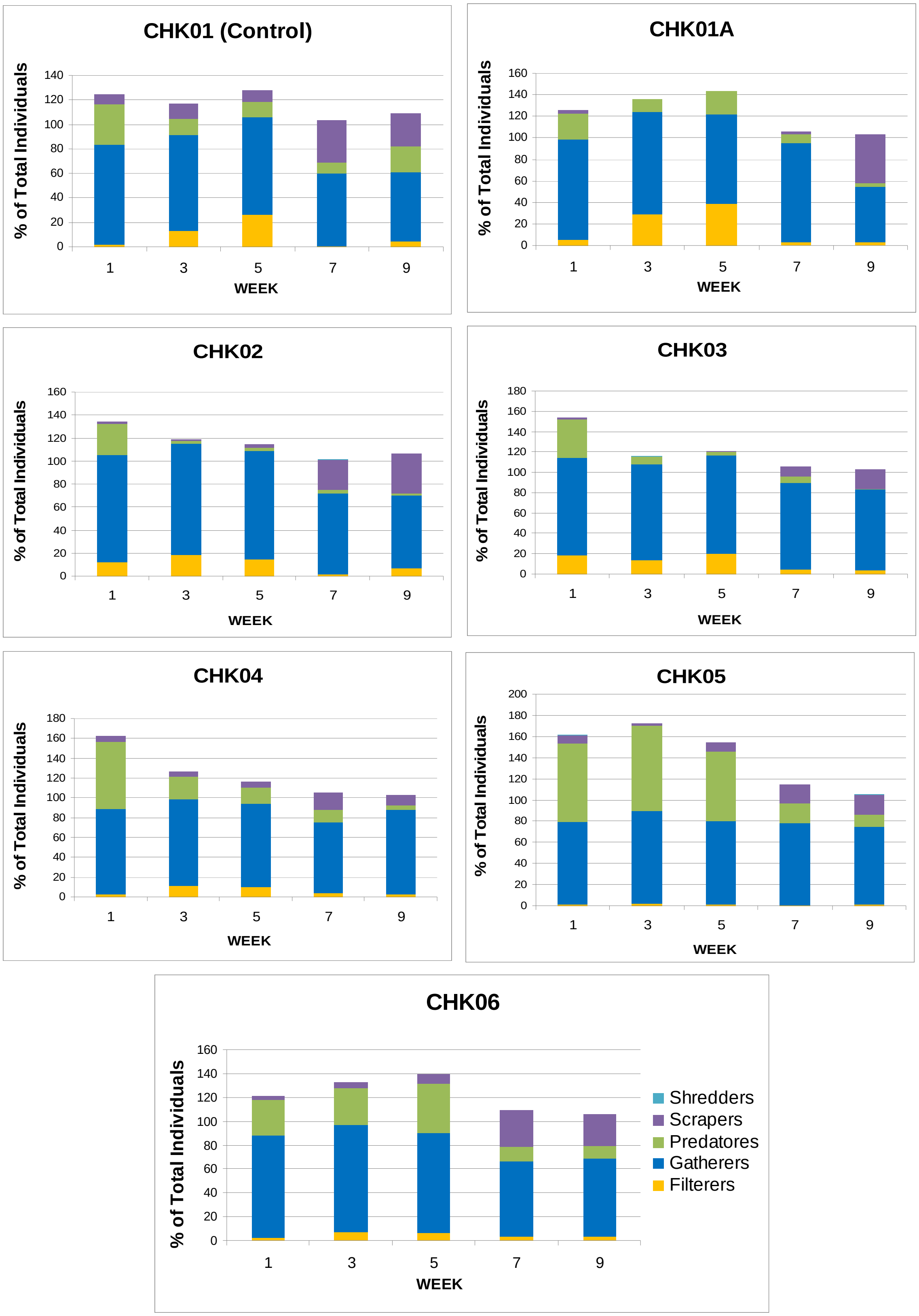


Notes: u/s of spill: Site 1 = d/s Daisy Lake Dam and Site 2 = Sandsheds; u/s of spill: Site 3 = u/s Culliton and Site 4 = d/s Cheekye. Areal density (number/m^2) can be calculated by multiplying total per sample by 25. Vertical line denotes the relative location of the NaOH spill.

Functional feeding groups (*i.e.*, shredders, predators, gatherers, clingers, scrapers and filterers) and their relative composition in kick net samples were reviewed over the five sampling sessions. The gatherers group (*e.g.*, mayfly Family: Baetid) were the most common group in all the kick net samples (Figure 4-6). The predator group appears to be more common in the three most downstream sites (CHK04, 05, 06). However, this may also be an artefact of the CABIN analytical tools classifying certain taxa groups as both gatherers and predators (see Section 3.0), which is reflected by the bars in the graph totalling >100% relative abundance (Figure 4-6). Most of the downstream sites also showed a declining percentage of predator composition over the sampling period. The percentage of predators at the upstream control site (CHK01) had a similar declining trend over time, with the exception of week nine (9) when the percentage of predators increased to approximately 20% of the total sample (Figure 4-6). Across all inter-site and inter-week comparisons of percent composition (Orders or functional feeding groups) in kick net samples there is only one sample per date, per site. The CABIN protocol ((Reynoldson *et al.*, 2001b) for comparing test sites to the Reference Condition database only requires a single sample per site because the inter-sample variability is accounted for in the database of reference site data. Comparisons using the 2005 kick net data which do not use the RCA have no sample-specific estimate of variation.

Several other groups were also found to be in greater relative abundance in downstream sites compared with the control site (CHK01). Site to site variation of instream conditions and tributary influences should be considered when explaining variations in feeding group relative

Figure 4-6. Percent composition of functional feeding groups, in kick net samples



abundance. For example, the sites located downstream of, and in close proximity to large tributary influences (i.e., CHK04 and Culliton Creek, CHK06 and Cheekye River) are expected to receive more immigration from downstream drift than sites upstream of these tributary influences (*e.g.,* CHK03). The abundance and biomass results from the colonization baskets, discussed in Section 4.2, provide more precise estimates of post-spill invertebrate quantities than kick net samples because the sampling area is more defined and there is greater replication (N = 5 vs. N = 1).

4.2 Colonization Baskets

Invertebrates collected in colonization baskets in 2005 comprised many of the same taxa groups as found in the kick net samples. In general, mayflies were dominant with the exception of baskets downstream of the Cheekye which contained approximately 15% mayflies. There was a greater percentage of mayflies in the 2005 samples compared to 2000 (Perrin, 2001, Figure 4-7). In sites 1 and 2 (upstream of the spill) the colonization baskets from 1996 had 60 to 70% mayflies followed by <20% in 2000 and 80 to 85% in 2005 suggesting the natural inter-annual variation of mayflies can be greater than 100% (Figure 4-7).

