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**Report Title:** Protocols for assessing water quality and aquatic biodiversity using macroinvertebrates

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**Year of project:** 1999

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**File Name:** Soil\_and\_Water\_1999\_Chiasson\_ Protocols for assessing water quality and aquatic biodiversity using macroinvertebrates

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# Protocols for assessing water quality and aquatic biodiversity using macroinvertebrates

Presented to the Fundy Model Forest  
June 1, 1999  
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## Introduction

Biomonitoring involves the use of living organisms to assess or monitor environmental conditions. In aquatic habitats, macroinvertebrates are frequently used. By definition, macroinvertebrates are those organisms that are retained by a 250: sieve. They have proven useful in detecting anthropogenic influences resulting from human activities. One of the particular strengths of the method is the ability to monitoring non-point sources of pollution such as storm-water runoff where chemical water quality testing is generally less informative.

Biomonitoring has several advantages over physical and chemical monitoring of water quality being more sensitive to a wide range of influences such as sedimentation, habitat degradation, chemical contamination and thermal pollution. There is also a heightened analytical sensitivity due to bioconcentration of certain contaminants and different scales of exposure can be investigated as invertebrates can integrate over meters and months and fish can integrate over kilometers and decades (Cuffney et al. 1993b). Biomonitoring can also provide historical information about past pollution, whereas chemical monitoring can only provide information at the time of sampling. Public interest and concern over loss of biodiversity and aquatic habitat are more readily understood in the context of biomonitoring as living organisms are affected (Barton and Metcalfe-Smith 1992, Cuffney et al. 1993b). However, biomonitoring is not without its drawbacks as it will often only detect impairment and to some extent severity, but additional chemical and biological toxicity testing is required to identify the exact nature of the problem.

Among the potential suite of habitats that might serve as monitoring sites, streams are one of the best. The life they support is directly linked to the instream physical and chemical characteristics of the watershed which frequently reflect land use practices and riparian conditions (Townsend and Scarsbrook 1997, Richards and Host 1994). According to Wilcoe et al. 1998, agriculture and forestry pose the biggest potential threat to aquatic ecosystems. These industries and urbanization, change watershed characteristics that influence runoff patterns, increase sediment supply to streams and decrease the supply of coarse woody debris. Radical shifts in the chemical and physical features of a watershed can dramatically affecting aquatic plant and animal communities (Richards and Host 1994, Wang et al. 1997, Delong and Brusven 1998).

Stream quality has been shown to decline when agriculture exceeds 50% of the land use activity along a stream (Wang et al. 1997). A positive correlation has been found between agricultural land use and nitrogen and phosphorous concentrations in streams (Wang et al. 1997).

In regards to forestry, a positive linear relationship has been found between upstream harvesting activities and downstream habitat quality and taxa richness (Fore et al. 1996, Wang et al. 1997).

Urban land use is associated with large areas of impermeable land such as parking lots, which dramatically increases runoff. Increased runoff breaks the dynamic equilibrium between the watershed and its streams, resulting in destruction of stream structure and habitat degradation. Urbanization can also introduce toxic materials and nutrients to streams, usually affecting biotic integrity more than habitat quality (Wang et al. 1997).

An increase in sediment load is a frequent result of many human activities that occur in watersheds. Substrate characteristics dictate to a large extent the distribution and richness of stream invertebrates. Fine sediments eliminate the spaces between coarser substrate, reducing substrate diversity, decreasing water flow through the substrate and inadvertently decreasing taxa richness (Richards and Host 1994, Rosenberg et al. 1997).

Benthic macroinvertebrates have been used extensively as indicators of water quality because they respond to a host of features such as altitude, width, depth, substrate, water velocity, vegetation, chemicals and other physical factors in addition to human activity (Bargos et al. 1990, Richards and Host 1994, Rosenberg et al. 1997). There are many qualities that make benthic macroinvertebrates ideal for biomonitoring: they are ubiquitous, usually present throughout the year, and they live in, on, or near the substrate, preferentially subjecting them to chemicals that are denser than water (Bargos et al. 1990, Cuffney et al. 1993b, Barbour et al. 1998). Benthic macroinvertebrates are relatively immobile compared to fish, making them good indicators of localized conditions and very practical for site specific impact studies (upstream - downstream studies) (Cuffney et al. 1993b). Benthic macroinvertebrates are useful long term monitors of stream health because they occupy different levels in an aquatic ecosystem and respond as functional groups and not as independent organisms.

There are some difficulties working with benthic macroinvertebrates. Their patchy distribution makes quantitative sampling difficult as large numbers of samples are needed to achieve reasonable precision in calculating population abundance. Processing and identification of samples can be time consuming and costly. The biodiversity of benthic macroinvertebrates can vary due to natural factors which can give misleading results and some groups are taxonomically difficult to identify. However, a proper study design can help minimize or control these sources of error (Rosenberg et al. 1997).

## **Selection of Sites**

Determining the area to sample ideally involves three elements: 1) locating a basic fixed site where chemical and flow data are normally taken, 2) establishing sampling reaches and 3) locating specific instream habitats from which samples are to be taken. The basic fixed site should be representative of the watershed. Sampling reaches are stream lengths that represent repeated geomorphic features such as a pool-riffle sequence. Reaches may be above, below or encompass a basic fixed site, but should not be established in areas of major discontinuity in the channel or riparian characteristics. Reaches should be located at least 100 m upstream from any road or bridge crossing and should not include within its boundaries any discharging tributaries. Identification and location of instream habitat types is broadly defined and based on a hierarchical grouping of three levels. The first level includes major geomorphic channel units, riffles, runs and pools; the second includes major channel boundaries, the main channel, channel and island margins; and the final level, the major channel features such as woody snags, macrophyte beds, natural beds and bars (Cuffney et al. 1993b).

Sampling reaches can represent specific conditions such as agricultural or industrial use. The location and length of sampling reaches are decided by a combination of geomorphic characteristics and sampling considerations. The length is determined by the repetition of two

geomorphic channel units such as a pool, riffle, pool, riffle sequence (Rosenberg et al. 1997). The geomorphic channel must cover 50 percent of the active channel width, the latter being defined as the mean stream width at maximum flow. Circumstances may be such that channel units may not repeat at every sampling site, in which case the length of the reach is based on twenty channel widths taken under normal flow conditions. A permanent reference point such as a bridge, stream gauge or survey marker can help relocate the site in addition to flag markers. Use of Global Positioning Satellite (GPS) coordinates is also recommended. In the event of multiple sampling reaches, they should be separated by a minimal distance of 150 m (Barbour et al. 1998).

## **Seasonal and Hydrologic Conditions for Sampling**

Macroinvertebrates are subject to many factors that influence distribution and abundance. Weather, nutrient supply, and interspecific competition all dictate the structure and function of the macroinvertebrate communities (Rosenberg et al. 1997). The optimal time for sampling is during low and stable flow, if compatible with life-history of the organisms being targeted. There are several advantages to sampling during this time, including easier access to the stream, a reduced need to use labor intensive deep water sampling techniques and a greater assurance that all parts of the wetted channel have always been submerged. However, regional characteristics must also be considered, particularly where streams are short-lived or where flow characteristics such as current velocity change significantly during normal summer low flows. In order to understand discharge effects it is recommended that all monitoring sites associated with basic fixed sites be continuously gauged for at least six months before sampling and throughout the sampling period (Cuffney et al. 1993b).

Ideally, sampling should occur at the time of year when the majority of insects are at or near maturity. In temperate climates, community maturity and richness are at their peak from late fall to early spring depending on factors influencing local temperatures such as elevations and latitude (Bargos et al. 1990, Cuffney et al. 1993b). It is best to sample in the spring after ice-out when late stage larval forms are present but have not begun their final maturation, or in the late fall after most species have mated and immatures have had time over summer to develop. Early instars lack morphological features and are small, making them difficult to identify and collect. The resting stage of invertebrates is also difficult to identify since there are no taxonomic keys, they are also difficult to collect because the pupae larvae may move into the stream bank where they are missed by standard sampling techniques (Cuffney et al. 1993b).

A single sampling period may miss some members of the macroinvertebrate community due to seasonal variability and unique short life histories (Rosenberg et al. 1997). The reproduction, development and the growth of macroinvertebrates is highly influenced by temperature. Specific species require a certain number of degree days to complete the aquatic portion of their life cycle. In certain instances where targeted sampling is conducted, life cycle traits must be taken into consideration.



The site must be accessible at all times during sampling. A high flow event can eliminate or redistribute a significant portion of the macroinvertebrate community. In general, a four week recovery period is required after a high flow event to avoid misinterpretation of channel habitat characteristics and sampling areas that were dry before the increased flow (Cuffney et al. 1993b).

There are many other considerations to be taken into consideration when deciding the best time to sample, including the life histories, other aquatic organisms and seasonal human activities. If possible sampling should be avoided during spawning and migration of fish, especially when dealing with threatened or endangered species. Seasonal agricultural practices can introduce nutrients, salts, sediment, fertilizers, pesticides and disrupt normal flow regimes of the stream (Barton and Metcalfe-Smith 1992). These influences should be included within the sampling design.

## **Sampling –general considerations**

Many different types of equipment exist for sampling macroinvertebrates. The choice of what equipment to use is controlled by depth, velocity, type of substrate and objectives of the study. Most samplers used in wadeable coarse-grained substrate depend on disturbing the substrate, dislodging the organisms and letting the current sweep them into a downstream net (Cuffney et al. 1993b). These samplers share some common problems. Frequently, the sampler will sit on a rock instead of being flat against the substrate. The rock should only be included in the sample if 50 percent of its area lies within the sampling area. If the rock is included, it is removed, held in front of the net and brushed clean.

Many sampling techniques require the substrate to be disturbed to a certain depth, usually 10 cm. Guide rods are useful as they can be set to a required depth and require less effort than moving substrate by hand. When it is impossible to reach the required depth, digging is done as deeply as possible. Sampling can be based on area, time, or number of substrate units investigated and is usually conducted with 400-600  $\mu$  mesh (Resh et al. 1995). Sampling methods can produce different estimates of relative abundance of even the most common species at the same site (Diamond 1996). Sampling conducted by Williams (1998, personal experience) in Humphreys Brook revealed that the Surber was more efficient than the Kick-Net in sampling oligochetes.

Sampling fast flowing rivers can be dangerous and require safety precautions. There should be a member of the sampling team on shore at all times, individuals should not go deeper than one meter, life vests should be worn and a safety rope should be within reach (Rosenberg et al. 1997).

## Sampling Equipment

### ***Artificial substrates:***

Artificial substrate samplers are generally made of metal wire, mesh bags, netting, or burlap. The substrate itself can include rocks, glass beads, packed leaves, plastic discs and twigs.

### *Advantages:*

They allow easy collection in locations that are typically hard to sample effectively (eg. bedrock, boulder or shifting substrates, deep or high water velocity).

Passive sample collection. Sampling is standardized by eliminating variation in the collection technique, therefore standardization is only required for the setting and retrieving the device (Pashkevich et al. 1996).

Confusing effects of habitat differences are reduced by providing a standardized microhabitat that may promote selectivity for specific organisms if the artificial substrate provides a microhabitat different from that occurring at the site.

Less skill or training is needed compared to disturbance-removal techniques. Assistants can place substrates but an experienced biologist should be responsible for site selection.

One person can set and retrieve samplers (Williams 1998, field experience).

### *Disadvantages:*

Two trips to the sampling site are required, compared to one with other direct methods.

Artificial substrates must be submerged for a minimum of six weeks, decreasing their utility for certain rapid biological assessments.

They may not fully represent the benthic assemblage at a station if the artificial substrate offers a different microhabitat than the naturally occurring one (Barbour et al. 1998).

Specific taxa can be favored, falsely portraying a high relative abundance in the natural substrate (Cuffney et al. 1993b, Barbour et al. 1998).

Sampler loss can occur from sedimentation, extreme high or low flows or vandalism (Barton and Metcalfe-Smith 1992).

