# The rapid bioassessment of lakes: protocol design and testing in Manitoba's boreal shield

by

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**ABSTRACT**

Rapid bioassessment (RBA) methods have largely been used for streams and rivers, with little development of equivalent methods to be used in lakes. This has restricted the assessment of lakes because traditional methods are time- and cost-intensive. Here I show that a newly designed RBA protocol can be used to monitor a wide range of boreal shield lakes effectively. Seventy per cent of lakes with over 25% of their shoreline developed with cottages were assessed as impacted using a multimetric index. This research has built on previous knowledge, placing new emphasis on standardizing sampling efforts by depth, habitat type (cobble sediments) and sample area in lakes. My recommendations provide water resource managers with methods that can be used as a screening tool to monitor a large group of lakes affected by a variety of stressors.

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# CHAPTER 1. Introduction

## 1.1 Rapid bioassessment

Canada's boreal shield extends from Newfoundland to northeastern Alberta, with a landscape dominated by conifers, Archean rocks, thin soils and hundreds of thousands of lakes. The lake basins were formed during the Wisconsinan ice age, when movement of glaciers scoured depressions into bedrock, removing soil and rock from some areas and depositing them in others. Until relatively recently, the boreal shield was a vast, relatively uninhabited, wilderness area. Today, this area's resources are utilized on large scales (e.g., mining, forestry, agriculture, aquaculture) and greater access to remote areas has increased recreational development (e.g., motor boating, fishing) and urbanization. These impacts can affect lake ecosystems that are important aesthetically, recreationally, industrially, and as a source of drinking water for humans. However, the constraints of time and cost make assessing the impacts of increased development across this broad geographic area using traditional lake biomonitoring methods problematic.

Biomonitoring is 'surveillance using the response of living organisms to determine whether the environment is favourable to living material' (Cairns and Pratt 1993). Surveillance of biotic responses began in early twentieth century Europe when Kolkwitz and Marsson (1908, 1909) examined macrophyte and invertebrate communities in relation to the degree of organic pollution in streams (Cairns and Pratt 1993). Since then, aquatic biomonitoring has expanded to include a variety of impacts using a number of different biological assemblages including macrophytes, diatoms,

phytoplankton, periphyton, zooplankton, benthic invertebrates and fish (US EPA 1998).

Benthic macroinvertebrates are the most commonly recommended (Hellawell 1986) and used (Abel 1989; Rosenberg and Resh 1993) assemblage in biomonitoring because of their diversity, biology and responsiveness to impacts. The term benthic macroinvertebrate includes invertebrates that live near, on, or in the substratum and that are retained in a minimum mesh size of 200 to 500 μm during at least one of their life stages (e.g., molluscs, leeches, crustaceans, oligochaetes, insects) (Rosenberg and Resh 1993). High diversity has resulted from the adaptation of macroinvertebrates to nearly all freshwater habitats (Lenat *et al.* 1980), and different species respond to impacts in different ways and in varying degrees (Abel 1989; Hellawell 1986). Extensive research on benthic macroinvertebrates has provided researchers with valuable information on habitat requirements, as well as numerous taxonomic keys to facilitate their identification (Abel 1989; Hellawell 1986). Macroinvertebrate abundance as well as small body size allows them to be sampled easily (Abel 1989; Lenat *et al.* 1980), while their sedentary nature allows the spatial extent of impacts to be delineated (Abel 1989; Rosenberg and Resh 1993). Their lifespan is usually long enough to be affected by exposure to contaminants or pollution (Abel 1989; Reice and Wohlenberg 1993), yet short enough to allow changes in community structure to be observed (Rosenberg and Resh 1993). The proximity of benthic macroinvertebrates to sediments can also be advantageous for biomonitoring; sediments may act as a sink for certain contaminants, causing increased exposure through prolonged contact (Reice and Wohlenberg 1993). Lastly, benthic macroinvertebrates are an ideal choice for biomonitoring because of

their placement in the aquatic food web. Invertebrate herbivores feed on macrophytes and periphyton (Lamberti and Moore 1984; Merritt and Cummins 1996; Merritt *et al.* 1984), converting primary producers into a protein source for other macroinvertebrates as well as fish (Healey 1984), and invertebrate detritivores recycle large amounts of decaying matter (Merritt *et al.* 1984). Thus, monitoring benthic invertebrates can allow inferences to be made concerning the biomass of other trophic levels (Hellawell 1986; Lamberti and Moore 1984; Post and Cucin 1984) as well as the energy transfer that occurs between them.

Traditionally, benthic macroinvertebrate biomonitoring has required taxonomic expertise and many replicate samples for statistical analyses (e.g., ANOVA, BACI). The reliance on identifying invertebrates to species and sorting multiple samples likely led to the characterization of traditional methods as expensive and labour-intensive (Lenat and Barbour 1993). The resources needed to complete bioassessments using these methods would limit monitoring programs to only a few lakes.

Rapid bioassessment (RBA) protocols were developed as cost-effective alternatives to traditional labour-intensive, quantitative protocols. RBA is primarily intended to be used as a screening tool (David *et al.* 1998) to identify sites warranting further investigation (Resh *et al.* 1995). The use of RBA techniques is a relatively new stage of aquatic biomonitoring, having been introduced in the late seventies (e.g., Mason 1979) and more widely applied in the mid-1980s (Resh *et al.* 1995); however, interest in the field has been growing (e.g., Duggan *et al.* 2003; Metzeling and Miller 2001; Metzeling *et al.* 2006; Winger *et al.* 2005) (Figure 1.1). In the past 20 years RBA has been adopted by government agencies within Canada (e.g., David *et al.* 1998; Jones

*et al.* 2004), the United States (e.g., Barbour *et al.* 1999), Australia (e.g., Turak *et al.* 2004) and Europe (e.g., Wright 2000) as part of regional freshwater biomonitoring programs.