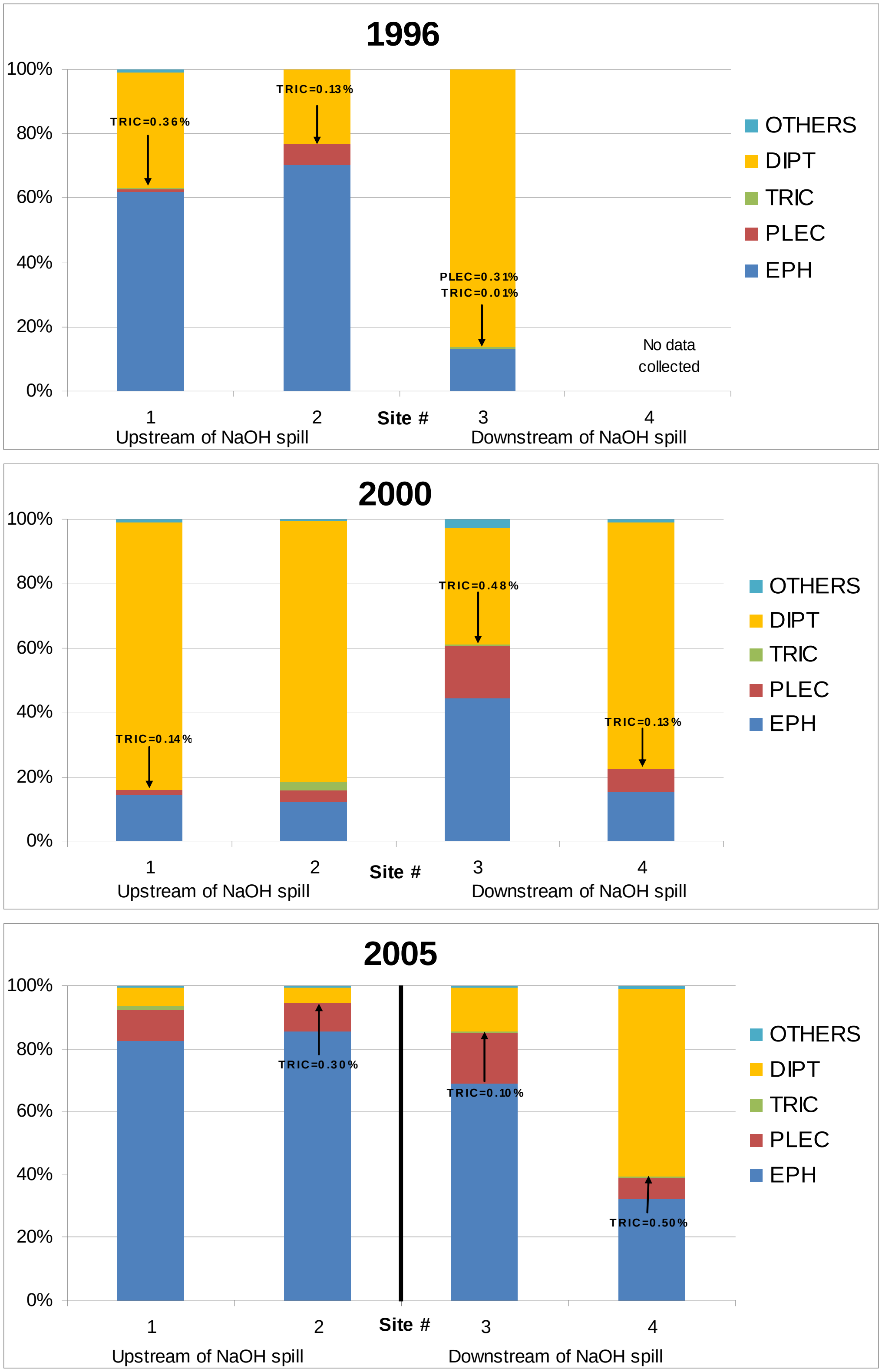
4.2.1 Community Structure

The dominant taxa groups from the 2005 colonization baskets were compared to data collected in 1996 and 2000 from the same sites (Figure 4-7). In upstream sites, there was variation in community structure among years. The mayflies were <15% of the total density in 2000, yet they comprised >80% of the 2005 benthos samples. Downstream of the spill, mayflies and species of the Chironomidae Family (Order: Diptera) made up the majority of invertebrates in the 2005 samples. The site downstream of the Cheekye confluence (Site 4) showed similar community structure in 2000 and 2005. In contrast, the site upstream of Culliton Creek (Site 3) had a majority of mayflies with the remainder being chironomids in 2005 and nearly the opposite trend in 1996.

The multivariate data analyses also indicate year to year differences in the community structure as shown in the non-metric multi-dimensional scaling (MDS) plot from the Bray Curtis similarities (Figure 4-8). These results indicate invertebrate community structure can change over time in sites above and below the spill. The MDS plot shows 2005 benthos community samples from baskets upstream and downstream of the spill to be more similar to themselves than to the 2000 or 1996 samples. This 2005 grouping is shown on the left half of the MDS plot (Figure 4-8) with the space between this group and the rest of the samples showing the distinction between sampling years. Spill-affected samples form the top half of the 2005 group on the MDS plot. It is not clear why all the 2005 samples are so different than previous year communities, although the 100 year flood event in October 2003 (BCCF, 2003) would have affected benthos communities, and may be one explanation contributing to the observed differences between 2000 and 2005 samples.

Given that year of sampling has a clear effect, multivariate analysis of the basket sample communities upstream versus downstream of spill was tested for similarities within 2005, 2000 and 1996 samples. The one-way Analysis of Similarities procedure (Clark and Gorley, 2006)

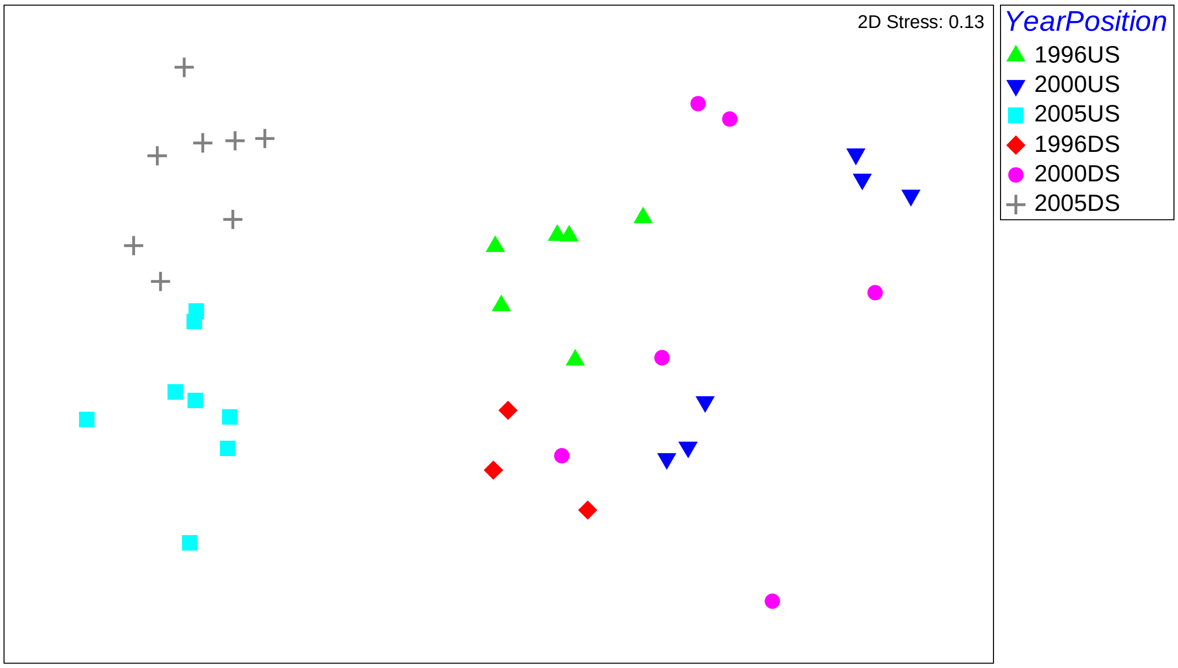
Figure 4-7. Percent composition of invertebrate taxa in colonization baskets



Data collected from colonization baskets in 1996, 2000 (Perrin, 2001) and 2005. DIPT=Diptera, TRIC=Trichoptera, PLEC=Plecoptera, EPH=Ephemeroptera. Vertical line denotes relative location of spill. Site 1 = d/s Daisy Lake Dam; Site 2 = Sandsheds; Site 3 = u/s Culliton; Site 4 = d/s Cheekye.

indicated communities from baskets upstream of the spill site were significantly different than downstream in all three sampling years. The upstream versus downstream difference was greatest in 2005 (P = 0.001), followed by 1996 (P = 0.024) and 2000 (P = 0.032). It is not clear if the greater significance of upstream versus downstream differences in 2005 is due to the effects of the spill or if they are a function of the greater number of samples collected in 2005 (N = 20) versus 2000 (N = 12) and 1996 (N = 12).