Transport and storage can be difficult especially if a large number of samplers are involved.

They may not be sensitive to changes in water quality associated with changes in land use (Cuffney et al. 1993b).

### ***Rectangular Dip Net***

The rectangular dip net's frame is 50 x 30 cm and attached to a long pole. The net is cone or bag-shape and sampling is conducted by jabbing, dipping, sweeping or by disruption of sediment upstream (Barbour et al. 1998).

#### *Advantages:*

Sampling requires one person.

Low maintenance.

Can be used in a variety of microhabitats.

#### *Disadvantages:*

Lack of precision in sampling area.

### ***Surber***

The Surber sampler consists of a 30 x 30 cm metal frame, which once unfolded delineates a 0.09 m<sup>2</sup> area of substrate. The current sweeps the dislodged organisms into a net that is fixed to the vertical arm of the frame. Large stones are first brushed clean within the delineated area prior to disturbing the substrate to a 10 cm depth (approximately finger depth) for a set time, usually between two to three minutes (Barbour et al. 1998).

#### *Advantages:*

Low maintenance, the Surber sampler is very simple requiring only occasional net repair.

Sampling requires only one person.

#### *Disadvantages:*

Sampling is restricted to depths below 30 cm (Barbour et al. 1998).

Drift contamination, organisms from outside the sample area can enter the net.

Invertebrates can escape between the sampler and the substrate if the seal is inadequate (Brooks 1994, Williams 1998, field experience).

A strong current or a clogged net can cause a pressure cone to develop, diverting organisms around the net (Brooks 1994).

### ***Hess***

The Hess sampler is a metal cylinder approximately 50 cm in diameter and has a sampling area of 0.8 m<sup>2</sup>. The wall of the cylinder is netted, allowing water to flow through the device, forcing the invertebrates into a cone net attached to the downstream side of the cylinder. Rocks within the delineated area are cleaned, removed, and the substrate disturbed to a depth of 10 cm for a set time, usually between two and three minutes.

#### *Advantages:*

No contamination from drifting invertebrates.

Absence of a pressure cone around the mouth of the collection net (Williams 1998, field experience).

Sampling requires one person.

#### *Disadvantages:*

Water velocity must be sufficient to force water through the netting and the invertebrates into the collecting cone, restricting the effective range of the sampler.

It is restricted to depths of less than 50 cm.

The cylinder may have difficulty penetrating cobble substrate, making it difficult to delineate a sample area, allowing water to flow under the sampler (Williams 1998, field experience).

### ***Stovepipe Core Sampler***

The Stovepipe sampler is best suited for depths of less than 75 cm. The sampler is a one meter length of polyvinyl chloride (PVC) pipe with a 30 cm diameter and a beveled bottom edge. The pipe is driven into the substrate deeply enough to produce a good seal around the bottom and then the substrate is removed from the sampler by hand or scoop and processed for invertebrates. In some situations the substrate will be too deep to reach, in this case the invertebrates are dislodged by using long handled brushes or poles and the water and suspended invertebrates are pumped into a net (Cuffney et al. 1993b). A cost effective design based on the stovepipe sampler can be constructed using a 20 liter pail with the bottom removed. A bilge pump

attached to the interior side of the pail is used to remove the suspended invertebrates (Chiasson 1998, field experience).

*Advantages:*

There is no dependence on current velocity to propel organisms into the net.

Samples can be collected in water 50 - 75 cm deep.

*Disadvantages:*

It is often difficult to handle the sampler in very fast-flowing water (Williams 1998, field experience)

The sampler is more complex, having more equipment (pump, battery, wires) making it awkward to operate and more likely to fail (Williams 1998, field experience).

Requires two people to effectively operate the sampler.

It is often hard to penetrate cobble substrate deep enough to seal the bottom allowing water to flow underneath the sampler (Williams 1998, field experience).

Sampling time is increased as the pump must clear the water within the cylinder after the substrate has been disturbed. Deeper water requires a longer pumping time, making it less efficient (Brooks 1994, Williams 1998, field experience).

***Kick-Net***

The Kick-Net is a 1.0 x 1.0 m sieve net attached to a pole at either end. The operator disturbs a 1.0 m<sup>2</sup> area immediately upstream of the net with the heel of their boot. The current then sweeps the invertebrates into the net. The Kick-Net is most efficient for sampling cobble substrate, such as riffles and runs and is used in approximately 75% of assessments in the United States (Resh et al. 1995, Barbour et al. 1998).

*Advantages:*

The Kick-Net can be used in depths of a few centimeters to just below one meter.

Simple design, requires only occasional net repairs.

Ease of use, multiple testing is not as tiring as other methods that require bending (Williams 1998, field experience).

*Disadvantages:*

There is no physical barrier defining the sampling area, therefore, sampling size may be inaccurate.

Strong currents cause a pressure cone to develop and the current flows around the net possibly removing invertebrates from the sample (Williams 1998, field experience).

There is a greater possibility of contamination from drifting organisms due to the size of the net.

Sampling requires two people.

***D-Frame Net***

The dimensions of the net are 30 x 30 cm and the upper frame is curved giving the net a "D" shaped appearance. A cylindrical or bag-shaped net is attached to the frame. Sampling occurs by jabbing, dipping, sweeping or using it as a Kick-Net (Barbour et al. 1998).

*Advantages:*

Sampling requires one person.

Low maintenance.

Can be used to effectively sample various microhabitats, making it good for complete taxa investigations.

*Disadvantages:*

No standardized sampling area.

Possible contamination from drifting invertebrates.

***Grabs (Ekman, Ponar, Peterson, Shipek and Van Veen)***

Grab samplers can be used to sample fine-grained substrates in wadeable or nonwadeable sites. A pole mounted Ekman grab operates best in fine sediment in wadeable water. Constructed from lightweight stainless steel, the Ekman grab has center pivot jaws that overlap, minimizing sample disturbance and loss. A messenger releases the mechanism. The Van Veen Grab is used both in freshwater and marine conditions. The jaws are pushed open and held in place by a hook. When retrieved, a set of leverage rods help to close the jaws. The Ponar grab is also constructed

from stainless steel and best suited for sampling fine-gravel substrate. One of its jaws has an underlip to push aside any substrate that may keep the jaws from closing. The Ponar grab closes on impact or when the line goes slack. The Ponar also has removable top screens equipped with rubber flaps to prevent wash-out which allows subsampling from within the scoops (Wildlife Supply 1997).

All grabs should be carefully lowered before release to avoid disturbing the substrate before contact. The recovered grab is carefully checked for sample loss due any obstruction that might be holding the jaws open. Individual samples can be combined in a suitable container prior to field processing or processed and then combined.

When sampling in nonwadeable water, the Ponar, Peterson, Shipek or Van Veen grabs can be used from boats. The Peterson grab is widely used in fresh water for taking samples of hard bottoms, such as sand, pebbles, and clay. It is hinged at the top and uses an automatic bayonet release mechanism. The Shipek is a heavy stainless steel grab that can be used for soft sediment to hard clay bottoms. Its center pivot allows 180° rotation of the scoop minimizing sample disturbance and it has an automatic release mechanism that opens when it strikes the bottom (Wildlife Supply 1997). To standardized the amount of force used to penetrate the substrate the grabs should be lowered to within 3 meters of the streambed, halted, and then allowed to drop to the streambed (Cuffney et al. 1993b).

## **Quality control of sampling equipment**

Sampling equipment must be properly maintained to avoid sampling error. All nets must be inspected for damage at least once daily and any damage immediately repaired. A glue gun provides a quick and durable seal for small holes or tears (Williams 1998, field experience). The damaged area should be cleaned and the glue applied to both sides of the hole. A torn net should first be sewn and then glued. Canvas material can be fixed by sewing a patch of similar material over the damaged area. A spare net should be included on longer sampling excursions.

## **Sampling Procedures**

There are two types of sampling, qualitative and semi-quantitative. Qualitative sampling involves collecting invertebrates from as many different instream habitats as possible and is intended to provide a list of taxa present in the sampling reach. Semi-quantitative sampling is intended to provide a measure of the relative abundance of each taxa present in two contrasting habitat types within the sampling reach. These sampling procedures along with the corresponding chemical and physical data are used to characterize the macroinvertebrate community within the sampling reach. Many streams vary chemically and physically, making no single sampling technique or device appropriate for all sites and instream habitats. It is recommended that a variety of equipment be available based on the type of information sought and on the site conditions such as water depth, current velocity and substrate composition (Cuffney et al. 1993b).

Semi-quantitative samples supplement qualitative samples by providing data on the presence and relative abundance of invertebrates in contrasting habitats. One site is relatively free of human influences and normally contains the richest assemblage of invertebrates within a given stream or region. In an investigation of sediment-borne contaminants a depositional area such as a pool is selected. A cumulative effect in depositional areas explains an earlier response by macroinvertebrates to contaminants than the more sensitive but less exposed rich habitats of course grained, fast-flowing riffles (Barton and Metcalfe-Smith 1992).

## **Semi-Quantitative Sampling**

The single habitat sampling technique is well suited for assessing differences between streams, particularly in riffle or run habitat where invertebrate diversity and abundance are highest. Riffles are easier for field crews to identify than pools and are more uniform in composition than other instream habitats making them more suitable for between site comparisons (Fore et al. 1996).

Most macroinvertebrate sampling programs record additional information on weather conditions and adjacent land use. A map or video of the sampling reach including instream attributes and any important characteristics of the bank and riparian zone can assist in later data interpretation. A video of the sampling reach can help document habitat changes between sampling periods, provide a visual record of seasonal changes in land use practices, save extensive writing and can be commented with audible notes (Rosenberg et al. 1997). Direction of stream flow and geographical location should be noted. GPS coordinates are usually provided for the furthest downstream point of the sampling reach. Sampling always commences at the lower end of the reach and proceeds upstream. Samples are transferred from the net to sample containers and preserved in enough 95 percent ethanol to cover the sample. While sampling, a field data sheet is completed, recording the percentage of each habitat type in the reach, the sampling gear used and the conditions of sampling (high flow, difficult access or anything that would suggest adverse sampling conditions). Depending on the researcher's preference field data sheets can be found in Barbour et al. (1998) and from The New Brunswick Department of Natural Resources and Energy.

## **Qualitative Sampling**



A different approach is required for qualitative sampling. Many streams have multiple habitats making it necessary to have a method that can sample a variety of habitats. A solution to this problem is to use a D-frame dip net as a Kick-Net or as jabbing device. In “jabbing” the dip net is poked into the gravel to dislodge organisms. The field sampling procedures are the same as the semi-quantitative method, except that all instream habitats are sampled. Once the map is drawn, the proportions of different habitat are determined and weighted sampling is conducted. Barbour et al. (1998) suggest that habitat types that make up less than 5 percent of the surface area should not be sampled, but if intolerant species occupy these habitats failure to sample would give a misrepresentation of stream condition.

## **Composite samples**

In instances where an overall picture of water quality within an ecoregion or ecodistrict is desired, samples from within a single habitat type within a single reach are grouped together to form a composite sample. The same procedure is applied to test sites. The number of samples that go into a single composite sample is variable but ranges from four to six. The statistical distribution of the reference site samples are then used as a comparison basis for the test samples

## **Sample processing**

Processing begins by removing debris such as rocks, leaves and twigs and inspecting this material for attached invertebrates. The remaining material is then inspected for any large, obvious or rare organisms that could be lost during sample splitting. It can be argued that these organisms should be removed and included in the sample due to their biological significance and high probability of them being left out of the subsample (Barbour et al. 1998). These organisms can be placed in a separate container labeled as "large rare".

The rest of the sample is washed onto a 425  $\mu$  sieve for semi-quantitative samples and a 212  $\mu$  sieve for qualitative samples to separate light organic mater from the heavier sand and gravel. The sample is washed in a deep container half filled with water and swirled to suspend as much material as possible (Rosenberg et al.1997, unpublished data). The contents of the container are decanted onto an appropriate sieve until the sediment reaches the lip of the bucket.