RBA protocols can increase the geographic scope of a biomonitoring program by using time saving techniques. Time is saved in the field by collecting fewer samples per site (Lenat and Barbour 1993) and processing time is reduced by sorting samples live in the field, subsampling, or reducing taxonomic resolution. For example, Eaton and Lenat (1991) were able to reduce processing time by more than 50% by altering their standard bioassessment protocol into an RBA protocol. This was done by collecting four samples instead of eight at each stream site, as well as by processing only Ephemeroptera, Plecoptera and Trichoptera (EPT). Reduced collecting and processing time, and the ability to use less skilled volunteers reduces employment costs for RBA programs (Beauchene 2003; O' Leary *et al.* 2004). Data analysis has generally been simplified through the use of indices and predictive models that can provide a 'score' indicating degree of impairment; this simple output has allowed non-specialists to interpret results (Fore *et al.* 2001). In essence, RBA protocols have been designed to allow biomonitoring programs to be maintained in areas with limited funding and human resources. RBA methods have become useful in monitoring freshwater resources in developing nations (e.g., Hart *et al.* 2001; Sudaryanti *et al.* 2001) and have also been used to monitor remediation efforts (e.g., Besley and Chessman 2008; Wesolek *et al.* 2010).

Reduced sampling, processing and analytical effort associated with RBAs have led to criticism of their sensitivity in detecting impact. Variability has been observed in

site descriptions made by different non-specialists (e.g., Hannaford and Resh 1995), macroinvertebrate metric scores (e.g., Ferring *et al.* 2010; Hannaford and Resh 1995; Resh and Jackson 1993), and the ability to detect impacts using RBA methods (e.g., Resh and Jackson 1993). Because lakes and streams are heterogeneous environments, the reduced number of samples collected from them can increase the variability of macroinvertebrate assemblage data (Lenat and Barbour 1993), thereby reducing assessment sensitivity (Resh *et al.* 1995). Subsampling also reduces the information gathered because rare taxa may be missed (Moulton *et al.* 2000) and sorting biases have been observed in the comparison of samples sorted live in the field with those preserved and sorted under magnification in the laboratory (typical of traditional, quantitative protocols) (e.g., Gillies *et al.* 2009; Nichols and Norris 2006). Using index scores to assess water quality has been criticized because information is reduced to a single score and they may be used without considering the biological relevance between the index used and the system being studied (Norris and Georges 1993; Washington 1984). These criticisms may cast doubt on the results of RBAs; however, for the most part, they appear to be acceptable as a preliminary screening tool.

RBA of streams is widespread; however, there is a need for further development of lake RBA protocols. RBA protocols have undergone less development in lentic *versus* lotic systems (Figure 1.1), and before these methods can be applied to lakes, a number of factors that can influence the accuracy of results require consideration.

The purpose of this thesis is to design an RBA protocol for boreal shield lakes. This will be accomplished by first reviewing literature related to effects of sampling

season, sampling design, sampler choice, sample area, sample processing and data analysis methods on the assessment of impacts (this chapter). I discuss the potential influences these factors could have on the RBA of boreal shield lakes and, whenever possible, I identify methods that had the most potential to maximize assessment sensitivity. Based on the results of this literature review, the effects of different sampling choices on estimates of benthic macroinvertebrate community composition were investigated in Malloy Lake, MB (Chapter 2). The findings from the Malloy Lake study were then used to refine the RBA protocols tested in a large group of boreal shield lakes (Chapter 3). Protocols were tested in lakes with and without cottages to determine how effective RBA methods were for the detection of this impact in lakes. The conclusions made in each of these investigations were used to determine if there is potential to use RBA protocols to monitor a wide range of boreal shield lakes, and which methods were most effective for the detection of impacts. Final recommendations for the RBA of lakes are summarized in Chapter 4.

A literature search was first performed on published benthic invertebrate RBA's to determine which methods were most common. This literature survey was limited to publications meeting the following criteria: (a) publication prior to July 2011, (b) assessment of freshwater impact(s) using rapid or qualitative benthic invertebrate biomonitoring methods (i.e., no review papers or marine studies) and, (c) use of a suitable control or reference system. Seventy-six articles met these criteria, and because one reference (Bonada *et al.* 2006) described two distinct sets of methods, a total of 77 protocols were used to summarize trends in RBA using benthic invertebrates. The references used for this literature survey are listed in Appendix 1. Most of these RBAs

were performed in Australian, U.S. and European streams (Figure 1.2); however, it is expected that a number of the techniques are applicable to lakes. Reference to these RBAs will be made throughout this chapter along with other pertinent literature describing how sampling, processing and analytical techniques affect our ability to detect impacts.

## 1.2 Sampling season

The temporal variation of benthic macroinvertebrate communities requires careful consideration before deciding when samples should be collected. Life stages and the numbers of individuals present within each species are variable through time. Throughout each year, emergence patterns, food supply, predation and weather patterns contribute to the temporal variability of benthic communities. This temporal variability can obscure community changes associated with impacts, and the early life stages of some benthos are more difficult to identify beyond order or family as they are small in size or distinguishing features have not developed (Harper and Cloutier 1986; Jones *et al.* 2004).

Benthic samples provide differing estimates of community composition when they are collected at different times of the year, largely because of the emergence of adult insects (e.g., Johnson 1998; Kashian and Burton 2000; Trigal *et al.* 2006). Many aquatic insects (e.g., Diptera, Ephemeroptera, Plecoptera, Trichoptera, Odonata) live in the water for only part of their life cycle. For example, nearly all Trichoptera spend their larval and pupal life stages in the water before emerging from their pupal case, swimming to the water surface and adopting a terrestrial lifestyle as a winged adult

(Wiggins 1996b). Once insects that spend only their immature life stages in the water emerge, they are no longer members of the benthic community and when they do so in large numbers at the same time (e.g., mayflies), community composition may be affected.