Figure 4-8. MDS plot of benthic invertebrate communities from colonization baskets



Note: Non-metric multidimensional scaling (MDS) plot of the Log (X+1) transformed abundance data for all invertebrate taxa from 1996, 2000 (Perrin 2001), and 2005 samples from upstream (US) and downstream (DS) of the spill site. Dissimilarity measure used is Bray-Curtis. Stress value = 0.13

4.2.2 Abundance

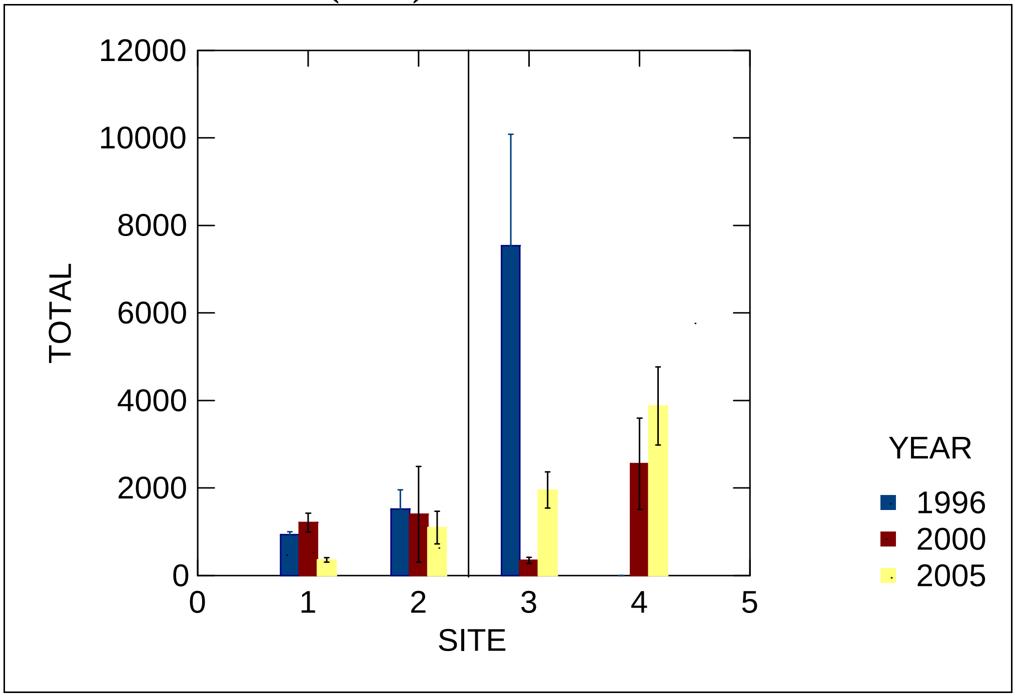
In 2005 the density of invertebrates in colonization baskets increased in sites further downstream Figure 4-9 and Figure 4-5). Year to year variation of within-site abundance at the Daisy Lake Dam and Culliton Creek sites also showed a change of >100% in invertebrate abundance can occur without the influence of a spill.

The variability (*i.e.*, coefficient of variation) of the 2005 basket sample density was found to be similar to the variability observed in 1996 and 2000 basket samples in upstream and downstream sites (Table 4-2,
Figure 4-9).

Table 4-2. The coefficient of variation for invertebrate abundance for 1996, 2000 and 2005 basket samples (N = 5)

SITE	1996	2000	2005
d/s Daisy Lake Dam	0.092	0.233	0.234
Sandsheds	0.375	1.022	0.570
u/s Culliton	0.443	0.262	0.354
d/s Cheekye	No data	0.534	0.385

Figure 4-9. Density (individuals/sample) +/- standard error (SE) of benthic invertebrates collected in colonization baskets (N = 5)



Note: Site 1= d/s Daisy Lake Dam and Site 2= Sandsheds; Site 3= u/s Culliton and Site 4=d/s Cheekye. Density (number/m^2) can be calculated by multiplying total per sample by 25. Vertical line denotes the relative location of the NaOH spill.

Two-way, single factor Analysis of Variance (ANOVA) was completed with SYSTAT 11 software (Systat Software Inc., 2004) to compare the variance of invertebrate abundance (*i.e.*, number of individuals) in samples collected from the four basket sites in 2005. The null hypothesis (H_0) that the means across all sites were equal was rejected, with the probability (*P*) of committing a Type I error (α) being <0.001 (F = 19.12; $F_{0.05,(2), 3, 12}$ = 3.86). This two-tailed ANOVA was also run on the basket data from 2000, whereby the intra-year abundance across all sites was compared and again found to be significant at *P* <0.05. Pairwise comparisons were then conducted to determine which sites were more or less similar based on intra-year abundance.

The results of pairwise comparisons (Table 4-3) showed the probability of the Sandsheds site (Figure 1-2, upstream of spill) and the Culliton Site (downstream of spill) being equal is 0.278, which is the same probability as that of the Cheekye site (downstream of spill) vs. the Culliton site. These pairwise comparisons suggest a linear trend in site similarity in which invertebrate abundance changes along with the site location distance downstream.

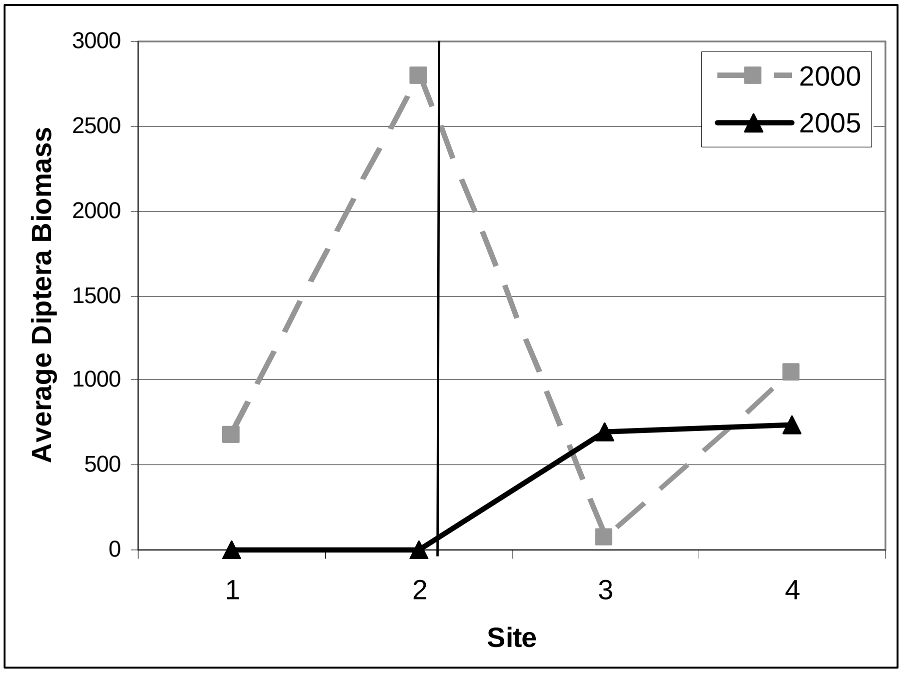
Table 4-3. The probabilities from Scheffe's pairwise comparisons of the invertebrate abundance from 2005 basket samples

SITE	Daisy Lake Dam	Sandsheds	U/S Culliton	D/S Cheekye
Daisy Lake Dam	1			
Sandsheds	0.064	1		
U/S Culliton	0.002	0.278	1	
D/S Cheekye	0.000	0.011	0.278	1

The variation in density of mayflies and chironomids was tested against the effect of year and location using ANOVA. The interaction of year and location effects was found to be significant ($P < 0.05$) and hence the effects of year and location could not be examined in isolation. Also, these year and location effects on mayflies and dipterans were plotted for the 2000 and 2005 samples from colonization baskets (Figure 4-10 and Figure 4-11). If the spill alone was responsible for the variation in density, it is unlikely the interaction between year and location would remain statistically significant due to the fact it would only affect 2005 samples.