A second container placed under the sieve catches any spilled material which is returned to the washing container for the next wash. The water in the backup container is discarded if it does not contain any spilled material, although it is convenient to reuse this water until washing is completed. By retaining this water, no new organisms are introduced and spilled material is not lost. Washing is continued until no organic material is left in the washing container. Finally, the material left in the washing bucket is inspected for invertebrates before being discarded. The most efficient way to sort this material is to place small quantities into a white pan filled with a small amount of water and to visually examine the material for any invertebrates (Rosenberg et al. 1997). In some instances the material is examined with a dissecting microscope to reveal any small organisms. If required, the inspected material can be used for quality control testing of

the washing efficiency, otherwise it is discarded at this point (Cuffney et al. 1993b). The organic material retained on the sieve is washed by swirling the sieve in a container of water and any large rare organisms within the sample are placed in the appropriate container. Samples to be immediately identified require no preservative. However, if sorting is to take place at a later date then ethanol should be added to preserve the sample and to kill any predatory species that might alter the species composition of the sample (Rosenberg et al. 1997).

There are other sorting techniques that can be useful in the recovery of organisms from sample material. Vital stains, flotation and elutriation have all been used successfully to separate organisms. Vital stains, such as Rose Bengal and Eosin B/Biebrich Scarlet can be used to stain organisms so as to stand out better against the white sorting pan. Flotation and elutriation work best for inorganic samples. Flotation works by putting the sample in a liquid with a high specific gravity, causing the organisms to float to the surface. Common flotation solutions include sugar, sodium chloride, and calcium chloride. The process of elutriation relies on the organisms being carried up in a column of bubbles and allowed to overflow into a trough or net. One problem with the last two techniques is that invertebrates with heavy shells or cases will not float. Even if these techniques are used it is recommended that a visual inspection of the material be performed (Rosenberg et al. 1997).

## **Subsampling**

In order to reduce the number of organisms to be identified and to save time and money, subsampling may be performed. The most abundant species can be subsampled while less abundant ones can be counted in full (Bargos et al. 1990). Subsampling is best conducted under laboratory conditions. The number of times a sample was split and the percentage of the original sample used for analysis should be recorded (Cuffney et al. 1993b).

Samples in multiple containers should be combined before subsampling and inspected for any large organic matter which is picked free of organisms and discarded. Samples preserved in alcohol should be rinsed and soaked for at least 15 minutes to rehydrate the organisms, which prevents floating during sorting (Barbour et al. 1998).

There are three basic methods for splitting: 1) the sieve splitter, 2) the sieve diameter splitter and 3) a tray with a grid overlay. The sieve splitter consists of a plexiglass box with a mesh bottom and two equally sized compartments tightly latched together. The sample is placed on the sieve and placed in water to help distribute the sample evenly over the surface of the sieve. The splitter is then taken from the water, drained, and unlatched to produce two subsamples. The sieve diameter splitter is simply a 20 cm diameter sieve that is transected by six equally spaced lines (diameters). The dividing lines extend up the sides of the sieve. Splitting is accomplished by placing the sample on the sieve, submerging it in water to uniformly distribute the sample and using a die to select which diameter line to use. A metal straight edge or a small scraper such as a putty knife, and a water bottle can be used to help split the sample along the selected diameter line. The selected half is analyzed and the other half is discarded unless it is required for quality control purposes (Cuffney et al. 1993b). The third method uses a pan marked into equal squares. The sample is spread throughout the pan and a random number table or dice are used to select the squares for analysis. Organisms that lie on a boundary are

including if the head lies within the selected area. In instances where the head is unidentifiable, the organism is included if more than 50% of its body lies within the selected area. Splitting is complete if the number of invertebrates is within twenty percent of the desired number of organisms which ranges from 100 to 300 (Barbour et al. 1998).

Subsamples to be immediately identified are placed in petri dishes, otherwise they are placed in glass vials and preserved in ethanol. Again attention to labeling is important and must include the sample identification number, date, stream name, and sampling location and number of splits (Barbour et al. 1998).

### ***Quality Control for Subsampling***

Quality control checks used in subsampling ensures that the estimates of number of taxa and proportion of each taxon meet the minimum requirements. A sample is subsampled until the subsample meets the criteria or until the entire sample has been processed. There are two checks used to evaluate subsampling. The first is that two subsamples must have at least 90 percent of the combined number of taxa in common and secondly, there must be a 90 percent similarity between the two communities represented by the subsamples, determined by the percentile similarity coefficient (PSC) (Cuffney et al. 1993a).

When the samples arrive at the lab they should be dated and recorded in a logbook. This entry will verify that all samples have arrived at the lab and are in proper condition for processing. During subsampling, notes should be taken on any relevant information, such as number of grids used to make up the sample or any difficulties. Any unused material from a sample should be labeled as "sorted residue" and identified with the appropriate labels. This material should be kept until personal in charge has given permission to discard it. Once all of the sorting has occurred for a given sample, all equipment used must be rinsed thoroughly, examined carefully, and picked free of organisms or debris. Organisms that are found are added to the sample residue (Barbour et al. 1998).

## **Taxonomy**

The taxonomic level of identification depends on the objectives of the monitoring program and cost. However, once decided the taxonomic level should be held constant between samples. The genus/species level requires more expertise but provides the most information on ecological-environmental characteristics and a more accurate assessment (Resh et al. 1995, Rosenberg et al. 1997). Identification made to the family level requires less expertise, is often more accurate and results are obtained faster (Barbour et al. 1998).

Dichotomous keys are used to identify organisms based on morphological characteristics. Any difficulties encountered during identification, such as a missing identification feature, should be recorded. For each sample, the identity, quantity, life stage, conversion factors (if subsampled or number per m<sup>2</sup>), taxonomist's initials and the taxonomic certainty rating (TCR), a measure of confidence, should be recorded. Specimens that are saved for future reference should be placed in jars with a small amount of denatured 70% ethanol and tightly capped. There must

be a proper label on the outside of the bottle, including the identification, date and type of preservative used (Rosenberg et al. 1997; Barbour et al. 1998).

### ***Quality control for taxonomy***

A taxonomist not responsible for the original identification should spot check all reference collection entries, spot check at least ten percent of the sample identifications and check the bench sheet for any errors (Barbour et al. 1998, Cuffney et al. 1993a).

A record of any specimens that are sent to private labs for validation should be made. The record should include label information, the date sent and any other relevant information. When the specimen returns, the date received and its identification should be recorded along with the person(s) who did the identification.

The science of taxonomy is ever changing with new species being identified and others being renamed. For this reason the lab should have an assortment of taxonomic keys and references that should be updated periodically. It is also recommended that taxonomists continue to educate themselves by reading new material and attending any training available on specific taxa groups (Barbour et al. 1998).

### **Statistical Analysis**

Biological data analysis should be relatively straightforward making it easy for policy makers to understand and allowing data to be a factor in decision making regarding the management of a resource (Environment Canada, 1998). Two common approaches are used to analyze macroinvertebrate data, the multimetric approach and a multivariate approach (Barbour et al. 1998).

Multimetric and multivariate methods depend on indices that gauge the condition of the stream. These indices are biological characteristics that change in an expected way with increased anthropogenic disturbance. There is a vast number of indices that have been developed for benthic invertebrate assessments, including taxa richness and composition measures, population attribute measures, tolerance/intolerance measures and functional feeding measures (Fore 1996, Resh 1994, Barbour et al. 1992, Barbour et al. 1998, Resh et al. 1995). Diversity and biotic indices demonstrate different qualities of the benthic invertebrate community. Diversity indices summarize the taxa's richness, evenness and abundance, so lower values typically represent impairment whereas biotic indices are based on specific indicator organisms to monitor a specific type of pollution (Barton and Metcalfe-Smith 1992). It can be argued that ratio metrics should not be used in assessments due to the possibility that the numerator and denominator can shift in a similar manner. It is presumptive to attribute a different biological meaning when these values are large than when they are small, because the same ratio and therefore the same score is obtained. Variance is also higher when two variables are combined to form a ratio than either one alone (Hannaford and Resh 1995, Fore et al. 1996). There are no clear recommendations on these problems.

Taxa richness represents the diversity of the benthic community at a given site and

usually consists of species level identifications but can also be evaluated at the genus, family or order level (Resh et al. 1995, Barbour et al. 1998). Increasing diversity correlates with increasing health of the assemblage and implies niche space, habitat, and food sources are satisfactory to support survival and reproduction of many species. Taxa richness and the Ephemeroptera, Plecoptera, Trichoptera (EPT) measure are successfully used in the United States and are taxonomically cost effective. The EPT index is more consistent in detecting impairment than the Simpson's, Shannon-Weiner composition indices, making them logical candidates for new water monitoring programs (Resh et al. 1995, Barbour et al. 1992, Resh 1994, Barton and Metcalfe-Smith 1992). Richness metrics detect heavy pollution with great success but do not clearly respond to slight organic pollution, in fact richness often increases (Cao et al. 1996, Metcalfe-Smith 1992). Resh (1994) found that after an acid spill all richness measures tested were accurate in indicating that an impairment had occurred and did not give false signals in areas that were not affected. A study by Stone and Wallace's (1998) on long term stream recovery from clear cutting found that the EPT measure and percent dominant taxa measure did not detect any initial difference between reference and clear cut streams, indicating that these metrics may not be useful for monitoring recovery from forest harvesting.

Composition measures provide information on the makeup of the streams invertebrate assemblage and the relative contribution of the individual populations to the total fauna. Ecologically important taxa provide information that is essential in describing the condition of the assemblage. Taxa comprising healthy and stable assemblages exist in proportional balances that are relatively stable, though absolute numbers may vary to some extent. In the absence of specific information on interactions between taxa, relative abundance is more informative than absolute abundance (Barbour et al. 1998). The most suitable measure of community similarity may be the Pinkham and Pearson index, however the biotic index is also used extensively with success (Barbour et al. 1992, Resh 1994, Hannaford and Resh 1995).

Tolerance/intolerance indices portray the relative sensitivity to impairment and can be independent of taxonomy or be specifically tailored to taxa that are associated with pollution tolerances. Tolerance measures are most efficient when expressed as percentages of total abundance and intolerance metrics should be expressed as taxa richness (Fore and Karr 1996). Tolerant organisms inhabit most streams but their abundance increase as conditions decline. The intolerant taxa are never abundant, making accurate estimates of abundance difficult with reasonable sampling efforts, but the presence of these taxa alone provides critical information to the streams biological condition (Fore and Karr 1996). Tolerance is generally non-specific to the type of stressor, but metrics that are sensitive to specific degraders, such as sedimentation, habitat modification or low dissolved oxygen will be useful in detecting initial impairments (Resh et al. 1995). The Hilsenhoff biotic index (HBI) was developed to detect organic pollution and the Biotic Condition Index is useful for evaluating sedimentation (Resh 1994). Oligochaeta are very tolerant to organic enrichment and low oxygen concentrations making the metric % Oligochaeta a good indicator of sewage pollution, increasing with increased levels of impairment. The dominance of Chironomidae can be correlated with metal contamination. In heavy pollution, Chironomidae may represent 75% of the population whereas in unpolluted streams they typically represent under 20% (Barton and Metcalfe-Smith 1992). However, Oligochaeta and Chironomidae are usually well represented in most streams because they have species that represent pristine and impaired conditions (Bargos et al. 1990). Fore et al. (1996)

found that Plecoptera may be more sensitive to human influence than Ephemeroptera and Trichoptera because its abundance dropped more at sites with lower levels of disturbance than did the others. Including only tolerance metrics in an assessment is discouraged because defining the tolerance of every species is difficult and a strong response by some taxa may be overlooked because most assemblages are dominated by taxa that are neutral, neither sensitive or insensitive. This problem can be avoided by only concentrating on the most and least tolerant organisms (Fore and Karr 1996).