The emergence of aquatic insects is mostly influenced by environmental factors such as temperature, food and photoperiod, that for the most part change seasonally in relatively predictable ways (Sweeney 1984). These seasonal changes cause a relatively consistent rise and fall in insect densities throughout the year; in temperate freshwater, insect density peaks between late summer to winter and drops to a minimum during spring and summer when a large number of insects are emerging (e.g., Ball and Hayne 1952; Jónasson 1965). The emergence patterns of aquatic insects are variable among different taxa as well as in different climates (Masteller 1993).

Shifts in the amount and quality of food contribute to the temporal variability of benthic macroinvertebrate communities throughout the year. In temperate climates, the primary production of algae and macrophytes peaks in spring and summer, and is the main source of food for macroinvertebrate communities (Minshall 1978). In fall and winter, food supply becomes more detritus based after primary producers die off and leaves fall into lakes and streams (Sweeney 1984). When more food becomes available, the density of some species increases, whereas others remain unaffected (e.g., Moore 1980). When there is not enough food present, emergence can be delayed and mortality increases (e.g., Danks 1978). The quality of food available can also affect community composition through effects on development time and reproductive success; food of higher quality can increase development time and reproductive success of benthic

invertebrates (e.g., Anderson and Cummins 1979; Eisenberg 1970). Thus, shifts in food resources affect what macroinvertebrates are present and their numbers.

Predation of benthic macroinvertebrates by fish and birds can vary seasonally and further contribute to the temporal variability of benthic communities. Feeding rates of some fish are much higher in summer *versus* winter (Gilinsky 1984; Hurlbert and Mulla 1981; Moffett and Hunt 1945). Because benthic macroinvertebrates can be an important food source for fish, increased summer predation can reduce the density of prey species (e.g., Gilinsky 1984). The intensity of bird predation on benthic macroinvertebrates also varies seasonally; however, this is largely influenced by the migration of birds. In a Scottish lagoon, the abundance of invertebrates, particularly molluscs, decreased during fall and winter after a large number of migratory shorebirds began feeding in the area (Mendonça *et al.* 2007). Similar seasonal reductions in the abundance of polychaetes in an estuary in Portugal (e.g., Rosa *et al.* 2008), and in the densities of annelids, molluscs and crustaceans in a California mudflat (e.g., Quammen 1984) have been observed in response of bird predation.

Weather patterns can affect benthic macroinvertebrates through fluctuations in water-level and temperatures. Water-level fluctuations affect benthic communities directly through changes in the littoral habitat available, and indirectly through the effects that these fluctuations can have on macrophytes and sediments (Dole-Olivier *et al.* 1997; Gathman *et al.* 1999). Benthic macroinvertebrates that live within littoral sediments may be particularly affected by decreasing water levels because they are at risk of becoming trapped and drying out (Matthaei and Townsend 2000). Fluctuations in the water levels of lakes can affect the community composition of benthic

macroinvertebrates (e.g., Scheifhacken *et al.* 2007) as well as the biomass of macroinvertebrates (e.g., Palomäki 1994). Temperature increases and decreases, relative to those optimal for invertebrate growth and development, can result in altered growth rates and reduced reproductive success (e.g., Sweeney and Vannote 1978).

Regardless of the source, temporal variability can confound the use of community measures for water quality assessments when samples are collected at different times of the year (Johnson 1998). Luckily, most sources of temporal variability follow seasonal trends, allowing this variability to be minimized by restricting sampling to a short period of time. RBA methods have been used to produce repeatable results when sampling was completed in three to four weeks at the same time each year (e.g., Hose *et al.* 2004; Reid *et al.* 1995).

There are benefits and drawbacks to sampling benthic macroinvertebrates in any one season that should be considered because they can affect sample processing and the quality of data. Jones *et al.* (2004) provided a summary of the pros and cons of sampling benthic invertebrates from Ontario streams, lakes and wetlands during each season. For instance, a benefit of collecting benthic samples in winter, spring or fall was that macroinvertebrate richness is high during these times. Many flies, mayflies, caddisflies and odonates emerge from boreal lakes at different times over the course of the summer, sporadically lowering richness estimates of benthic macroinvertebrate samples. Richness estimates need to be as consistent and accurate as possible because they are one of the most effective community measures used in benthic macroinvertebrate biomonitoring for the detection of impacts (e.g., Kerans and Karr 1994; Resh and Jackson 1993). Because aquatic insects are emerging sporadically

throughout the summer, other macroinvertebrate metrics based on the relative proportions of taxa collected during this season are also expected to be more variable. Many freshwater impacts are expected to be more pronounced in summer (e.g., agriculture, fishing, motorized watercraft), the second most common sampling season for RBAs (Figure 1.3); however, to ensure community estimates are consistent, benthic sampling should be performed during winter, spring or fall in north temperate regions. Other seasonal considerations include the added difficulty and potential safety hazards of sampling during cold winter months, a shorter time frame for sampling in spring (i.e., after ice-off and before insect emergence), and the abundance of small, immature invertebrates in fall that can be more difficult and take more time to identify (Jones *et al.* 2004). The logistical constraints of winter sampling appear to be a valid concern as no north temperate RBAs have been performed during this season (Figure 1.3). The most popular season for RBAs is spring (Figure 1.3), indicating that the shortened sampling period may only be a problem for large scale biomonitoring programs. Fall RBAs are less common (Figure 1.3), an unexpected trend considering that the technique of using larger mesh sizes in RBAs would limit the number of small, immature invertebrates that are collected. Even more unexpected is that sampling in the majority of RBAs was not stratified to one season (Figure 1.3). Sampling throughout multiple seasons will reduce the ability to detect community changes caused by impact because community changes will be occurring naturally through time (Johnson 1998). Sampling the same place in multiple seasons will also lengthen the time needed to complete assessments, reducing the value of rapid protocols. For large scale RBA programs in the boreal shield, sampling in fall is recommended because there is enough

time to sample many lakes, impacts are expected to be more pronounced immediately following summer (in comparison with winter and spring), and this season provides comparable richness estimates to those obtained in spring (e.g., Reid *et al.* 1994b), the most commonly used sampling season for RBA.