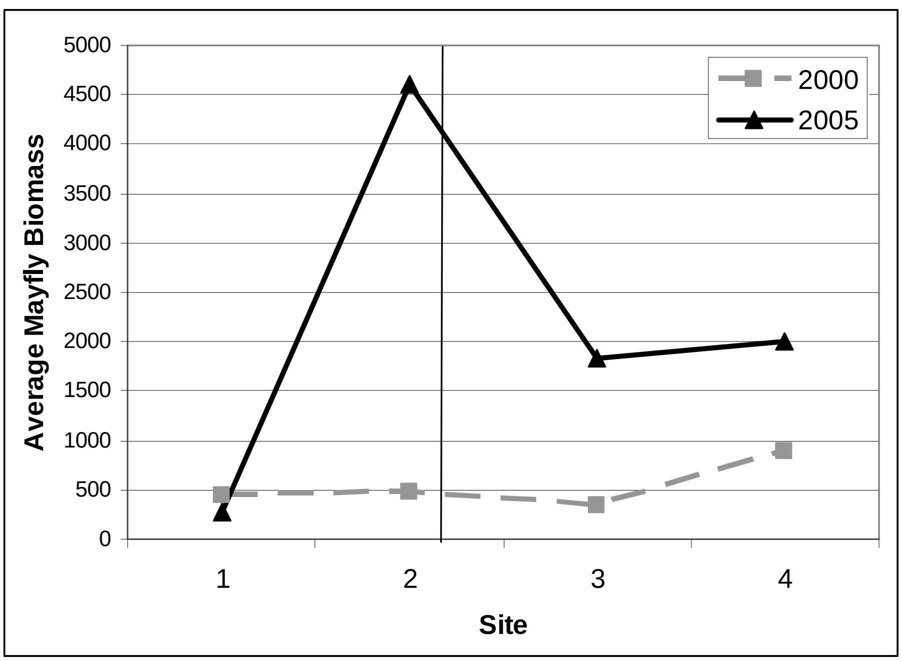
Benthic invertebrate community structure in kick net samples was further examined using the Bray-Curtis distance from the calculation of the similarities in the ANOSIM procedure (Section 3.0). A dendrogram plot of the similarities was constructed using the Site Blocks (Figure 4-12). The results show the control site (CHK01) benthos (across all dates) was distributed in several different clusters without clear separation from other Blocks of sites exposed to NaOH (Figure 4-12). The sites located downstream of the major tributaries (Site Block 3) were observed to cluster together. In the ANOSIM procedure, a test of the null hypothesis that there was no difference between Site Blocks was completed. The ANOSIM test statistic, R, would be near zero if there were no differences between Site Blocks. When all pairwise comparisons are considered, the overall test statistic is called the “Global R”. In the Cheakamus invertebrate abundance across Site Blocks, the Global R was just -0.001 ($P > 0.46$, 999 permutations), meaning there was no suggestion of effect between the Site Blocks in the Global Test.

Figure 4-10. Average (N = 5) biomass of Diptera insects collected in colonization baskets



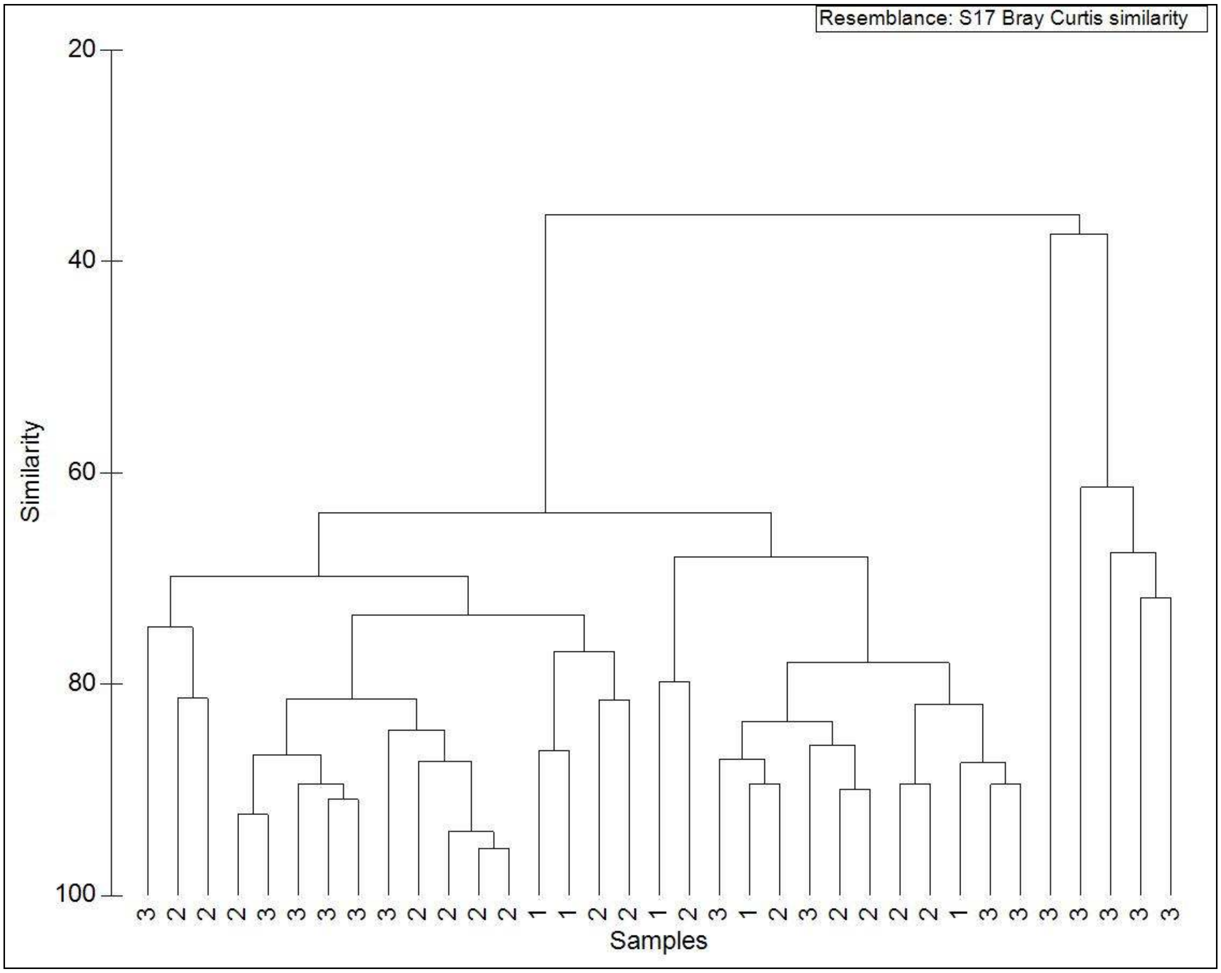
Site 1= d/s Daisy Lake Dam and Site 2= Sandsheds; d/s of spill: Site 3= u/s Culliton and Site 4=d/s Cheekye.
Note: vertical line denotes relative location of the spill.

Figure 4-11. Average (N = 5) biomass of Mayfly (Ephemeroptera) insects collected in colonization baskets.



Site 1= d/s Daisy Lake Dam and Site 2= Sandsheds; d/s of spill: Site 3= u/s Culliton and Site 4=d/s Cheekye.
Note: vertical line denotes relative location of the spill.