Functional feeding measures provide information on the balance of feeding strategies. These metrics include scrapers, shredders, gatherers, filterers, and predators. Trophic dynamics also fall within this category and include the relative abundance of herbivores, carnivores, omnivores and detritivores. A pristine stream contains organisms with different feeding strategies including specialized feeders such as scrapers, piercers, and shredders which are more sensitive to pollution (Barbour et al. 1998). A popular feeding metric and a good choice for designing a water monitoring program is the percentage of individuals in the scraper functional group (Resh et al. 1995, Barbour et al. 1992)

The multimetric approach is informative because it takes biological information from individual, population, community, and ecosystem levels and uses this to produce a single water quality rating system. The success of multimetric assessments is depended on the following considerations: 1) what population and/or community measures are relevant?, 2) what are the baselines against which these measures are to be compared? and 3) how much deviation from the baseline indicates impairment? (Rosenberg et al. 1997)

There are two phases to developing a multimetric biomonitoring program. The first phase is to select and calibrate metrics and then to create an index according to homogenous site classes which will form the basis of the assessment. The second phase is to assess the biological condition of each site and make a judgment on its level of impairment. The development of appropriate metrics is determined by the type of taxa to be sampled, the biological characteristics at reference conditions and the anthropogenic influences being assessed. Once the metrics are selected, they are evaluated for effectiveness and validity and poor indicators are eliminated as well as metrics with little or no relation to stressors. The remaining core metrics will provide useful information in determining relatively pristine sites or impaired biotic characteristics (Barbour et al. 1998).

When developing a multimetric index for an assessment there are five steps to be followed. The first step is to classify the stream resources, evaluating the natural differences among streams. The multimetric approach classifies streams by their geographic, physical, or chemical properties. Confirmation is done using biological data and should be done by comparing the sites with the most natural reference sites (Reynoldson et al. 1997). One approach is to use ecoregions which bases the biotic characteristics of streams on their physiographic features. However, most ecoregions are large, have greater internal variability than small ones and they assume that test site attributes exactly match ecoregion reference sites. There is little evidence that invertebrate communities demonstrate high levels of similarity within an ecoregion and local conditions may have a bigger impact on benthic invertebrate communities than regional ones. Local conditions can be important determinants of aquatic ecosystem characteristics, the instream, and riparian characteristics should be included in classification because aquatic invertebrates may exhibit a direct response to changes in these without water

quality problems (Resh et al. 1995). Wang et al. (1997) found that watershed land use was a better predictor of habitat quality and biotic integrity than riparian land use patterns with the exception of localized agricultural practices. For example, riparian zones used to pasture cattle showed increased erosion and sedimentation. Alternative classification methods such as, subecoregion, stream type, and elevation may be tested by using multiple biological characteristics including species composition and metrics (Barbour et al. 1998, Resh et al. 1995).

When establishing the reference condition it is desirable to use multiple reference stations to identify the variability in naturally occurring communities and the best attainable conditions within the area (Barbour et al. 1992, Reynoldson et al. 1997). It is important that classification puts reference sites into groups with similar habitat and invertebrate communities because comparisons need to be made where site characteristics are expected to have the same communities without any disturbance (Reynoldson et al. 1997).

The second step in the multimetric approach is to identify potential measures for each stream class. There are two technical qualities metrics that must be met, it must be ecologically relevant to the invertebrate community, meeting the program objectives and it must be sensitive to stressors, clearly making a distinction between the response and natural variation (Barbour et al. 1998). Successful multimetric indexes include a balance of metrics that respond across a wide range of impairment (Fore and Karr 1996). An index should include metrics from (1) taxonomic richness measures for diversity or variety of the assemblage, (2) composition measures for identity and dominance, (3) tolerance and intolerance measures that represent sensitivity to disturbance, and (4) trophic measures for information on feeding strategies (Fore and 1996, Barbour et al. 1998). Taking metrics from all four of these categories ensures that all elements of the benthic community are considered. Out of the immense number of metrics that may qualify, many will be discarded for various reasons, such as inadequate data or the range of data is not sufficient for discrimination between natural variability and human influences (Barbour et al. 1998).

Step three in the multimetric approach is to select the best metrics. The most accurate way to test the validity of metrics is to study their scores in both impacted and unimpacted streams (Brussock 1993). The metrics left after screening that distinguish between known reference and impaired conditions are called the core metrics. Core metrics are vital to the success of the biomonitoring program.

Step four is to further reduce the number of qualified metrics, selecting the best metrics to form the index. One difficulty in developing an index is that individual taxa may not be equally sensitive to all types of disturbance (Chessman and McEvoy 1998.) Standardization will convert each metric into an unitless score and makes the assumption that each metric is equal in importance (Barbour et al. 1998). Metrics are tested at reference sites to determine its distribution in pristine conditions which in turn is used to score sites that will undergo assessment. The 25th percentile (lower quartile) of reference expectations is often used, this assumes that 25 percent of sites in the reference database may be below the expectations for a certain metric. For metrics in which the score increases with added disturbance the upper quartile (75th percentile) is used. Values that are below the upper quartile of the reference distribution receives the highest score (Barbour et al. 1998). By using the suitable quartile as the threshold, scoring can be achieved by assigning five points to the maximum value of the reference population, three points to represent a lower condition and a score of one point would be

assigned to the most impaired. When there are no reference sites defined in the assessment, then aspects of all sites are used representing a gradient of conditions and an upper percentile such as the 95th can be used to determine scores (Barbour et al.1998).

The final step in establishing a multimetric assessment involves applying the index to determine the condition of the stream. The multimetric index combines all individual measures into one score, providing a way to judge if the stream is impaired. When a score is greater than the preset criteria the system is labeled pristine or excellent in condition. Streams that do not meet the criteria are impacted in some aspect, being pollution, suffering from habitat destruction or sedimentation. The multimetric index will not determine the cause of the problem but if the metrics making up the index and the raw data are investigated the problem often becomes clear (Barbour et al.1998).

The multimetric index approach can involve a lot of work initially, though once established assessment is quick, understandable and cost effective. There are some potential problems with the multimetric approach, often not all information collected is used, there can be redundancy in a combination index causing errors to be compounded and it is difficult to find current procedures (Reynoldson et al. 1997).

Multivariate methods are very commonly used in aquatic biological monitoring. They take each species as a variable, enabling the detecting of very small changes in community structure. In addition there are no prior assumptions required or establishment of reference sites and test sites. (Reynoldson et al. 1997, Cao et al. 1996). The multivariate approach does not assume that test sites exactly match reference groups but uses multivariate analysis to calculate the probability of a site belonging to a reference group based on its fauna (Reynoldson et al. 1997). Fore et al. (1996) argue that multivariate statistics are better for exploratory analysis when there is limited knowledge of the ecological system and testable hypothesis are to be generated.

One of the most common multivariate techniques to analyze monitoring data is Principal Components Analysis (PCA). This technique uses taxa lists and abundances to interpret differences between stream sites. PCA calculates the line that extracts the maximum amount of statistical variance from a cluster of points. For example, each point could represent a stream site and the number of dimensions through which the line passes is equal to the number of taxa collected. The method does not always work,Fore et al. (1996) found that PCA was unable to detect clear differences between their most and least disturbed sites.

Other ordination techniques, such as correspondence analysis are useful when analyzing species abundance, or count data. This technique is similar to PCA in that it extracts maximum statistical variance from the variance-covariance matrices of species and/or sites (Ludwig and Reynolds 1998). Another multivariate technique that can be useful is canonical correlation analysis, which can be used to relate two sets of variables to each other, for example, land use variables and taxa abundance (Fore and Karr 1996)

There are some problems with multivariate approaches, most are unable to show if the conditions are improving or deteriorating (Cao et al. 1996). Additional information is needed on species tolerance levels, physical and chemical properties to make a judgment on stream condition. Multivariate methods are often hard to understand so they are frequently overlooked by managers and the public (Reynoldson et al. 1997). Multivariate methods leave out important information on how animals feed, reproduce, or respond to human impairment and they are not



easily adapted to rank streams on their level of impairment (Fore and Karr 1996).

## **1998 field study**

In 1998, a study was conducted to evaluate four common sampling methods on three streams representing different degrees of human impact. All streams were located in southern New Brunswick, Canada. The site locations were: 1) the Pollet River, approximately 1 km upstream from its confluence with the Petitcodiac River, 2) Gorge Brook, located on the outskirts of the city of Moncton, approximately 1 km upstream from its intersection with the Trans Canada Highway and 3) Humphreys Brook located in the city of Moncton, approximately 50 meters downstream of a spillway at the intersection with Mill Road. Respectively these three habitats represent relatively pristine conditions, mild degree of impact from urbanization and direct impact from storm sewer effluent. The New Brunswick Department of the Environment has further water quality information on these sites which is currently unavailable.

The sampling methods consisted of Surber, Hess, Pump and Kick-Net. With the exception of the pump sampler which does not use a net, mesh size in all cases was 500 $\mu$ . The Surber sampler was of standard design, sampling a 30 cm sq area. The Hess sampler was constructed from a plastic pail with a diameter of 26 cm. The pump sampler was also constructed from a 26 cm diameter pail equipped with a 12 volt bilge pump. The Kick-Net was ordered from Wards Biological Supply Company and met EPA specification. The Kick-Net samples a 1 m<sup>2</sup> area.

A total of 10 samples were taken at each site and subsequently identified to family level in the laboratory using the taxonomic keys of Merritt and Cummins (1996). Collection dates are in Table 1. Electrofishing was conducted at all sites and covered an area of 500 m<sup>2</sup>, single pass with no barrier nets. Water temperature, oxygen and temperature were recorded on the day of electrofishing (Appendix 4). Two artificial substrate basket were placed at each site but trapped by ice conditions and not retrieved as part of this study.

### ***Results of pilot macroinvertebrate sampling***

Invertebrates counts for each site are listed in Appendix 1. The index used for analysis was total number of taxa. All data analysis were performed with Systat. Data were distributed normally and Levene's test detected equal error variance across groups ( $F=1.149$ ,  $P = 0.332$ ). The assumptions for analysis of variance were therefore met. A two way analysis of variances was used to detect difference among sites and methods. The paired difference method was used to detect difference among individual sites and methods (Systat). Anova and paired comparison results are given in Appendix 2. The level of significance in all tests was  $\alpha = 0.05$ .

Both sites and samples methods were found to be significantly different (Figure 1). There was no significant interaction between sites and methods. The total number of taxa in Humphreys Brook was significantly below values recorded in either the Pollet or Gorge Brook.

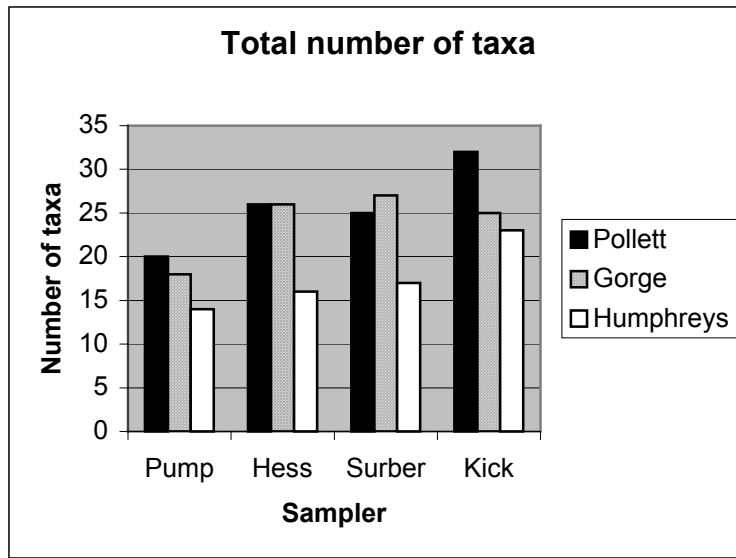


Figure 1. Total number of taxa identified in the Pollett River, Gorge Brook and Humphreys Brook. Total number of samples per method is 10.

This met with our hypothesis that proximity to a storm sewer effluent would depress the invertebrate community in Humphreys Brook. Pollett and Gorge Brook did not differ significantly in the total number of taxa captured .