### 1.3 Method of sampling

A variety of samplers, sampler modifications and sampling methods have been used to collect benthic macroinvertebrates. Samplers are often designed for specific habitats and can be quantitative or qualitative. Quantitative samplers collect invertebrates from a standardized volume or area. Knowing the volume or area of the sample can allow macroinvertebrate density (number per square metre) to be calculated. In contrast, qualitative samplers, typically some form of hand-net, collect invertebrates without any delineation of area and these samples are used to determine what taxa are present and their relative abundances (Mackey *et al.* 1984; Merritt *et al.* 1996; Storey *et al.* 1991). Qualitative samplers can be considered semi-quantitative when used in a standardized way (e.g., standardized sampling time, number of kicks, sample area) that allows rough density estimates to be made (Merritt *et al.* 1996). Based on the literature search of RBAs, qualitative samplers are more commonly used than quantitative samplers (Table 1.1). The most commonly used quantitative samplers in RBA were Surber and Hess samplers (Table 1.1), designed for use in lotic systems. When deciding what sampler to use in a RBA program for lakes, it is important to consider both the sampling habitat and the program's design (i.e., what type of community estimates are desired) (Downing 1984).

### *1.3.1 Quantitative samplers*

Quantitative samplers are used to collect benthic macroinvertebrates from a constant volume or area and include grabs, corers, suction samplers and artificial substrates. Most of these samplers were made to sample a specific habitat type and have limited use in other environments as well as bias towards the collection of certain macroinvertebrates (e.g., Flannagan 1970).

Grabs are usually brass or stainless steel boxes with jaws that collect sediment from a standardized surface area. The effectiveness of grabs is influenced by how deeply they enter the sediment (influenced by grab weight, shape, sediment grain size, and sediment compaction), the creation of a bow-wave from water resistance as the sampler is lowered, and jaw closure (Hellawell 1978; Resh 1979). From 1970 to 1981, the Ekman grab was used in 44% of surveyed studies and it was the most commonly used benthic invertebrate sampler of lentic habitats during this time period (Downing 1984). It has been modified in a number of ways and can be used in the littoral or profundal zones (Downing 1984). The Ekman grab can effectively sample soft sediments, but cannot be used in grain sizes equal or exceeding sand, hard sediment, mixed sediment, or in the presence of macrophytes or coarse woody debris because these will impair closure of its spring-loaded jaws (Downing 1984; Merritt *et al.* 1996). The heavier PONAR grab with levered jaws is better for sampling benthos from hard sediments, but is less efficient than the Ekman in soft sediments (Flannagan 1970; Howmiller 1971). The inefficiency of the PONAR in comparison with the Ekman in soft sediments is in part because of its greater weight which can cause the sampler to sink too deeply into the sediment and the shock wave that is created by its screened top

(Howmiller 1971). The descent of an Ekman does not disturb the sediment surface to the same extent as a PONAR because the top covers open while the sampler is lowered (Howmiller 1971). No single grab appears to be efficient in all habitats (Flannagan 1970). The use of grabs in RBA has been rare (Table 1.1) with fewer than 4% of studies using them in combination with qualitative samplers. The heterogeneity of sediments, macrophytes and coarse woody debris in boreal shield lakes as well as transportability limits the utility of grabs for a RBA protocol to be applied to remote lakes.

Core samplers, or open core tubes, are driven vertically into sediments before capping the top of the tube to allow the sediment within to be raised to the water surface without being lost. In a literature review performed on lentic studies published from 1970-1981, corers were reported to have been used in 22% of investigations and were the second most commonly chosen sampler (Downing 1984). Corers can be used in littoral or profundal habitats, but not in grain sizes coarser than sand (Downing 1984). In spite of their bulkiness, another benefit of using core samplers instead of grabs is that they are lighter and may be more easily transported to remote areas. Corers have only been used in 2.6% of RBAs (Table 1.1) and are likely impractical for the RBA of boreal shield lakes because of their restriction to soft sediments. The prevalence of coarse sediments and debris in boreal lakes warrants the use of a more versatile sampler.

Suction samplers, also referred to as hydraulic or airlift samplers, encompass an area or volume of sediment before compressed air is used to flush lighter material (including invertebrates) into a net (Downing 1984). They are relatively expensive to

use and require a SCUBA diver in deeper water (Downing 1984). Suction samplers are less affected by sediment size (e.g., Brooks 1994; Gale and Thompson 1975; Mackey 1972; Pearson *et al.* 1973) and could be an effective way to sample the hard, coarse sediments common in the boreal shield. Invertebrate samples collected with suction samplers have been compared to those collected using grabs and corers in fine sediments and Surber samplers in gravel sediments and no significant differences in the total number of benthos per sample area were observed (Mackey 1972; Pearson *et al.* 1973). Suction samplers can be used effectively to sample different habitat types; however, suction sampling can be affected by water depth, air pressure and the amount of time air is flushed into the sample (Pearson *et al.* 1973). Suction samplers were not used to sample benthos in any of the RBAs examined in the literature search (Table 1.1), perhaps in part because their use requires more equipment and training than other samplers. While potentially useful for sampling boulder habitats in the littoral zone, or sediments with macrophytes or coarse woody debris present, they are not a practical choice for sampling remote lakes because of the added equipment needed (e.g., compressed air cylinders, diving equipment).