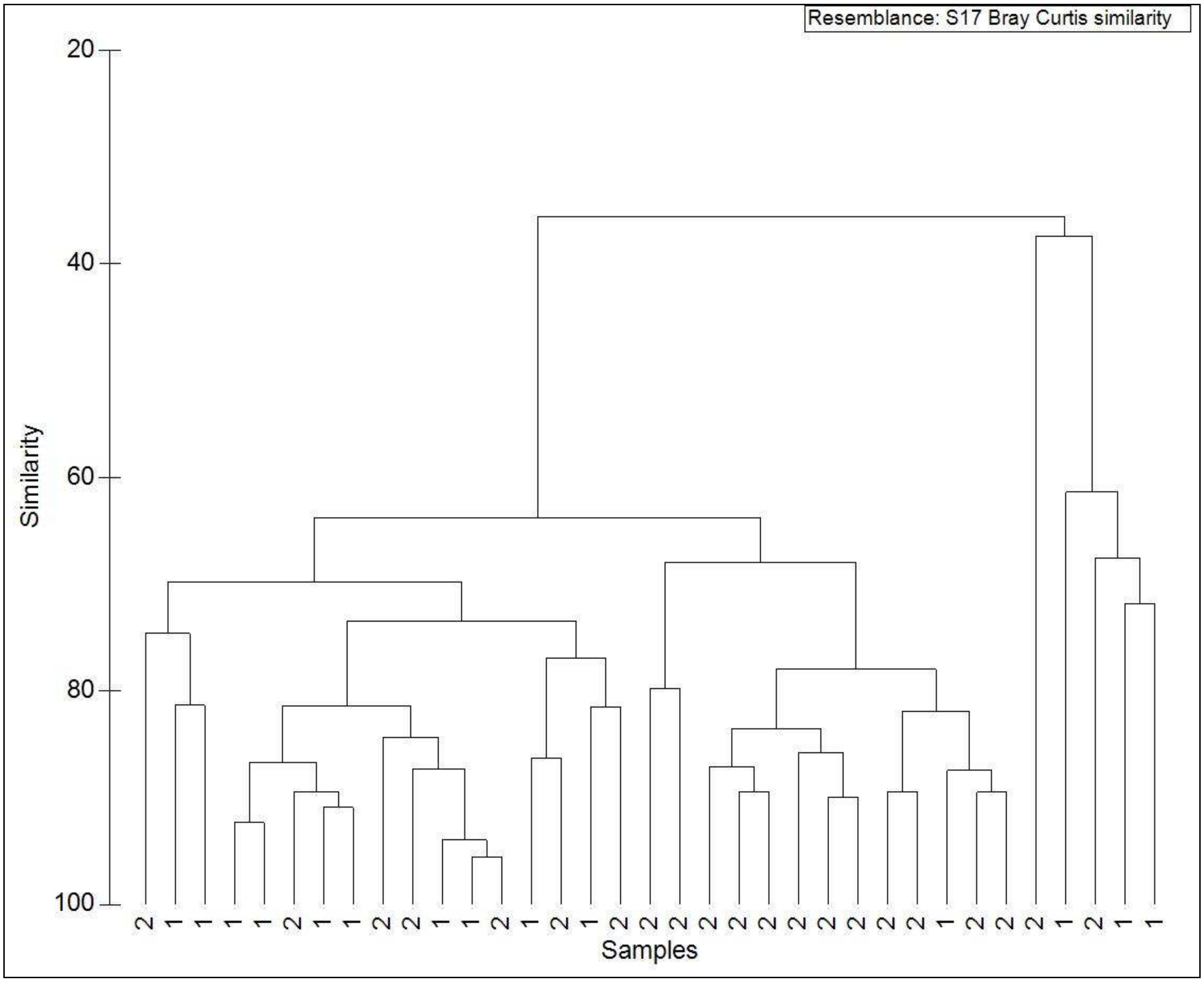
Figure 4-12. Dendrogram plot showing Site grouping of 2005 Cheakamus River benthos kick net samples using the Bray-Curtis distance measure of sample similarities.



Note: Sample labels indicate Site Block # [1 = CHK01 (Control – upstream of the spill); 2 = CHK01A, 02, 03 (downstream of the spill but upstream of Culliton Creek); and 3 = CHK04, 05, 06 (downstream of Culliton Creek)]. Samples are clustered based on the group average whereby any one cluster group should not contain less than 10% of the total number of samples.

To study the timing of sampling effect using Bray-Curtis distance measure from the group average, a second dendrogram of Week Blocks was plotted (Figure 4-13). This is a similar dendrogram structure as Figure 4-11, however sample labels are by Week Block. There are three basic groups formed in this dendrogram; one of the groups is dominated (*i.e.*, 12 of 13 samples) by samples from the last three sampling sessions. Overall the effect of the Week Block factor (Global R = 0.027 (P >0.28) was greater than the Site Block effects [Global R = -0.001 (P >0.46)]. Based on these results, there appears to be more differences among 2005 Cheakamus benthos from the first two sampling sessions (*i.e.*, Week Block 1) and the last three sampling session than the differences among sampling sites. In essence, the effect of time was greater than location when measuring differences among benthos abundance from samples collected after the NaOH spill.

Figure 4-13. Dendrogram plot showing sampling period grouping of 2005 Cheakamus River benthos kick net samples using the Bray-Curtis distance measure of sample similarities.



Note: Sample labels indicate the post-spill sampling date by Week Block # (1 = first two sampling sessions; 2 = last 3 sampling session) across all sites. Samples are clustered based on the group average whereby any one cluster group should not contain less than 10% of the total number of samples.

Using the resemblance matrix of Bray-Curtis similarities, a two-way Analysis of Similarities (SIMPER) procedure was run for the 2005 kick net benthos sample data using the *PRIMER-E* software. The contribution of the taxa groups (*i.e.*, top five families for percent abundance) to the group similarity was cut off at 90% (*i.e.*, once 90% of the group similarity was explained, no more taxa groups were compared). The output of the ANOSIM and SIMPER procedures with pairwise comparisons needed to construct the dendrogram plot is provided in Appendix 7. Some key points of the SIMPER results as related to the spill are:

- Baetid mayflies explained the majority of the similarities across Week Blocks for the Control (CHK01) as well as spill affected sites;
- The average dissimilarity between impacted Site Blocks was higher than the average

dissimilarity between the Control and the two impacted Site Blocks, across all weeks; and,

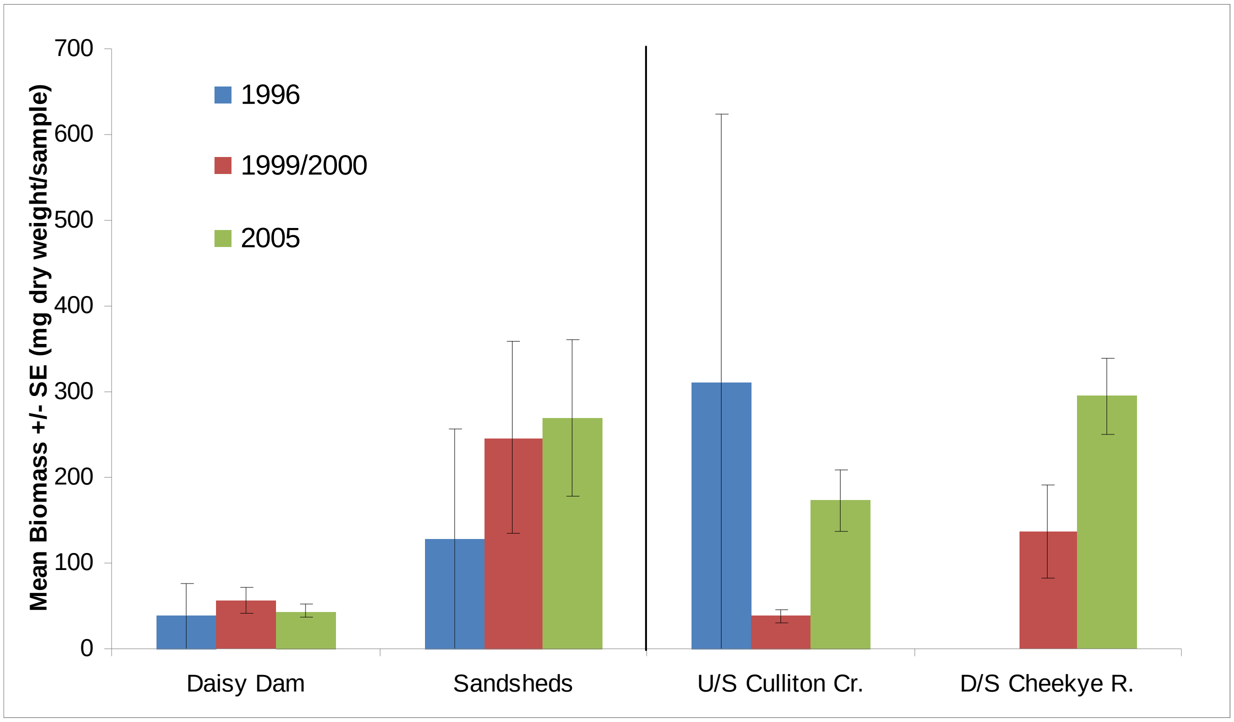
- Heptagenid mayflies stand out as taxa contributing to the composition of the control site benthos community across all weeks, however these mayflies had less influence on the composition of impacted sites.

4.2.3 Biomass

The biomass (dry weight) of invertebrates from colonization baskets was examined to determine if different taxa types showed any differences in mass between sites (Figure 4-14 and Figure 4-15) compared to previous studies (Perrin 2001). Downstream sites across all years had a higher proportion of species in the Chironomidae Family (Order: Diptera) than sites upstream of the spill (Figure 4-7) and because chironomids were smaller than mayfly, caddisfly, and, stonefly individuals, the mass per individual is lower in downstream sites. Site 2 had a higher proportion of mayfly/stonefly/caddisfly individuals across all years and hence its mass per individual is the greatest of all sites. This variation in mass per individuals points to the importance of looking beyond density when assessing the available food for invertebrate predators (*e.g.*, salmon and trout).