Comparison of sampling methods was done on an individual site basis. In regards to the best method it was judged that data from the Pollett River should be used. This conclusion was based on the premise that the Pollett contained the greatest diversity, whereas the other two sites contained less. A large number of few taxa would be less likely to show differences among methods compared to higher diversity and fewer numbers. The goal in all cases is to select the sampling method that captures the largest number of macroinvertebrates.

### Comparison of methods by site

In descending order, the total number of taxa captured in the Pollett River showing a significant difference among methods were : Kick > Hess and Pump, Surber > Hess and Pump, and Hess > Pump (Figure 2). There was no significant difference between the Kick Net and the Surber at the Pollett River site.

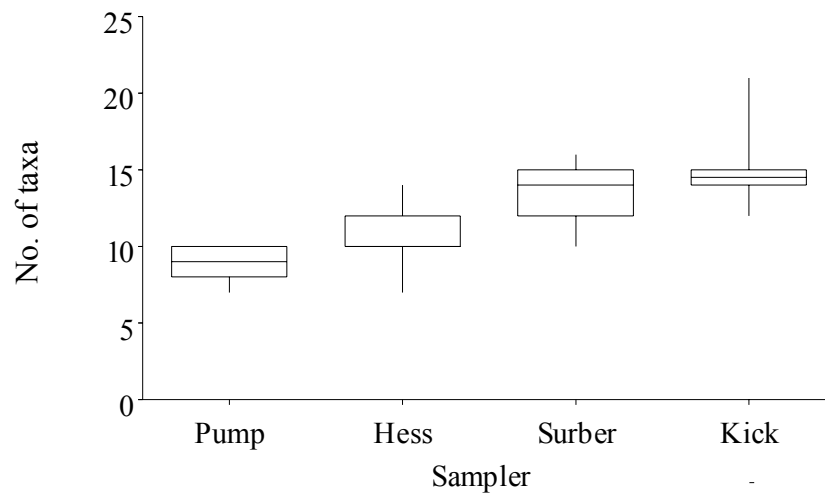


Figure 2. Boxplots of the total number of taxa identified at Pollett River. Sample size is 10. Central line is the median, other divisions represents quartiles.

At the Gorge Brook site the following methods were significantly different: Kick > Pump, Surber > Pump, and Hess > Pump (Figure 3). No other significant differences were found.

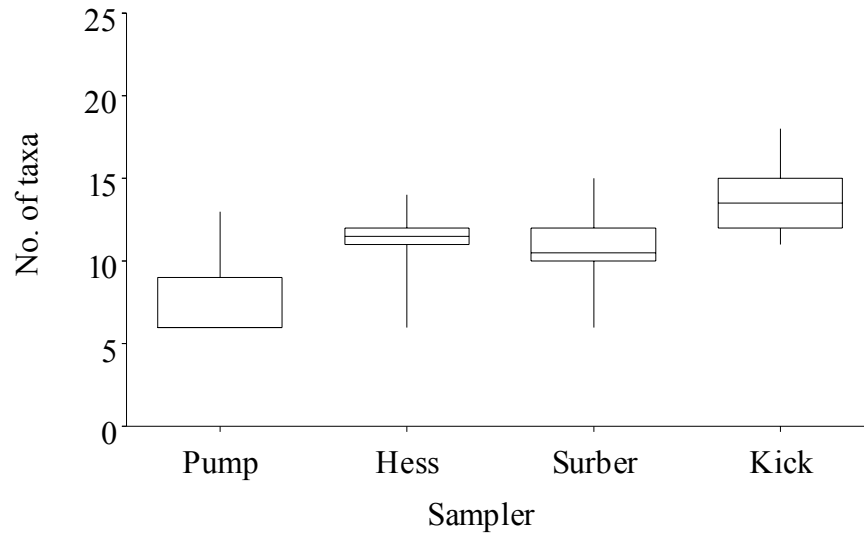


Figure 3. Boxplots of the total number of taxa identified at Gorge Brook. Sample size is 10. Central line is median other divisions are quartiles

Results at the Humphreys Brook site were identical to Gorge Brook where the following methods were significantly different: Kick > Pump, Surber > Pump and Hess > Pump (Figure 4). No other significant differences were found.

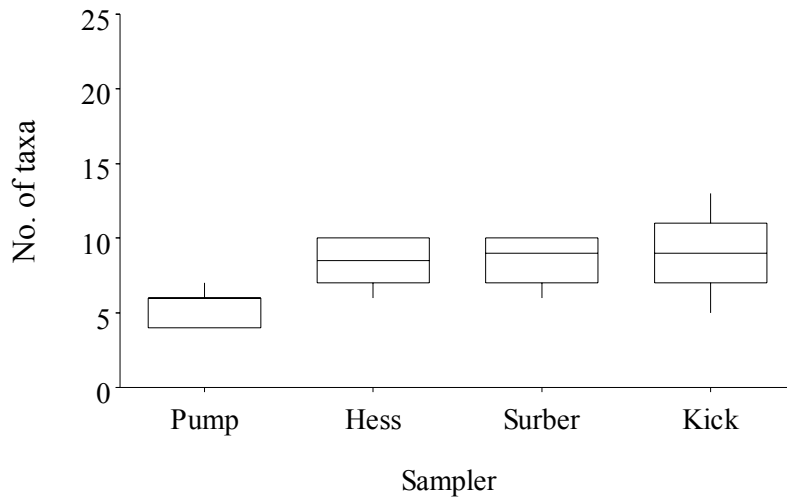


Figure 4. Boxplots of the total number of taxa captured in Humphreys Brook by method. Number of samples is 10. Central line is median, other divisions are quartiles.

### ***Comparison among sites***

A significant difference between the Pollett and the remaining two sites, Gorge and Humphreys Brook, was found for all methods except Hess. The Pollett River and Gorge Brook sites were not significantly different.

### ***Sample size***

The effect of sample size was investigated by examining the cumulative sum of the number of taxa identified with the additional of each sample replicate. In theory, the total number of taxa should stabilize as the number of taxa in the samples approaches the true population value. With the exception of the Kick-Net there appears to be a plateau reached at eight samples (Figure 5). Based on all sites it would appear to be ill-advised to take less than 6 samples. Again, the basis for number of samples should be draw from the site containing the highest diversity. In the case of Humphreys, the number of samples appear to reach a plateau at 4 with the exception of the kick net. This might be anticipated when the number of species is relatively small but abundant, in these circumstances fewer samples are more likely to represent the true population than the case of many different species but each being comparatively rare.

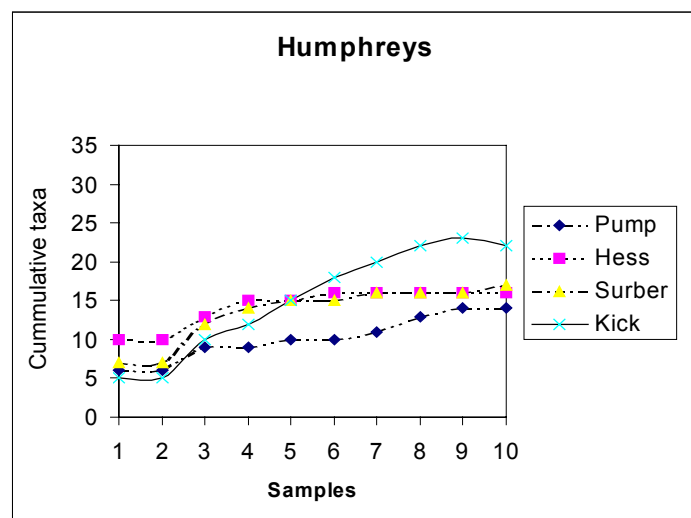
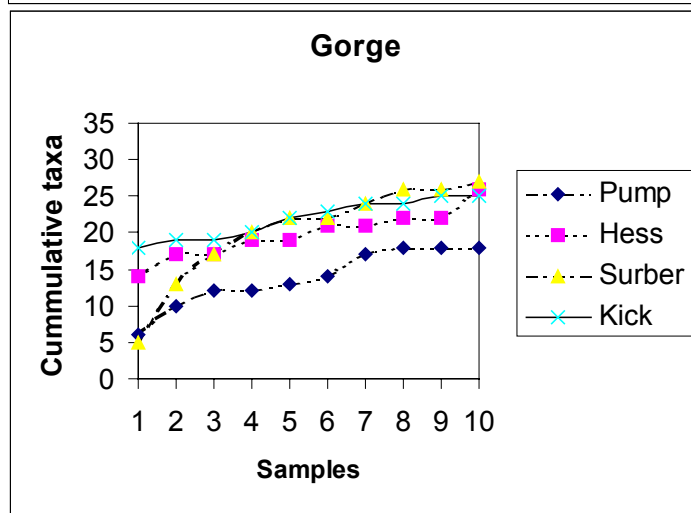
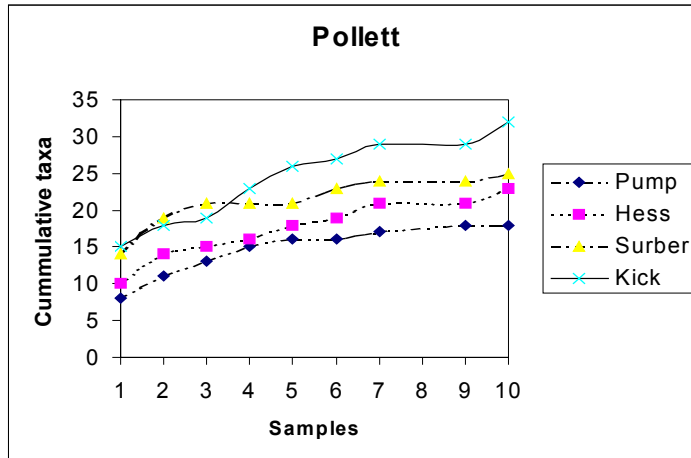


Figure 5. Cumulative number of taxa with increasing number of samples.

## ***Electrofishing results***

In general counts were low with the exception of Gorge Brook. The large number of brook trout (Salvelinus fontinalis) captured in Gorge Brook supports the contention that despite its urban location, impact is not severe. Counts were surprising low in the Pollett , perhaps because of the riffle location and the late date. In Gorge, the stream is sufficiently small that both pools and riffles are included in a 500 m<sup>2</sup> area. In Humphreys only mummichog (Fundulus heteroclitus), American eel (Anguilla rostrata) and golden shiner (Notemigonus crysoleucas) were captured. Mummichog and American eel are particular well know for there resistant to pollution..

## **Recommendations for water quality and biodiversity monitoring in the Fundy Model Forest**

- 1) There is considerable more information on using macroinvertebrate to assess water quality then there is to assess biodiversity. However, the two are strongly related as taxa richness is a frequently used index in assessing water quality yet equally qualifies as a measure of biodiversity.
- 2) The Kick-Net performed well in all habitat types. The ease of operation, requiring no bending makes it the method of choice. In addition, data collected by this method allows comparison with many data sets in the US. The method will also be used in Maine to evaluate the effects of forestry practices on water quality.
- 3) A minimum of eight samples is recommended.
- 4) In evaluating water quality over a geographical area, the ecodistrict unit of classification is recommended. Furthermore, samples may be combined to form a single composite. The total number of samples to comprise the reference sites as well as the test sites should be no less than 20 and ideally 30.
- 5) Quality control on taxonomic identification requires additional funds and at the moment the Huntsman is the only local institution other than universities in New Brunswick possessing the required expertise. However, this can be supplanted by an internal check provided by a university professor not involved in the initial identification.