Artificial substrata include plates (e.g., Benoît *et al.* 1998; Hester and Dendy 1962), artificial plants (e.g., Gerrish and Bristow 1979; Olomukoro and Nduh Tochukwu 2006) and baskets filled with rocks or other objects (e.g., Casey and Kendall 1996; Dickson *et al.* 1971) placed underwater for a period of time before collecting the benthic macroinvertebrates that colonize them. Artificial substrate samplers could be an asset to rapid protocols because they lower the ratio of organic material to invertebrates, allowing processing times to be reduced (e.g., Hilsenhoff 1969; Muzaffar

and Colbo 2002) and they can allow sampling of habitats such as large boulders that cannot be efficiently disturbed by kick sampling or deep areas that cannot be reached using hand-held nets (Rosenberg and Resh 1982). Artificial substrata are usually left in the water from four to eight weeks to allow time for colonization before they are removed and the macroinvertebrates inhabiting them are collected (Benzie 1984; De Pauw *et al.* 1994; Mason *et al.* 1973). Removing artificial substrata too early can result in a sampling bias towards macroinvertebrate species that colonize the samplers more quickly than others (Olomukoro and Nduh Tochukwu 2006), and some species are unlikely to colonize them at all (Rosenberg and Resh 1982). Artificial substrata can also provide a standardized substrate for colonization, reducing the effects that habitat differences will have on macroinvertebrate samples (Dickson *et al.* 1971; Thorne and Williams 1997) as well as reducing the variability associated with the collector (e.g., level of effort, time spent sampling) ( De Pauw *et al.* 1986) as long as they are used in similar habitats (Rosenberg and Resh 1982). Among the drawbacks of using artificial substrata for a RBA protocol is the need to visit each lake twice, having to leave the samplers submerged for multiple weeks, the risk of tampering or vandalism (e.g., De Pauw *et al.* 1994) and the loss of invertebrates when the sampler is collected (Hilsenhoff 1969). The amount of algae that colonizes artificial substrate samplers (e.g., Casey and Kendall 1996), their structural complexity and interstitial spacing (e.g., De Pauw *et al.* 1986; Erman and Erman 1984; Khalaf and Tachet 1980; Schmude *et al.* 1998), their volume (e.g., De Pauw *et al.* 1986; Khalaf and Tachet 1980) and how closely they mimic natural habitats (e.g., Gerrish and Bristow 1979; Soszka 1975) can all influence their efficiency in collecting benthic macroinvertebrates. Because of

differences between natural substrata and artificial ones, and the chance that artificial substrata were not left in the water long enough for complete colonization by benthos, these samplers may assemble communities that are uncharacteristic of the local environment (e.g., Casey and Kendall 1996; Hellawell 1986). To minimize this effect, rocks from the study area of interest can be used in baskets and samplers can be left in the water for longer periods of time; however, both of these methods would limit the ability to complete an assessment quickly. The time required for effective sampling with artificial substrata is likely the reason they have not been used in many RBAs (i.e., < 3% of studies; Table 1.1).

### *1.3.2 Qualitative samplers*

Qualitative samplers allow multiple habitat types to be sampled in a cost-efficient manner (Rosenberg *et al.* 1999a) and include seine nets and hand-held nets. The versatility of qualitative samplers across different habitat types allows the collection of benthic samples with higher richness, allowing more complete taxa lists to be made (Mackey *et al.* 1984; Storey *et al.* 1991). When qualitative samplers are used in a standardized way, reproducible results can be obtained (Rosenberg *et al.* 1999a; Storey *et al.* 1991) and samples can be made semi-quantitative if estimates of density are important for assessment. Qualitative samples have been standardized by sampling time in streams (e.g., Chessman *et al.* 2011; Hämäläinen and Huttunen 1996; Oliveira and Cortes 2006), wetlands (e.g., Carew *et al.* 2007), ponds (e.g., Proctor and Grigg 2006) and lakes (e.g., Reid *et al.* 1995; Somers *et al.* 1998; Wesolek *et al.* 2010); however, standardizing sample size by area is more appropriate because it can reduce

the variability from different collectors and from differences in micro-habitat (Reid *et al.* 1994a). Both seine nets and hand-held nets have been recommended for the RBA of lakes (e.g., David *et al.* 1998; Jones *et al.* 2004).

If two people are available for sample collection, seine nets can be used to collect littoral macroinvertebrates after sediments have been disturbed by kicking. One person disturbs the sediment by kicking while the other collects the dislodged invertebrates and debris with the net (David *et al.* 1998). Seine nets can effectively capture large, fast moving invertebrates that swim quickly through the water column (Cuffney *et al.* 1993); however, this type of net may be more difficult to maneuver than simple hand held nets, potentially allowing more macroinvertebrates to escape the net. The need for two collectors would also increase the sampling time required; however, this is expected to have minimal effect on total assessment time. Seine nets were recommended by (David *et al.* 1998) for the RBA of lakes; however, they were not used in any of the RBAs evaluated in the literature search (Table 1.1).

Hand-held nets (pond, kick and D-) are swept through the water column, macrophyte stands and along the sediment surface after it has been disturbed by kicking. Often hand nets have one flat edge (i.e., the net opening is shaped like a D) so that it can be held more closely to the substratum while following the collector's foot that is disturbing the sediments (David *et al.* 1998; Reid *et al.* 1994a). This flattened edge held near the substratum and continuous forward motion in lakes (i.e., the current in streams will keep invertebrates in the net) ensures that fewer macroinvertebrates are missed during sampling. Hand nets can be used in nearly all habitat types (e.g., woody debris, rocky shores, soft sediments, macrophytes) and as a result can be used to collect

a diverse assemblage of benthic macroinvertebrates. These samplers are also relatively easy to transport and mend, which can be an asset for sampling in remote areas. Drawbacks of sampling with hand nets include collecting more non-invertebrate material in comparison with artificial substrates (Muzaffar and Colbo 2002), and sampling sediments less effectively beyond 10 cm of sediment depth than corers (Keegan and Könnecker 1973). In spite of these drawbacks, hand nets are the most commonly used sampler for RBAs (used in > 72% of RBAs; Table 1.1).