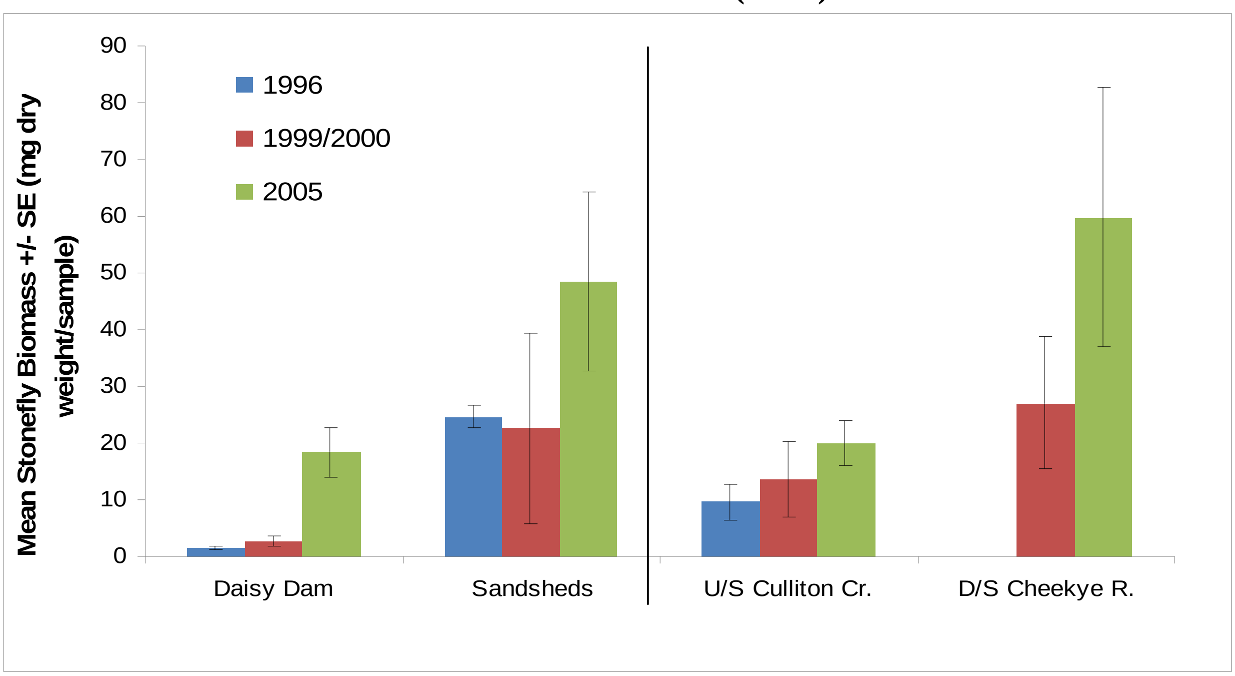
Biomass variation among colonization baskets was also tested using a two-factor, one way ANOVA (Appendix 6) and as with density, the effect of the interaction between year and location on mayfly and diptera biomass variation was found to be significant (P = 0.019, 0.019 respectively). In addition, the microbenthos (*i.e.*, benthic invertebrates with a maximum dimension < 1mm) variation related to year effect was not considered to be related to the spill because of the significant interaction between the year and location variables. In contrast, stoneflies did not have a significant interaction term (P = 0.427) and the effect of year and location was found to be significant (P = 0.001 and 0.01, respectively). This suggests stonefly biomass (averaging ~15% of total biomass in 2005 samples) varied in biomass due to factor(s) related to year to year or location differences. In the case of stoneflies, the 2005 samples had significantly greater stonefly biomass than 2000 samples in upstream and downstream sites.

Figure 4-14. Mean Biomass +/- SE (mg dry weight per sample) of benthic invertebrates collected from Cheakamus River colonization baskets (N = 5).



Note: Vertical line denotes the relative location of the NaOH spill. Biomass per unit area (mg dry weight/m^2) can be calculated by multiplying biomass per sample by 25.

Figure 4-15. Mean Stonefly Biomass +/- SE (mg dry weight per sample) of samples collected from Cheakamus River colonization baskets (N = 5).



Note: Vertical line denotes the relative location of the NaOH spill.

5.0 Discussion

Comparison to the Georgia Basin Reference Condition

The reference sites of the Georgia Basin have not yet been compared to an episodic disturbance such as a NaOH spill. However, the RCA tools are sensitive enough to detect deviations in communities represented by mass mortalities or missing taxa groups in the Cheakamus River. In fact, the same upstream control site had both an "unstressed" and "potentially stressed" rating on different dates over the sampling period. A benthos mortality rate commensurate to the scale of the fish kill (McCubbing *et al*., 2006) would be expected to have resulted in most or all downstream sites receiving a "severely stressed" rating in the BEAST RCA tool. The data collected in this study did not indicate a consistent "severely stressed" rating, but instead the median rating for downstream sites was "potentially stressed", which is similar to the median rating of the upstream control.

Based on results from RCA and BEAST analyses the Cheakamus River may not have been in an "unstressed" condition in all locations prior to the spill. The results of this study show the control site had an overall BEAST rating of "potentially stressed" and results from Stamford *et al*., (2007) also showed evidence of stress at this location using the same analyses. The presence of >75% mayflies/stoneflies/caddisflies (EPT taxa) in kick net samples may have contributed to the two instances of "severely stressed" ratings (Figure 4-1), but these taxa groups are not a traditional indicator of disturbance. During the week of the spill, Stamford *et al.* (2007) also collected samples with a high abundance of Ephemeropterans. EPT taxa are typically used as a metric having a positive relationship with good water quality (Lenat, 1988), hence their high abundance in the week of the spill and in this study corroborates information from other studies indicating toxicity did not persist after 48 hours (McCubbing *et al*., 2006; Triton, 2007a; and Triton, 2007b) and these groups were able to recover rapidly.

Post-Spill Community Similarities

The multivariate comparison of post-spill kick net samples did not suggest there was a greater difference between control and downstream samples than the difference observed among downstream sites. The dissimilarity among impacted sites as compared to their contrast with the control site suggests in-river variability (*i.e*., site-specific physical habitat conditions) had a greater influence on determining benthos community structure than the spill. The upstream versus downstream communities of basket samples were found to significantly differ in each year of sampling, also suggesting there is a background influence resulting in upstream/downstream differences in community structure that are not related to the spill.

Effects on Cheakamus River Fish

The Cheakamus River benthos provides a primary food source for fish species in the river. Consideration of the effects of the spill on benthic food supply also took into account the following:

1. Do fish species primarily consume specific aquatic invertebrate taxa or do they consume the most available species?
2. If fish consume specific taxa groups, were those taxa affected by the spill?
3. What was the duration and severity of the declines in these taxa, if any?

4. Were there less, more or the same amount of consumers seeking invertebrates during the weeks and months after the spill?

The stomach contents of juvenile coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*), as well as steelhead trout (*O. mykiss*), from the Cheakamus have been examined in the past (Perrin, 2001). Perrin (2001) concluded these fish are indiscriminate foragers, and rearing salmon and other species prey on available invertebrates; hence the quantity of invertebrates is important, not the quantity of specific taxa.

The colonization basket work provided the most precise tool for measuring the relative quantity (*i.e.*, biomass and number of individuals) of invertebrates present. The 2005 post-spill results suggest the abundance of invertebrates increased downstream of the spill (Figure 4-9). When density was compared to previous years, the 2005 upstream samples had fewer individuals per square metre than in 1996 or 2000 (Figure 4-9). This variation in above-spill benthos density reflects year to year variation. The largest year to year variation in invertebrate density was at Site 3 (u/s Culliton Creek) where there were 188,375 individuals/m^2 in 1996 samples, while in 2000 the same site had <5% of this density (8,675 individuals/m^2) in collected samples (Figure 4-9). The contrast between observed invertebrate density in 1996 and 2000 indicates natural conditions can lead to changes greater than 100% without the influence of an event such as the NaOH spill.