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## **Appendices**

## Appendix 1

### ***Data sheets of macroinvertebrate counts***

Pollett River, New Brunswick.  
Pump Sampler  
Collected November 9,1998

	PP1	PP2	PP3	PP4	PP5	PP6	PP7	PP8	PP9	PP10	Total	%
Oligochaeta	3	13	17	17	27	8	3	3	20	2	113	27.83
Heptageniidae	12	5	13	3	4	8	12	2	6	3	68	16.75
Ephemeridae	0	0	0	0	0	0	0	0	0	1	1	0.25
Ephemerellidae	0	1	1	2	2	1	1	0	3	0	11	2.71
Isonychiidae	0	0	2	0	0	1	0	0	0	1	4	0.99
Baetidae	0	0	0	0	0	0	1	0	0	0	1	0.25
Leptophlebiidae	6	7	12	6	8	7	6	7	10	1	70	17.24
Pteronarcyidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlodidae	1	6	7	1	2	8	4	3	0	0	32	7.88
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Taeniopterygidae	1	1	3	0	6	1	2	2	1	0	17	4.19
Capniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Nemouridae	2	0	2	0	0	0	0	1	0	0	5	1.23
Chloroperlidae	0	1	0	0	0	0	0	0	0	1	2	0.49
Hydropsychidae	0	2	0	0	2	0	1	1	0	0	6	1.48
Brachycentridae	0	0	0	1	0	0	0	0	1	0	2	0.49
Polycentropodidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Phryganeidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Psychomyiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Odontoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Limnephilidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Rhyacophilidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Glossomatidae	1	0	0	0	0	0	0	0	0	1	2	0.49
Corydalidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Elmidae	0	0	0	1	0	0	0	0	0	1	2	0.49
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Chironomidae	5	1	13	1	10	0	6	3	3	7	49	12.07
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Tipulidae	0	0	0	0	1	0	0	1	0	0	2	0.49
Empididae	0	0	1	1	0	0	0	0	0	0	2	0.49
Simuliidae	0	0	0	0	0	0	0	1	0	0	1	0.25
Gomphidae	0	0	0	0	0	0	0	0	0	1	1	0.25
Cordulegastridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Isotomidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Gastropoda	0	0	0	0	0	0	0	0	0	0	0	0.00
Nematoda	0	0	0	0	0	0	0	0	0	0	0	0.00
Arachnida	0	0	0	0	0	0	0	0	0	0	0	0.00
Isopoda	0	0	0	0	0	0	0	0	0	0	0	0.00
Density/m <sup>2</sup>	747	892	1711	795	1494	819	867	578	1060	458	9422	
Total # Taxa	8	9	10	9	9	7	9	10	7	10	20	

Pollett River, New Brunswick.  
Hess  
Collected November 9, 1998

	PH1	PH2	PH3	PH4	PH5	PH6	PH7	PH8	PH9	PH10	Total	%
Oligochaeta	0	2	5	22	1	28	2	5	4	8	77	12.18
Heptageniidae	25	37	22	8	8	5	15	13	13	26	172	27.22
Ephemeraeidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Ephemereillidae	4	3	3	1	0	0	2	1	3	1	18	2.85
Isonychiidae	1	4	1	0	0	0	1	0	3	0	10	1.58
Baetidae	0	0	0	0	1	0	0	0	0	0	1	0.16
Leptophlebiidae	8	26	15	6	8	12	16	8	6	17	122	19.30
Pteronarcyidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlodidae	9	9	9	5	4	9	2	1	5	3	56	8.86
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Taeniopterygidae	0	5	0	2	0	2	2	3	7	2	23	3.64
Capniidae	0	0	0	0	0	0	0	0	0	1	1	0.16
Nemouridae	0	0	0	0	0	0	2	1	0	0	3	0.47
Chloroperlidae	9	7	2	1	0	0	4	0	3	0	26	4.11
Hydropsychidae	2	1	1	1	0	0	2	2	0	0	9	1.42
Odontoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Limnephilidae	0	0	0	1	0	0	0	0	0	0	1	0.16
Rhyacophilidae	0	0	0	0	0	0	0	0	1	0	1	0.16
Glossosomatidae	1	2	0	0	0	0	0	0	0	0	3	0.47
Philopotamidae	0	1	0	0	0	0	0	0	0	0	1	0.16
Corydalidae	0	0	0	0	0	0	1	0	0	0	1	0.16
Sialidae	0	0	0	0	0	1	0	0	0	0	1	0.16
Elmidae	1	0	0	1	0	0	2	0	1	0	5	0.79
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Chironomidae	6	20	10	10	4	16	2	10	5	9	92	14.56
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Tipulidae	0	0	1	0	0	0	0	2	0	0	3	0.47
Empididae	0	1	0	0	0	0	1	0	0	0	2	0.32
Simulidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Dolichopodidae	0	0	0	0	0	0	0	0	1	0	1	0.16
Athericidae	0	0	0	0	0	0	0	0	0	1	1	0.16
Pelecorhynchidae	0	0	0	0	0	0	0	0	0	1	1	0.16
Gomphidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Cordulegastridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Isotomidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Gastropoda	0	0	0	0	0	0	0	0	0	0	0	0.00
Nematoda	0	0	0	0	1	0	0	0	0	0	1	0.16
Arachnida	0	0	0	0	0	0	0	0	0	0	0	0.00
Density/m <sup>2</sup>	1590	2843	1663	1398	651	1759	1301	1108	1253	1663	15229	
Total # Taxa	10	13	10	11	7	7	14	10	12	10	26	

Pollett River, New Brunswick.  
 Surber  
 Collected November 9, 1998

	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8	PS9	PS10	Total	%
Oligochaeta	0	9	4	5	7	2	16	1	3	6	53	6.21
Heptageniidae	10	7	9	34	11	36	13	33	30	37	220	25.76
Ephemeridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Ephemerellidae	1	2	2	6	1	4	2	4	1	2	25	2.93
Isonychiidae	0	3	0	5	5	14	5	4	13	3	52	6.09
Baetidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Potamanthidae	0	0	0	0	0	2	0	0	0	0	2	0.23
Leptophlebiidae	10	17	18	14	9	30	12	11	33	15	169	19.79
Pteronarcyidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlodidae	8	3	9	6	7	14	6	1	13	10	77	9.02
Perlidae	0	0	0	0	0	2	0	0	1	0	3	0.35
Taeniopterygidae	1	1	2	17	2	12	2	5	9	5	56	6.56
Capniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Nemouridae	0	0	1	0	0	0	0	1	0	0	2	0.23
Chloroperlidae	1	0	0	2	0	4	0	4	3	2	16	1.87
Hydropsychidae	2	2	1	3	1	4	4	3	4	5	29	3.40
Brachycentridae	1	0	0	0	0	0	0	0	0	0	1	0.12
Polycentropodidae	1	0	0	0	0	0	0	0	0	0	1	0.12
Phryganeidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Psychomyiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Odontoceridae	0	1	0	0	0	0	1	0	3	0	5	0.59
Limnephilidae	4	1	0	1	0	2	1	3	1	5	18	2.11
Rhyacophilidae	1	0	0	0	0	0	0	0	0	0	1	0.12
Glossosomatidae	0	1	0	0	0	0	1	0	0	0	2	0.23
Philopotamidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Elmidae	4	0	0	0	0	2	1	1	1	2	11	1.29
Psephenidae	0	0	0	0	0	0	1	0	0	0	1	0.12
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Chironomidae	31	5	4	10	5	2	9	8	8	11	93	10.89
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Tipulidae	2	1	0	1	1	0	1	1	0	1	8	0.94
Empididae	0	0	0	0	0	0	0	0	0	0	0	0.00
Simuliidae	0	0	0	0	0	0	0	0	1	1	2	0.23
Dolichopodidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Athericidae	0	1	2	0	0	0	0	0	0	0	3	0.35
Pelecorhynchidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Gomphidae	0	0	1	0	0	1	0	0	1	0	3	0.35
Desnity/m <sup>2</sup>	770	540	530	1040	490	1310	750	800	1250	1050	8529	
Total # Taxa	14	14	11	12	10	15	15	14	16	14	25	



Pollett River, New Brunswick.  
 Kick-Net  
 Collected November 16,1998

	PK1	PK2	PK3	PK4	PK5	PK6	PK7	PK8	PK9	PK10	Total	%
Oligochaeta	26	10	6	10	16	8	18	10	2	4	110	3.53
Heptageniidae	54	116	64	58	56	90	34	130	145	180	927	29.73
Ephemeraeidae	0	1	0	0	8	0	0	0	0	2	11	0.35
Ephemereillidae	4	4	0	8	16	6	0	12	15	14	79	2.53
Isonychiidae	24	16	40	34	38	30	2	16	17	26	243	7.79
Baetidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Baetiscidae	0	0	0	0	6	0	0	0	0	0	6	0.19
Potamanthidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Leptophlebiidae	104	84	96	148	88	88	38	66	54	72	838	26.88
Pteronarcyidae	0	0	0	0	0	0	0	0	1	0	1	0.03
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlodidae	54	32	10	58	50	28	16	26	28	30	332	10.65
Perlidae	2	4	0	0	0	4	0	0	0	4	14	0.45
Taeniopterygidae	18	8	12	46	48	6	4	12	9	12	175	5.61
Capniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Nemouridae	0	0	2	0	4	0	0	2	0	0	8	0.26
Chloroperlidae	2	10	0	0	0	0	0	10	9	10	41	1.31
Hydropsychidae	4	6	12	8	26	16	0	4	10	12	98	3.14
Brachycentridae	0	0	0	0	6	4	2	0	1	0	13	0.42
Polycentropodidae	2	0	0	0	2	0	0	0	0	0	4	0.13
Odontoceridae	0	0	0	2	0	0	2	2	0	0	6	0.19
Limnephilidae	4	6	2	2	4	0	0	0	1	2	21	0.67
Rhyacophilidae	0	0	0	0	2	0	0	0	0	0	2	0.06
Glossosomatidae	0	0	0	0	0	4	0	0	0	0	4	0.13
Philopotamidae	0	0	0	2	0	0	0	0	0	0	2	0.06
Salidae	0	0	0	1	0	0	0	0	0	0	1	0.03
Corydalidae	0	0	0	2	2	0	2	2	0	0	8	0.26
Sialidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Elmidae	0	8	0	0	6	10	4	0	3	4	35	1.12
Psephenidae	0	0	0	0	0	0	0	0	1	0	1	0.03
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Chironomidae	6	6	6	6	4	18	6	12	11	16	91	2.92
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Tipulidae	0	2	0	0	2	0	0	0	1	0	5	0.16
Athericidae	8	0	4	0	2	6	0	2	1	0	23	0.74
Tabanidae	0	0	0	0	0	0	2	0	0	0	2	0.06
Stratiomyidae	0	0	0	0	0	0	4	0	0	0	4	0.13
Pelecorhynchidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Gomphidae	1	0	1	0	1	0	1	0	1	0	5	0.16
Arachnida	0	0	0	0	0	0	0	0	1	1	2	0.06
Density m <sup>2</sup>	313	313	255	385	387	318	135	306	311	389	3112	
Total # Taxa	15	15	12	14	21	14	14	14	19	15	32	

Gorge Brook, Moncton, New Brunswick  
 Pump, November 5, 1998

	GP1	GP2	GP3	GP4	GP5	GP6	GP7	GP8	GP9	GP10	Total	%
Oligochaeta	0	1	0	0	0	3	1	2	0	0	7	2.51
EphemereUidae	5	2	8	5	4	18	20	6	3	11	82	29.4
Baetidae	0	3	0	3	1	4	4	4	1	7	27	9.68
Perlodidae	0	0	0	0	0	0	2	0	2	0	4	1.43
Perlidae	0	0	0	0	0	0	2	0	0	0	2	0.72
Taeniopterygidae	0	0	0	0	0	0	3	0	0	1	4	1.43
Capniidae	0	0	0	0	0	0	0	1	0	0	1	0.36
Nemouridae	0	0	0	0	1	0	6	2	0	0	9	3.23
Chloroperlidae	0	1	0	2	0	2	3	0	0	1	9	3.23
Hydropsychidae	1	0	0	5	1	4	6	0	0	1	18	6.45
Leptoceridae	0	0	1	0	0	0	0	0	0	0	1	0.36
Glossosomatidae	0	0	1	0	0	0	0	0	0	0	1	0.36
Elmidae	3	9	12	2	1	8	12	6	1	0	54	19.4
Chironomidae	0	1	2	0	0	4	8	4	1	3	23	8.24
Ceratopogonidae	1	0	0	0	0	0	0	0	0	0	1	0.36
Tipulidae	2	0	2	2	2	8	5	8	2	0	31	11.1
Nematoda	0	0	0	0	0	1	3	1	0	0	5	1.79
Arachnida	1	0	0	0	0	0	0	1	0	0	2	0.72
Density/m <sup>2</sup>	313.3	409.6	626.5	457.8	241	1253	1807	843.4	241	578.3	6771	
Total # Taxa	6	6	6	6	6	9	13	10	6	6	18	

Gorge Brook, Moncton, New Brunswick.