The use of qualitative sampling gear can be restricted to certain habitats; however, for the most part these samplers are more versatile than quantitative samplers and have been used more often in RBAs (Table 1.1). Hand nets have been recommended for the RBA of lakes by the Ontario Benthos Biomonitoring Network (OBBN) (Jones *et al.* 2004) and the Ontario Ministry of Environment and Energy (OMEE) (Reid *et al.* 1994a) and have been used during the RBA of impacts from cottages, acidity and metals in Ontario lakes (e.g., Somers *et al.* 1998; Wesolek *et al.* 2010). For the RBA of streams, hand nets collect the majority of taxa present (Furse *et al.* 1981; Gillies *et al.* 2009), and the samples collected with them allow us to discriminate impacted from unimpacted sites as effectively as those collected using quantitative samplers (Metzeling *et al.* 2003), and provide repeatable results (Bradley and Ormerod 2002; Stark 1993). Hand-held nets are the most practical way to sample hard and soft substrata effectively in the littoral zone of lakes. Different samplers will collect certain community members over others (e.g., Cheal *et al.* 1993; O' Connor *et al.* 2004); however, no sampler appears as versatile, effective and practical as hand nets across different littoral zone habitats.

## 1.4 Sampling design

The heterogeneity of benthic habitat in lentic and lotic systems can naturally influence macroinvertebrate communities and affect the community estimates used to assess impact. The underlying source of spatial variability in lotic systems is flow, which is the primary determinant of channel shape and substratum (Allan 1995). In lakes, habitat differences are also primarily influenced by water flow in the form of currents and waves that sort sediments into different size classes. Differences in sediments, macrophytes, wave or current velocity have all been found to affect the distribution of benthic macroinvertebrate communities (e.g., Barton and Carter 1982; James *et al.* 1998; Weatherhead and James 2001). For sampling design to be effective, the community variation caused by natural environmental differences requires consideration.

In a heterogeneous environment, placement of sampling sites is an important consideration. If multiple locations are sampled, sampling sites can be selected randomly. When only a few samples are collected and there is an underlying pattern of heterogeneity, sampling should be stratified to reduce the variability of community estimates (Johnson 1998). Large, single, qualitative samples are more common than the collection of multiple replicates of smaller sized samples in RBAs (Figure 1.4). Either this sampling method provides reliable samples for the description of a site, or there are multiple failures to do so.

The influence of spatial variability on stream bioassessment has been minimized by using sampling techniques that maximize the richness of samples collected. This has been accomplished by maximizing the number of habitats or

microhabitats (i.e., minor habitat differences within one habitat type) sampled by collecting benthos along one long zig-zag transect using qualitative or semi-quantitative sampling methods (e.g., Environment Canada 2010; James *et al.* 2010). In most RBAs, samples have been collected across multiple habitat types (Figure 1.5); however, when one habitat type has been sampled in streams it was usually riffle. Stream riffles generally have coarse sediments (pebble, cobble) with many interstitial spaces used as benthic habitat (Brusven and Rose 1981; Williams and Hynes 1974; Zanetell and Peckarsky 1996) and well oxygenated water. Riffle habitat generally has higher invertebrate richness and riffle samples are consequently more informative for the assessment of impacts than depositional sites such as pools where fewer taxa are present (Barbour *et al.* 1999).

Sampling designs that maximize benthic richness are also applicable to lakes. The few RBA protocols designed for lakes have restricted benthic sampling to the littoral zone where richness is higher and have semi-quantitatively sampled multiple habitat types (David *et al.* 1998; Jones *et al.* 2004). Within the littoral zone, habitats without many taxa (e.g., bedrock, sand) can be avoided (e.g., David *et al.* 1998; Wesolek *et al.* 2010); by collecting benthic samples (and pooling them) across diverse habitat types, the informative value of the sample is maximized. Nevertheless, few researchers have applied RBA methods to lakes, perhaps because the spatial variability of littoral zone benthos has been considered by some to be too large to detect impact (e.g., Moss *et al.* 2003; Rasmussen and Kalff 1987). Because the littoral zones of lakes are heterogeneous, it may be wise to limit sampling to one habitat type to reduce the variability of community estimates across lakes.

Stratifying sampling to one habitat type is an effective way of reducing the variability of a variety of community estimates, and consequently facilitating impact detection (Johnson 1998; Resh and McElravy 1993; White and Irvine 2003). Based on published RBA literature, stratification by habitat type is less common than sampling multiple habitat types (Figure 1.5); in some cases the influence of impacts on benthic communities may be large enough to be observed in spite of high natural variability. For the RBA of lakes, it has been recommended in protocol manuals to sample habitats in proportion to their abundance along the lakeshore (e.g., David *et al.* 1998); however, this method requires that the proportion of habitat types are known, requiring a large initial investment in time surveying each lakeshore. This can be particularly time consuming when sampling large, remote lakes that can be accessed only by canoe. This method can also produce variable results if the dominant habitat types present in lakes differ (i.e., because different benthic communities are present in different habitat types). When the variability of community estimates is high, more samples will be required to detect community changes associated with impact. To maximize the cost-efficiency of RBA protocols, sampling one habitat type that supports a diverse community of macroinvertebrates (e.g., cobble or mud shorelines in boreal shield lakes) is recommended. While none of the RBAs reporting habitat stratification were performed in lentic habitats, this method may facilitate the collection of fewer samples per lake because of decreased sample variability.