The specific mechanisms of recovery for benthic invertebrates have not been identified although downstream drift is considered a contributor. In addition, the extent to which the spill affected numbers and biomass is also not known, nor do we know what percentage was killed due to NaOH exposure. Moore (2005) found some invertebrate groups (*i.e.*, Hydropsychid caddisflies, Ephemerellid mayflies) could be tolerant of exposure to pH 12 for brief periods of time (*e.g.*, 24 hours). After the spill in early morning the NaOH moved through the Cheakamus River in a matter of hours (McCubbing *et al*., 2006, Triton 2007a, Triton 2007b) and invertebrates were exposed to unknown levels of NaOH. Studies in BC streams (Lancaster, 1992) found invertebrate drift rates are four times higher in the overnight period from dusk to dawn than during the daylight hours. In the scenario where drifting organisms receive more exposure to the spill than those in the cover of the benthic zone, it is plausible a reduced number of the invertebrate community avoided acutely lethal exposure.

Catastrophic events related to invertebrate mortality and recovery have been studied in the past. Badri *et al.* (1987), for example, measured changes in invertebrate abundance from a massive flood in a Moroccan stream system in the Atlas Mountains, where upstream regions saw density declines from 4,360 individuals m^2 to just 95 individuals m^2 and reductions in taxa from 26 to 9 taxa groups. Within one month benthic invertebrates in this watershed returned to pre-flood levels of abundance and taxa richness. Rapid recovery in freshwater invertebrates after a NaOH spill was also reported by Moore (2005) who cited the rapid dispersal mechanisms (*i.e.*, downstream drift) as the likely reason for the recovery.

The results reported here represent the product of a number of potential mechanisms (*e.g.*, downstream drift, change in predation pressures, reduced/increased competition for resources) contributing to benthic invertebrate diversity, abundance and recovery. The overall net result is recovery of the benthic community was occurring shortly after the spill.

5.1 Representative Sampling

Benthic invertebrate sampling with colonization baskets and kick nets was confined to sections of the Cheakamus River safely accessed by wading. This sub-sample of the community is considered representative of the benthic fauna in deeper sections of the river as well as areas of the river outside the sampling sites. Efforts have been made to minimize sampling bias by replicating the methods used in past years; hence errors resulting from sub-sampling are not considered to be proportionally greater in one year over another.

5.2 Changes to Specific Taxa

The 2005 mayfly data from colonization basket and kick net samples showed selected taxa were less abundant in downstream samples than the upstream control. The Heptagenids from samples collected in the week after the spill (Stamford *et al*. (2007) were also found to be lower in abundance at CHK02 to CHK05 in comparison to the control site and Squamish River sites. Return of Heptagenids to CHK02 to CHK05 was observed over the nine week kick net sampling periods (Figure 4-4) with the results from the last week of sampling being similar to the control site values (median = 15.6 vs. 16.6% for the affected sites and control, respectively).

Baetid mayflies in kick net samples from the week of the spill (Stamford *et al*., 2007), showed sites CHK02 to CHK05 had a range of 0 to 4.6% of total mayfly abundance, whereas the upstream control samples had 50 to 80%. In contrast, this study showed the median (N = 20) for Baetid Mayflies was over 64% (range 5 to 93%) for sites CHK02 to CHK05 across all sampling sessions, while the control median was 63% (N = 5). Baetids displayed the greatest relative abundance across all samples. An increase in Baetids was observed in the first weeks of sampling and like the Heptagenids, indicates a trend of rapid recovery after the spill. Baetid mayflies have been ranked the highest for fish forage (Radar, 1997) and may signal rapid return of available food for fish after the spill.

The abundance of stoneflies from the *Sweltsa* Genus (Family: Chloroperlidae) was shown to be less than the upstream control site. In contrast, samples collected from directly below the spill on August 10 to 11, 2005 (*i.e*., 5 to 6 days after the spill) by MoE show Chloroperlids to be one of the top three dominant taxa groups in at least one sample (Stamford, 2007 pers. comm.). The Chloroperlidae stoneflies were also studied in 219 reference sites in the Fraser River catchment (Rosenberg *et al*., 1999) where they comprised 1.7% of the total individuals found in kick net samples. In this study, the 2005 downstream kick net samples from the Cheakamus River had an average Chloroperlid abundance of 1.8% of total individuals, while the upstream control was approximately 5.3%.

The relative amount of Chloroperlid stoneflies in 2005 kick net samples from NaOH exposed Cheakamus River sites (1.8%) was similar to the average for Fraser River reference sites (1.7%). The relative abundance of this stonefly in the control site kick net samples (5.3%) appears to be more than 50% greater than both the affected sites and the typical sites in the reference database.

The high relative abundance of Chloroperlid at the control site does not provide clear conclusions about the affects of the spill on downstream sites. The results from the August 10 to 11, 2005 sampling did not find the same reductions in downstream Chloroperlids (Stamford, 2007 pers. comm.), providing further evidence this spatial variability of stonefly relative abundance may not be related to the spill.

Higher overall invertebrate abundance was consistently observed at this control site in two other studies (Stamford *et al*., 2007; Perrin, 2001). The reasons for these differences are unknown although Perrin (2001) noted river nutrient levels were exceptionally high at the CHK01 control site, and Stamford *et al*. (2007) observed excessive filamentous algae in kick samples from this site, but not in downstream samples. These studies suggest CHK01 is located in a more productive part of the river compared to downstream sites and this could explain some of the observed differences.

The importance of *Sweltsa* stoneflies as forage for salmonids, relative to other invertebrate taxa, was studied by Radar (1997), whereby *Sweltsa* were assigned 12 out of a possible 23 ranks (1 is best for fish, 23 is worst). Hence the reduction in *Sweltsa*, a group which typically has low (<2%) abundance and a medium rank in terms of fish food (Radar, 1997), is not likely to result in a measurable decline in fish food availability, especially when one considers the high abundance of post-spill Baetids in spill-affected samples (Section 4.2.2).

5.3 Recovery Mechanisms

Based on the results of this work it is difficult to identify the principal mechanism(s) contributing to community recovery observed in this study. There is substantial (*e.g.*, 50% change) inter-annual variation in invertebrate abundance in the Cheakamus River unrelated to the spill (*e.g.*, upstream sites; 1996 vs. 2000 comparisons). The intra-annual variation in community structure between upstream and downstream sites in colonization basket samples was also shown to be significantly different in each year of sampling. Inter-annual changes to environmental conditions in the Cheakamus River watershed such as snow pack levels, rainfall, temperature and discharge could also be affecting abundance and community structure in the benthic invertebrate community. For example, in October 2003 a 100 year flood event resulted in morphological changes to the channel bed (BCCF, 2003) which may also have affected benthic invertebrates through alteration of physical habitat or losses from stranding as water levels receded. Inter-site variation from site-specific conditions must also be considered when reviewing and interpreting sampling results. While sampling methods employed in 2005 were designed to reduce inter-site variation by sampling a broad area (*i.e.*, travelling kick net) or using replicate samples (*i.e.*, colonization baskets), influence of site-specific factors (*e.g.*, tributaries, substrate size and velocities) on community structure and abundance cannot be eliminated.

River regulation as part of BC Hydro’s power operations is another source of potential variation for benthic invertebrates, as flow rates and wetted area can affect aquatic habitat. Cheakamus River mainstem sections above large tributary confluences are more susceptible to the effects of water regulation and constraints on downstream drift. These locations (CHK01A, 02, 03), not surprisingly, were the most different from the reference condition. If downstream drift is considered to be the primary mechanism for invertebrate recovery in the Cheakamus River, then it would follow sites 01A, 02, and 03 would recover more slowly than sites further downstream in the initial weeks after the NaOH spill.

The cause of the stress recorded at sites CHK02 and 03 which had the "stressed" and "severely stressed" rating was further investigated by examining the relative abundance of taxa groups found in samples from those sites. These samples contained a high relative abundance (*e.g.*, 79, 95 and 96%) of pollution-sensitive EPT taxa. A disproportionately high abundance of a particular taxa group, as compared to the relative abundance levels of taxa groups in reference site samples, can result in an increased stress rating by the analytical tools used to rate site condition (*i.e.*, BEAST analysis, Reynoldson *et al*., 1995). This result may signal these pollution-sensitive taxa were the first groups to recolonize sites CHK02 and 03 in the weeks following the spill.