Hess

Collected November 5, 1998

	GH1	GH2	GH3	GH4	GH5	GH6	GH7	GH8	GH9	GH10	Total	%
Oligochaeta	2	0	1	0	4	0	0	0	0	0	7	1.2
Ephemereillidae	18	24	30	15	34	10	5	24	7	14	181	31.2
Baetidae	15	14	2	6	11	10	0	5	3	5	71	12.2
Leptophlebiidae	0	0	0	0	0	0	0	0	0	1	1	0.17
Perlodidae	3	1	0	3	3	2	0	6	0	1	19	3.27
Perlidae	2	0	1	0	0	0	0	4	1	0	8	1.38
Taeniopterygidae	1	0	1	1	4	3	2	0	2	0	14	2.41
Capniidae	1	0	0	1	0	0	0	2	0	0	4	0.69
Nemouridae	1	0	0	0	1	10	0	0	4	1	17	2.93
Chloroperlidae	0	5	4	0	3	0	0	0	4	0	16	2.75
Hydropsychidae	2	8	1	4	6	8	1	4	2	4	40	6.88
Phryganeidae	0	1	0	0	0	0	0	0	0	0	1	0.17
Psychomyiidae	0	0	0	0	0	1	0	0	0	0	1	0.17
Leptoceridae	3	0	0	1	0	0	0	0	1	0	5	0.86
Odontoceridae	0	0	0	1	1	0	0	0	0	0	2	0.34
Limnephilidae	0	0	0	0	0	0	0	0	0	1	1	0.17
Rhyacophilidae	0	0	0	0	0	0	0	0	0	1	1	0.17
Glossosomatidae	1	0	1	0	0	0	0	0	1	1	4	0.69
Elmidae	1	5	4	24	13	2	8	5	4	17	83	14.3
Chironomidae	2	1	3	1	4	3	2	4	1	2	23	3.96
Ceratopogonidae	0	0	0	5	0	0	0	1	0	0	6	1.03
Tipulidae	8	11	4	19	11	2	6	5	5	7	78	13.4
Cordulegastridae	0	0	0	0	0	0	0	1	0	0	1	0.17
Gastropoda	0	1	0	0	0	0	0	0	0	0	1	0.17
Nematoda	0	0	0	0	0	0	0	0	0	1	1	0.17
Arachnida	0	0	0	0	0	0	0	0	0	0	0	0
Isopoda	0	0	0	0	0	1	0	0	0	0	1	0.17
Density/m <sup>2</sup>	1446	1711	1253	1952	2289	1253	578.3	1470	843.4	1349	14145	
Total # Taxa	14	10	11	12	12	11	6	11	12	13	26	

Gorge Brook, Moncton, New Brunswick.  
 Surber  
 Collected November 5, 1998

	GS1	GS2	GS3	GS4	GS5	GS6	GS7	GS8	GS9	GS10	Total	%
Oligochaeta	0	4	0	3	13	0	0	0	1	0	21	2.69
Ephemerellidae	35	44	49	37	44	31	10	75	16	13	354	45.3
Baetidae	14	8	12	8	4	12	1	7	10	0	76	9.72
Leptophlebiidae	0	1	0	1	1	0	0	0	0	0	3	0.38
Peltoperlidae	0	0	0	0	0	0	0	1	0	0	1	0.13
Perlodidae	4	2	4	3	1	1	0	0	2	0	17	2.17
Perlidae	0	0	9	1	0	0	0	0	0	0	10	1.28
Taeniopterygidae	1	1	0	0	3	0	1	1	0	0	7	0.9
Capniidae	0	0	1	0	0	1	0	0	0	0	2	0.26
Nemouridae	0	1	4	1	1	0	1	0	0	2	10	1.28
Chloroperlidae	0	0	1	1	0	1	0	3	0	0	6	0.77
Hydropsychidae	7	8	17	6	6	22	4	5	1	0	76	9.72
Brachycentridae	0	0	0	0	0	0	0	0	0	1	1	0.13
Polycentropodidae	0	0	0	0	0	0	0	1	0	0	1	0.13
Phryganeidae	0	0	0	0	0	0	0	0	0	0	0	0
Psychomyiidae	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0
Odontoceridae	0	0	0	0	0	0	0	0	0	0	0	0
Limnephilidae	0	0	0	0	3	0	0	0	0	0	3	0.38
Rhyacophilidae	2	0	2	0	0	3	0	0	1	0	8	1.02
Glossosomatidae	0	0	1	0	1	0	0	0	0	0	2	0.26
Elmidae	5	12	9	13	14	7	1	6	2	1	70	8.95
Staphylinidae	0	0	0	1	0	0	0	0	0	0	1	0.13
Chironomidae	2	1	0	1	2	2	5	1	1	1	16	2.05
Ceratopogonidae	0	0	0	1	0	0	0	0	0	0	1	0.13
Tipulidae	11	12	3	9	40	3	11	2	2	6	99	12.7
Cordulegastridae	0	0	0	0	0	0	1	0	0	0	1	0.13
Isotomidae	0	0	0	1	0	0	0	0	0	0	1	0.13
Gastropoda	1	0	0	0	3	0	1	0	0	0	5	0.64
Nematoda	0	0	0	0	4	0	0	0	0	0	4	0.51
Arachnida	0	0	0	0	0	0	1	0	0	0	1	0.13
Isopoda	0	0	0	0	0	0	0	0	0	0	0	0
Density/m <sup>2</sup>	1976	2265	2699	2096	3373	2000	891.6	2458	867.5	578.3	19205	
Total # Taxa	10	11	12	15	15	10	11	10	9	6	27	

Gorge Brook, Moncton, New Brunswick.  
 Kick-Net  
 Collected November 16, 1998

	GK1	GK2	GK3	GK4	GK5	GK6	GK7	GK8	GK9	GK10	Total	%
Oligochaeta	4	5	8	13	5	4	10	10	0	8	67	2.2
Ephemerellidae	68	34	122	70	64	64	132	270	31	246	1101	36.1
Baetidae	40	6	38	26	26	44	32	46	4	16	278	9.11
Leptophlebiidae	0	0	0	2	0	2	2	4	0	12	22	0.72
Pteronarcyidae	0	0	0	0	0	1	1	0	0	0	2	0.07
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0
Perlodidae	4	2	18	4	6	8	12	26	5	18	103	3.37
Perlidae	4	0	0	0	4	6	1	12	1	8	36	1.18
Taeniopterygidae	4	1	2	2	4	6	0	0	1	12	32	1.05
Capniidae	2	0	0	0	2	0	0	0	0	0	4	0.13
Nemouridae	0	2	2	0	1	0	2	0	0	4	11	0.36
Chloroperlidae	0	0	0	0	1	0	0	2	0	0	3	0.1
Hydropsychidae	16	6	8	13	36	40	40	96	20	34	309	10.1
Brachycentridae	0	0	0	0	1	0	0	0	0	0	1	0.03
Polycentropodidae	0	0	0	0	0	0	2	4	2	2	10	0.33
Phryganeidae	0	0	0	0	0	0	0	0	0	0	0	0
Psychomyiidae	1	0	0	0	0	2	0	0	0	0	3	0.1
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0
Odontoceridae	0	0	0	0	0	0	0	0	0	0	0	0
Limnephilidae	0	0	0	0	0	0	0	0	0	0	0	0
Rhyacophilidae	4	0	6	2	6	10	6	16	2	8	60	1.97
Glossosomatidae	2	0	0	1	0	0	0	0	0	0	3	0.1
Corydalidae	0	0	0	0	0	0	0	0	1	0	1	0.03
Elmidae	60	71	174	83	83	74	90	74	34	30	773	25.3
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	6	4	0	3	0	0	2	0	0	0	15	0.49
Ceratopogonidae	2	1	2	2	1	2	0	0	2	2	14	0.46
Tipulidae	14	6	12	25	20	10	28	28	8	16	167	5.47
Cordulegastridae	0	0	0	0	0	0	0	0	0	0	0	0
Isotomidae	0	0	0	0	0	0	0	0	0	0	0	0
Gastropoda	1	0	0	0	0	0	0	0	0	0	1	0.03
Nematoda	2	0	2	0	0	0	0	0	0	0	4	0.13
Arachnida	1	0	0	0	0	1	1	0	0	0	3	0.1
Isopoda	0	0	0	0	0	0	0	0	0	0	0	0
Density/m <sup>2</sup>	5663	3325	9494	5928	6265	6602	8699	14169	2675	10024	72843	
Total # Taxa	18	11	12	13	15	15	15	12	12	14	25	

Humphreys Brook, Moncton, New Brunswick.  
Pump Sampler  
Collected October 28, 1998

	HP1	HP2	HP3	HP4	HP5	HP6	HP7	HP8	HP9	HP10	Total	%
Oligochaeta	0	0	0	0	0	0	0	0	0	0	0	0.00
Glossiphoniidae	0	0	0	0	0	0	1	0	0	0	1	0.32
Gammaridae	2	2	5	8	1	2	7	6	1	8	42	13.25
Heptageniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Ephemeridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Ephemerellidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Isonychiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Baetidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Leptophlebiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Pteronarcyidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlodidae	0	0	0	0	0		0	0	0	0	0	0.00
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Taeniopterygidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Capniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Nemouridae	1	1	12	0	5	1	2	3	3	0	28	8.83
Chloroperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Hydropsychidae	0	0	1	1	1	0	0	0	0	0	3	0.95
Brachycentridae	0	0	0	0	0	0	0	1	0	0	1	0.32
Polycentropodidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Phryganeidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Psychomyiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Odontoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Limnephilidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Rhyacophilidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Glossosomatidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Sialidae	0	0	0	0	0	0	0	1	0	0	1	0.32
Corydalidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Elmidae	1	1	2	1	1	1	0	3	3	5	18	5.68
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Chironomidae	24	26	39	9	14	20	17	17	14	22	202	63.72
Ceratopogonidae	0	3	0	0	0	0	0	0	0	1	4	1.26
Stratiomyiidae	0	0	0	0	0	0	0	0	1	0	1	0.32
Tipulidae	1	0	0	0	0	0	0	0	0	0	1	0.32
Empididae	0	0	0	0	2	0	0	0	0	0	2	0.63
Gastropoda	2	0	0	0	0	0	0	0	0	0	2	0.63
Nematoda	0	1	3	0	2	0	0	0	1	4	11	3.47
Density/m <sup>2</sup>	747	819	1494	458	627	578	651	747	554	964	7639	
Total # Taxa	6	6	6	4	7	4	4	6	6	5	14	

Humphreys Brook, Moncton, New Brunswick.