## 1.5 Sample area

Traditional, quantitative sampling methods involved the collection of sufficient replicates to obtain good estimates of community composition and to allow certain statistical analyses to be used (e.g., ANOVA, BACI). There is a relationship between invertebrate density and variance that can be used to determine the number of samples required to achieve desired precision levels for community estimates (Elliott 1977c); however, the number of samples collected per site is largely dependent on the size of each sample (i.e., the sample area covered). To achieve a standardized error when macroinvertebrate densities are assumed constant, the most efficient way of sampling quantitatively is to collect more replicates with a smaller sampler (Downing 1979). Quantitative methods are effective for detailed analyses of lakes but are still very labour-intensive and cannot be applied to large RBA programs.

With qualitative sampling, the use of species-area curves becomes more important to ensure that an accurate description of the species present can be made. The relationship between sample area and richness has long been understood; as we sample larger areas incrementally, the probability of encountering rare taxa increases (Colwell and Coddington 1995; Magurran 2004b). Ecologists use species-area curves to determine the appropriate sample area for a study (Magurran 2004b). For example, by using sample areas near or at the asymptote of the curve, we can ensure that we have collected the majority of taxa present in a given habitat type, making our richness estimates less variable. It is uncommon for species-area curves to be considered in the design of RBA methods. A total sample area $\leq 0.6$ m² has been most commonly used in RBAs, with total sampling areas $> 10$ m² being rare (Figure 1.6). These figures are

primarily based on stream data and the prevalence of time-delineated sampling in lentic RBAs (e.g., Carew *et al.* 2007; Menetrey *et al.* 2011; Proctor and Grigg 2006; Somers *et al.* 1998; Wesolek *et al.* 2010) makes it difficult to know if similar sampling areas would be applicable to lakes. Of the lentic RBAs examined, three wetland studies provided enough information to determine their total sampling areas (e.g., Chessman *et al.* 2002; King and Richardson 2002; Stein *et al.* 2009). The total sample areas used in these studies ranged from 0.27 to 2.04 $m^2$, with a mean area of 1.27 $m^2$ sampled for wetland RBA. It is unclear from lotic and lentic studies whether or not the total sample area used in RBAs is arbitrary or based on preliminary studies where the relationship between sample area and richness was examined. Schreiber and Brauns (2010) may be the only researchers to publish species-area curves for lakes based on kick-sampling methods. They found that sample areas of at least 6.4 $m^2$ would be needed to collect all taxa; however, substantially smaller sample areas could be used if rare taxa were omitted (Schreiber and Brauns 2010). The relationship between sample area and richness should be examined in preliminary sampling surveys to determine the kick sample area to be sampled for the RBA of lakes, even though this practice is rare in benthic invertebrate biomonitoring.

### 1.6 Sample processing

Sample processing is a very time consuming part of benthic macroinvertebrate biomonitoring (Ciborowski 1991; Vlek *et al.* 2006), making the incorporation of rapid processing methods important. Time saving methods used during sample processing include separation of organic material by elutriation (Moulton *et al.* 2000), flotation

using sugar or carbon tetrachloride (Whitehouse and Lewis 1966), as well as the use of dyes (e.g., rose bengal, Lugol's iodine, phloxine B) that stain animals (Mason and Yevich 1967; Williams and Williams 1974). While these methods can facilitate sample sorting, it is more common to wash samples in a larger mesh size, process macroinvertebrates live in the field, subsample and reduce taxonomic resolution to allow RBAs to be completed more quickly.

### *1.6.1 Mesh size*

Mesh size affects the retention of invertebrates and non-invertebrate material in sampling gear and consequently affects community measures and sample processing times. The retention of invertebrates can be influenced by body shape (i.e., the retention of oligochaetes and chironomid larvae is more variable than round-bodied snails) as well as the non-invertebrate material within the sample (i.e., invertebrates can be caught in filamentous algae) (Morin *et al.* 2004). A given mesh size should retain all benthic macroinvertebrates with a body length ten times the length of its openings (Morin *et al.* 2004); however, more small invertebrates will pass through sieves if they are alive (Storey and Pinder 1985). The proportion of benthos retained in a sieve will depend on site characteristics and which invertebrates are present (Thompson *et al.* 2003).

The choice of mesh size used in a biomonitoring program sets a size constraint on the community collected (Bishop and Hartley 1986); however, this choice has been variable in benthic invertebrate biomonitoring. To focus benthic sampling on macroinvertebrates, mesh sizes of 200 μm or more should be used (i.e., to exclude

microinvertebrates) (Rosenberg and Resh 1993), but the mesh sizes used in RBAs range from 125 to 2000 µm (Figure 1.7). Two hundred and fifty micron (40% of studies) or 500 µm (34% of studies) mesh sizes were the most common choices for RBAs, and there does not appear to be a trend through time towards use of larger or finer mesh sizes (Figure 1.7). The mean and median mesh sizes used in RBAs were 408 µm and 290 µm, respectively. These fall within the ranges most commonly reported for quantitative studies in lentic environments; literature reviews performed by Resh and McElravy (1993) and Downing (1984) indicated that mesh sizes of 101 to 200 µm (fine) and 450 to 600 µm (medium) were most common, respectively. Studies using rapid assessment techniques in lakes have used medium (e.g., Jones *et al.* 2004; Wesolek *et al.* 2010) and large (≥ 1 mm) (e.g., David *et al.* 1998; Reid *et al.* 1995) mesh sizes. Using larger mesh sizes will result in the collection of fewer small species and immature invertebrates.