6.0 Conclusions

Based on the results of the 2005 benthic invertebrate recovery monitoring program and the above observations the following conclusions are drawn.

- Invertebrate exposure to increased pH and the NaOH spill was episodic and resulted in the mortality of an unknown number of invertebrates.
- The extent to which the community structure was affected is unknown.
- Confirming the mechanisms of recovery was complicated by natural site-specific and inter-annual variation in the abundance of benthic invertebrates not related to the spill.
- The Reference Condition Approach (RCA) and CABIN analytical tools along with associated and recognized statistical tests were reliable methods to evaluate the effects of the spill.
- Complementary benthic sampling using colonization baskets and statistical comparisons of abundance corroborated the findings from CABIN analyses of kick net samples.
- Sites furthest downstream of the spill were most similar to Reference Conditions.
- Re-colonization by EPT taxa was observed in the weeks following the spill, and Mayflies, particularly Baetids, were the largest taxa group in most samples.
- Some taxa (*e.g.*, Heptagenids) appeared to be more affected than other taxa, and Heptagenid recovery to levels comparable to the control site was observed by end of the nine (9) week sampling period.
- There was a rapid recovery in the overall abundance and biomass of invertebrates observed in kick net samples.
- Post-spill sampling with colonization basket suggested invertebrate abundance and biomass had recovered to levels similar to pre-spill conditions.
- Given the weight of evidence from kick net samples and colonization baskets showing rapid recovery in abundance and biomass of invertebrates, and the observed inter-annual variation, no further monitoring of benthic invertebrates is recommended.

7.0 References

Badri, A., J. Giudicelli, and G. Prévot, 1987. *Effets d'une crue sur la communauté d'invertébrés benthiques d'une rivière méditerranéenne, Le Rdat (Maroc).* Acta Oecologica; Oecologia Generalis 8(4):481-500. (In French)

Bailey, R.C., R.H. Norris, T.B. Reynoldson. 2004. *Bioassessment of Freshwater Ecosystems: Using the Reference Condition Approach*. Kluwer Academic Publishers. 170pp.

BCCF (British Columbia Conservation Foundation). 2003. *Habitat assessments and restoration prescriptions for recovery of steelhead trout in the Mamquam, Lower Cheakamus and Upper Squamish Rivers*. Prepared for Ministry of Water, Land and Air Protection, Surrey, BC.

Bray, J.R. and J.T. Curtis. 1957. An ordination of the upland forest communities of Southern Wisconsin. *Ecol. Monogr*. 27:325-349.

Clark, K.R. and R.N. Gorley. 2006. *Plymouth Routines in Multivariate Ecological Research* (PRIMER), software version 6.0. PRIMER-E Ltd, Plymouth Marine Laboratory, Plymouth, UK.

EC (Environment Canada). 1984. *Sodium Hydroxide. Enviro Technical information for Problem Spills*. Ottawa, Ontario.

Hauer, F.R. and V.H. Resh. 1996. *Benthic macroinvertebrates* in *Methods in Stream Ecology*, eds. F.R. Hauer and G.A. Lamberti. Academic Press, San Diego. p.339-367.

Hilsenhoff, W.L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. *Journal of the North American Benthological Society* 7: 65-78.

Kerans, B.L. and J.R. Karr. 1994. A Benthic Index of Biological Integrity (B-IBI) for rivers of the Tennessee Valley. *Ecological Applications* 4(4): 768-785.

Lancaster, J., 1992. *Diel variations in the effect of spates on mayflies (Ephemeroptera: Baetis).* Canadian Journal of Zoology 70:1696-1700.

Lenat, D. R. 1988. Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. J. N. Am. Benthol. Assoc. 12: 279-290

Marmorek, D.R. and I. Parnell. 2002. *Cheakamus River Water Use Plan - Report of the Consultative Committee*. Prepared by ESSA Technologies Ltd., Vancouver, BC on behalf of the Cheakamus River Water Use Plan. Consultative Committee, Vancouver, BC for BC Hydro.

McCubbing, D.J.F., C.C. Melville, G. Wilson, and M. Foy. 2006. *Assessment of the CN rail caustic soda spill August 5th, 2005 on the fish populations of the Cheakamus River*. Prepared for the BC Ministry of Environment, Land and Parks, Surrey, BC. 67 pp.

Moore, J.A. 2005. *The effects of a sodium hydroxide spill on the macroinvertebrates of a third order, southern Appalachian stream*. M.S. Thesis, Appalachian State University.

NHC (Northwest Hydraulic Consultants). 2000. *Analysis of channel morphology and sediment transport characteristics of the Cheakamus River*. BC Hydro Ref No CMS-MRPHLGY. Prepared for BC Hydro.

Perrin, C.J. 2001. *Tropic structure and function in the Cheakamus River for Water Use Planning*. Final Report, prepared for BC Hydro and the Resort Municipality of Whistler. Limnotek Research and Development Inc., Vancouver, BC. 67 pp.

Perrin, C. 2006. Limnotek Research and Development, Personal Communication

Radar, R.B. 1997. A *functional classification of drift: traits that influence invertebrate availability to salmonids*. Canadian Journal of Fisheries and Aquatic Sciences. 54: 1211-1234.

Reynoldson, T.B., R.C. Bailey, K.E. Day, and R.H. Norris. 1995. Biological guidelines for freshwater sediment based on BEnthic Assessment of SedimenT (the BEAST) using a multivariate approach for predicting biological state. *Australian Journal of Ecology* 20:198-219.

Reynoldson, T.B., R.C. Bailey, K.E. Day, R.H. Norris, and V.H. Resh. 1997. The reference condition approach: a comparison of multimetric and multivariate approached to water quality impairment using benthic macroinvertebrates. *Journal of the North American Benthological Society* 16:833-852.

Reynoldson, T.B. D.M. Rosenberg and V.H. Resh. 2001a. Comparison of models predicting invertebrate assemblages for biomonitoring in the Fraser River catchment, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 1395-1410.

Reynoldson, T.B., C. Logan, T. Pascoe. S.P. Thompson. 2001b. *Invertebrate biomonitoring field and laboratory manual for running water habitats*. Revised by S. Sylvestre, 2004. National Water Research Institute, Environment Canada. 51 pp.

Rosenberg, D.M., T.B. Reynoldson, and V.H. Resh. 1999. *Establishing reference conditions for benthic invertebrate monitoring in the Fraser River catchment, British Columbia, Canada*. Fraser River Action Plan, Environment Canada Report: DOE-FRAP 1998-32. 149 pp.

Stamford M. 2007. Stamford Environmental. Personal Communication

Stamford, M., S. Barrett, and G. Wilson. 2007. *Explanation of the invertebrate community in the Cheakamus River: the initial impacts from the CN Rail caustic soda spill*. Prepared for B.C. Ministry of Environment. 47pp.

Sylvestre, S., M. Fluegel, and T. Tuominen. 2005. *Benthic invertebrate assessment of stream in the Georgia Basin using the reference condition approach: expansion of the Fraser River invertebrate monitoring program 1998 – 2002*. Environment Canada, Vancouver, BC. 194 pp.

Sylvestre, S. Unpublished data from Fall 2005 Cheakamus River benthic site assessments (EC Site Codes CHK01, CHK04, and CHK05). Environment Canada, Pacific and Yukon Water Quality Monitoring Section.

Systat Software Inc. 2004. *SYSTAT 11 for Windows*. Richmond, CA, USA.

Teal Solutions. 2005. *Draft Environmental Emergency Remediation Plan, CN Rail Derailment Garibaldi Mile 58.5, August 8th 2005*. 16pp.

Triton Environmental Consultants Ltd. 2007a. Sodium Hydroxide Derailment Mile 56.6 Water Quality Assessment. Final Report submitted to Canadian National Railway. September 2007.

Triton Environmental Consultants Ltd. 2007b. *Cheakamus River sodium hydroxide derailment Mile 56.6, Screening Level Assessment of Ecological Effects*. Final Draft Report. Prepared for CN Railway Company. Richmond, BC. 78pp.

Wilson G., 2006. BC Ministry of Environment, Personal Communication, 12/12/06 CERTC Meeting.

Woodiwiss, F.S. 1964. The biological system of stream classificastion used by the Trent River Borard. *Chemistry and Industry* 11: 443-447.