Hess

Collected October 28, 1998

	HP1	HP2	HP3	HP4	HP5	HP6	HP7	HP8	HP9	HP10	Total	%
Oligochaeta	0	2	1	0	1	0	0	0	1	1	6	0.67
Glossiphoniidae	1	0	0	0	0	0	0	1	0	0	2	0.22
Gammaridae	26	7	6	5	14	25	7	7	13	15	125	13.90
Heptageniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Ephemeridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Ephemerellidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Isonychiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Baetidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Leptophlebiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Pteronarcyidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlodidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Taeniopterygidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Capniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Nemouridae	5	3	0	9	7	3	3	5	11	1	47	5.23
Chloroperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Hydropsychidae	0	1	0	0	0	2	3	4	1	0	11	1.22
Brachycentridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Polycentropodidae	0	0	0	2	0	0	0	0	0	0	2	0.22
Phryganeidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Psychomyiidae	1	0	0	6	2	2	3	3	0	0	17	1.89
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Odontoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Limnephilidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Rhyacophilidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Glossosomatidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Sialidae	2	1	0	1	1	0	0	0	1	0	6	0.67
Corydalidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Elmidae	10	12	11	9	5	23	7	30	24	14	145	16.13
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Chironomidae	54	52	26	57	39	45	54	85	51	29	492	54.73
Ceratopogonidae	1	0	0	0	0	0	0	0	1	1	3	0.33
Stratiomyiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Tipulidae	0	0	0	0	0	1	3	0	0	0	4	0.44
Empididae	3	0	0	0	1	0	0	0	0	0	4	0.44
Simuliidae	0	0	0	1	0	0	0	0	0	0	1	0.11
Isotomidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Gastropoda	3	0	1	10	8	3	1	0	1	0	27	3.00
Nematoda	0	1	2	1	3	2	0	0	0	1	10	1.11
Density/m <sup>2</sup>	2554	1904	1133	2434	1952	2554	1952	3253	2506	1494	21735	
Total # Taxa	10	8	6	10	10	9	8	7	9	7	16	

Humphreys Brook, Moncton, New Brunswick.  
 Surber  
 Collected October 28, 1998

	HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8	HS9	HS10	Total	%
Oligochaeta	0	0	0	1	0	0	1	0	0	1	3	0.40
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Gammaridae	5	13	3	19	18	15	6	12	22	5	118	15.67
Heptageniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Caenidae	0	0	0	0	0	0	0	0	0	1	1	0.13
Ephemeridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Ephemerellidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Isonychiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Baetidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Leptophlebiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Pteronarcyidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlodidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Taeniopterygidae	0	0	0	10	0	0	0	0	0	0	10	1.33
Capniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Nemouridae	0	3	2	0	0	4	4	2	1	1	17	2.26
Chloroperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Hydropsychidae	1	1	0	0	0	1	0	2	0	0	5	0.66
Brachycentridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Polycentropodidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Phryganeidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Psychomyiidae	1	1	3	1	0	0	0	4	0	0	10	1.33
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Sialidae	0	0	0	0	1	1	0	0	0	0	2	0.27
Corydalidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Elmidae	2	1	5	7	19	18	11	27	3	7	100	13.28
Staphylinidae	0	0	0	0	0	0	0	0	0		0	0.00
Chironomidae	31	41	48	49	38	30	40	51	30	30	388	51.53
Ceratopogonidae	0	1	1	0	0	1	0	0	0	2	5	0.66
Stratiomyiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Tipulidae	0	1	0	0	2	0	0	0	0	0	3	0.40
Empididae	1	3	4	3	0	1	1	4	0	0	17	2.26
Simulidae	0	0	1	0	0	0	1	0	0	0	2	0.27
Trichogrammetidae	0	0	0	0	0	0	1	0	0	0	1	0.13
Isotomidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Gastropoda	8	1	5	4	13	3	3	2	8	1	48	6.37
Nematoda	0	0	3	2	0	0	2	4	3	1	15	1.99
Density/m <sup>2</sup>	490	659	749	959	909	739	699	1079	669	490	7443	
Total # Taxa	7	10	10	9	6	9	10	9	6	9	17	



Humphreys Brook, Moncton, New Brunswick.  
 Kick-Net  
 November 16, 1998

	HK1	HK2	HK3	HK4	HK5	HK6	HK7	HK8	HK9	HK1	Total	%
										0		
Oligochaeta	0	1	1	2	2	1	0	1	0	1	9	3.37
Glossiphoniidae	0		0	0	1	0	1	0	0	0	2	0.75
Gammaridae	0	1	1	6	0	8	1	1	0	0	18	6.74
Heptageniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Caenidae	0	0	0	0	0	1	0	0	0	0	1	0.37
Ephemeridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Ephemerellidae	0	0	0	0	0	0	1	0	0	0	1	0.37
Isonychiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Baetidae	0	1	0	0	0	0	1	2	1	0	5	1.87
Leptophlebiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Pteronarcyidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Peltoperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Perlodidae	0	0	1	0	0	0	0	0	0	0	1	0.37
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Taeniopterygidae	0	0	0	0	0	3	0	1	1	0	5	1.87
Capniidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Nemouridae	1	3	5	1	4	4	2	2	0	1	23	8.61
Chloroperlidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Hydropsychidae	6	8	2	2	4	7	8	8	2	5	52	19.48
Brachycentridae	0	0	0	0	0	0	0	0	0	0	0	0.00
Polycentropodidae	0	0	0	1	0	0	0	0	0	0	1	0.37
Phryganeidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Psychomyiidae	0	0	0	0	0	0	1	0	0	0	1	0.37
Saldidae	0	0	0	0	0	1	2	0	0	0	3	1.12
Elmidae	1	4	8	1	1	7	2	1	1	3	29	10.86
Staphylinidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Chironomidae	12	5	6	8	1	20	5	11	5	7	80	29.96
Ceratopogonidae	0	0	0	0	0	0	0	1	0	0	1	0.37
Stratiomyiidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Tipulidae	0	3	1	2	1	1	1	1	0	0	10	3.75
Empididae	0	0	0	0	0	0	0	0	0	0	0	0.00
Simuliidae	2	1	1	0	2	3	2	4	2	4	21	7.87
Gomphidae	0	0	0	0	0	0	0	0	0	0	0	0.00
Coenagrionidae	0	0	0	0	0	0	0	1	0	0	1	0.37
Chaoboridae	0	0	0	0	1	0	0	0	0	0	1	0.37
Gastropoda	0	0	0	1	0	0	2	0	0	0	3	1.12
Nematoda	0	0	0	0	0	0	0	0	0	0	0	0.00
Arachnida	0	0	0	0	1	0	0	0	0	0	1	0.37
Isopoda	0	0	0	0	0	0	0	0	1	0	1	0.37
Density/m <sup>2</sup>	22	27	26	24	18	56	29	34	13	21	270	
Total # Taxa	5	9	9	9	10	11	13	12	7	6	23	

## Appendix 3

### ***Analysis of variance results and multiple comparisons***

#### Univariate Analysis of Variance

Source	df	F	Significance
Methods	3	37.507	< 0.000
Sites	2	42.632	<0.000
Methods x Sites	6	1.829	0.100

Pairwise difference between methods  
Matrix of pairwise comparison probabilities

#### Pollett

	Pump	Hess	Surber	Kick
Pump	1.000			
Hess	0.343	1.000		
Surber	10.3403	0.007	1.000	
Kick	0.00	0.000	0.736	1.000

#### Gorge

	Pump	Hess	Surber	Kick
Pump	1.000			
Hess	0.001	1.000		
Surber	10.004	1.000	1.000	
Kick	0.000	0.323	0.155	1.000

#### Humphreys

	Pump	Hess	Surber	Kick
Pump	1.000			
Hess	0.001	1.000		
Surber	0.000	1.000	1.000	
Kick	0.000	1.000	1.000	1.000

Pairwise difference between sites

Pump

	Pollett	Gorge	Humphreys
Pollett	1.000		
Gorge	0.135	1.000	
Humphreys	0.000	0.018	1.000

Hess

	Pollett	Gorge	Humphreys
Pollett	1.000		
Gorge	1.000	1.000	
Humphreys	0.128	0.021	1.000

Surber

	Pollett	Gorge	Humphreys
Pollett	1.000		
Gorge	0.058	1.000	
Humphreys	0.000	0.054	1.000

Kick

	Pollett	Gorge	Humphreys
Pollett	1.000		
Gorge	0.058	1.000	
Humphreys	0.000	0.058	1.000

## APPENDIX 4

### ***Water quality, weather conditions and electrofishing results***

**Location:** Humphreys Brook, Moncton New Brunswick.

**Date:** October 28, 1998.

**Time:** 11:30 - 16:30

**Crew:** Chris Williams and Darren

**Weather:** Overcast, 60% cloud cover

**Air Temperature:** 12°C.

**Water Temperature:** 5°C.

**Dissolved Oxygen:** 10.8

**Tests:** 10 Surber, 10 Hess, 10 Pump, 2 Artificial Substrate baskets.

#### **Notes:**

Ducks feeding above spillway and throughout study area.

Possible oxygen input with spillway above study area.

#### **November 16, 1998**

**Tests:** 10 Kick Net

**Time:** 9:30-11:30

**Crew:** Chris Williams and Darren

**Weather:** Sunny, 40% cloud cover.

**Air Temperature:** 1°C

**Water Temperature:** 2°C

#### **Electrofishing**

**Location:** Humphreys Brook, Moncton, New Brunswick.

**Date:** November 17, 1998.

**Time:** 9:30-11:30

**Crew:** Chris Williams and Darren

**Weather:** Sunny, 0% cloud cover.

**Air Temperature:** -6°C

**Water Temperature:** 2°C

**Species:** American eel 19

Mummichog 7

Golden shiner 1

**Location:** Gorge Brook, Moncton, New Brunswick.  
**Date:** November 5, 1998.  
**Time:** 9:30 - 14:00  
**Crew:** Chris Williams and Darren  
**Weather:** Sunny, 5% cloud cover.  
**Air Temperature:** 6°C.  
**Water Temperature:** 4.5°C.  
**Dissolved Oxygen:** 12.2  
**Tests:** 10 Suber, 10 Hess, 10 Pump, 2 Artificial Substrate baskets.

**November 16, 1998**

**Tests:** 10 Kick Net  
**Time:** 12:00-13:30  
**Crew:** Chris Williams and Darren  
**Weather:** Sunny, 60% cloud cover.  
**Air Temperature:** 3°C  
**Water Temperature:** 2°C

**Electrofishing**

**Location:** Gorge Brook, Moncton, New Brunswick.  
**Date:** November 17, 1998.  
**Time:** 12:00-13:30  
**Crew:** Chris Williams and Darren  
**Weather:** Sunny, 0% cloud cover.  
**Air Temperature:** -2°C  
**Water Temperature:** 1.5°C  
**Species:** Brook trout 20  
                  American eel 1

Brook Trout Measurements (cm)

1. 8.2	6. 7.3	11. 7.2	16. 5.4
2. 7.5	7. 7.4	12. 7.5	17. 8.5
3. 14.9	8. 8.4	13. 7.8	18. 6.8
4. 9.7	9. 7.7	14. 7.3	19. 6.0
5. 8.5	10. 6.0	15. 7.9	20. 5.8

**Location:** Pollet River, New Brunswick.

**Date:** November 9, 1998.

**Time:** 10:00 - 14:30

**Crew:** Chris Williams and Darren

**Weather:** Overcast, 90% cloud cover.

**Air Temperature:** 2.0°C.

**Water Temperature:** 3.0°C.

**Dissolved Oxygen:** 12.3

**Tests:** 10 Suber, 10 Hess, 10 Pump, 2 Artificial Substrate baskets.

### **November 16, 1998**

**Tests:** 10 Kick Net

**Time:** 14:00-15:00

**Crew:** Chris Williams and Darren

**Weather:** Sunny, 60% cloud cover.

**Air Temperature:** 4°C

**Water Temperature:** 1.5°C

### **Electrofishing**

**Location:** Pollet River, New Brunswick.

**Date:** November 17, 1998.

**Time:** 14:00-15:00

**Crew:** Chris Williams and Darren

**Weather:** Sunny, 60% cloud cover.

**Air Temperature:** -1.5°C

**Water Temperature:** 1.0°C

**Species:** White sucker (Catostomus commersoni) 4

Threespine stickleback (Gasterosteus aculeatus) 1

Blacknose dace (Rhinichthys atratulus) 5

Golden shiner 11

# **Appendix 5**

## ***Brochure***