Use of different mesh size can affect estimates of density, richness, diversity, biotic index scores and biomass. For kick samples collected in Ontario and Quebec streams, Morin *et al.* (2004) observed that estimates of macroinvertebrate density dropped sharply when using mesh sizes larger than 250 µm. Loss of smaller individuals through larger mesh sizes can affect estimates of the relative proportions of taxa (i.e., if many small chironomids are washed out of samples, % Chironomidae will be underestimated and % EPT will be overestimated) (e.g., Battle *et al.* 2007). Reduced abundance of invertebrates estimated using medium mesh sizes in comparison with smaller mesh sizes (≤ 250 µm) has also been observed in streams in British Colombia and Brazil. In both studies it was observed that retention of fewer invertebrates did not

significantly affect richness (Buss and Borges 2008; Rosenberg *et al.* 1999b). Barba *et al.* (2010) found that estimates of taxa richness were significantly reduced when using a 1 mm mesh for washing samples collected with a Surber sampler in North Iberian streams instead of a 500 µm mesh. Significant differences were also observed in biotic index scores between samples washed using 500 µm and 1 mm sieves, whereas no differences were observed for diversity (Barba *et al.* 2010). Biomass estimates appear to be less influenced by the use of larger sieves because the individuals that are lost make up only a small proportion of total biomass (Morin *et al.* 2004). Morin *et al.* (2004) observed that biomass estimates from kick samples decreased when using mesh sizes larger than 1 mm. Based on these results, the community measures used for assessment should have some influence on the choice of mesh size. For accurate estimates of density a mesh size of 250 µm should be used. Larger mesh sizes of 500 µm should be sufficient when richness and biotic indices are used, and mesh sizes of 1 mm can be used for biomass estimates.

Funding can also influence the choice of mesh size because a finer mesh will increase the time needed to process samples (e.g., Thompson *et al.* 2003; Wildish 1978). Using larger mesh sizes lowers sample processing times by reducing the fine sediment and organic matter that the macroinvertebrates need to be removed from as well as by reducing the number of small, early instar invertebrates that can require more expertise to identify (Jones *et al.* 2004). The time required to process marine grab samples was reduced by approximately 70% when using a 2.5 mm sieve in comparison with a 1 mm sieve (Wildish 1978), and by 38% for core samples washed in a 1 mm sieve *versus* a 500 µm sieve (Thompson *et al.* 2003). The amount of processing time

saved will depend on characteristics of the sampling site (i.e., presence of macrophytes or filamentous algae, amount of coarse debris, sediment grain size) as well as the type of sampler used (e.g., Wildish 1978) and total savings in time will vary between biomonitoring programs. Farara and Burt (1999) observed that using a 250 μm mesh instead of a 500 μm mesh would increase the cost of completing one Environmental Effects Monitoring (EEM) sample by $1,776.

For the RBA of lakes, using a mesh size of 500 μm may provide a good compromise between reliability of community measures and savings in time and cost. A mesh size of 500 μm has produced reliable estimates of richness, biotic indices and biomass (e.g., Buss and Borges 2008; Morin *et al.* 2004; Rosenberg *et al.* 1999b) and substantially reduces the effort and thus the cost of sample processing (e.g., Farara and Burt 1999). In cases where processing times must be further reduced, using a 1 mm mesh size may produce reliable results for diversity and biomass estimates, but this greatly limits the utility of benthic invertebrates for biomonitoring.

### *1.6.2 Live sorting*

Sorting macroinvertebrate samples live in the field can greatly reduce sample processing time. Live sorting usually involves placing a portion of the sample along with some water in a white pan and removing invertebrates without the aid of magnification. The main benefits of live sorting is that organisms can be found more easily within the sample material because they are moving, some invertebrates will be less damaged and can be more easily identified, and sites can be resampled on the same day if an insufficient number of macroinvertebrates was collected (Hilsenhoff 1982).

The transport and storage of large samples is also avoided because only the picked organisms are preserved. Completing sample processing in the field also allows researchers to collect the data they need for analysis before they leave the sample site, greatly decreasing the time needed to complete an assessment. These benefits have led to the incorporation of live sorting of samples into 41% of RBAs; however, the use of live sorting methods is not becoming more common (Figure 1.8).

The main argument against live sorting is that there is bias towards collecting benthos that move more quickly, are larger, or more conspicuous (e.g., brightly coloured) (Hilsenhoff 1982; Nichols and Norris 2006). In a comparison of macroinvertebrate samples sorted live in the field and those sorted dead in the lab, Nichols and Norris (2006) found that live sorted samples had higher numbers of Atyidae, Physidae, Dytiscidae, Gyrinidae, Psephenidae, Corixidae, Gerridae, Mesoveliidae, Notonectidae, Corydalidae and Odonata in comparison with the samples that were sorted after preservation. In the samples sorted dead in the lab using magnification, Nichols and Norris (2006) found higher numbers of Oligochaeta, Scirtidae, Orthocladiinae, Tanypodinae, Chironominae, Empididae and Psychodidae in comparison with samples sorted live. Unfortunately, cryptic taxa more common in samples sorted dead generally have higher tolerance values than the taxa in greater abundance in live-sorted samples (Hilsenhoff 1988). Tolerance values are used to rank the sensitivity of a species to a specific impact (e.g., organic pollution, mining) and to calculate biotic index scores. Consequently, a biased collection of organisms can affect our interpretation of whether or not a site is impacted (i.e., higher tolerance values will result in higher biotic index scores). Relative proportion metrics such as %

Chironomidae or % EPT may be similarly affected because their use is based on those groups being generally tolerant and intolerant to pollution, respectively. Sorting bias can also affect richness estimates because some taxa are entirely missed during sorting. Smith *et al.* (1999) found that when samples were sorted dead, 90% of the families present were identified, whereas only 76% of the families were identified by live sorting. Collecting fewer taxa in live-sorted samples is probably because small species will be missed without the aid of magnification. For comparisons of live-sorting in the field by eye *versus* sorting samples dead in the lab using magnification, it is expected that fewer taxa and fewer tolerant individuals will be collected using live-sorting methods.

If sorting biases were reduced, live sorting could be an effective way of keeping sample processing times low. Keizer-Vlek *et al*. (2011) did not report any differences in processing costs or in the time required to sort benthos live *versus* dead; however, this was likely because the same processing methods (i.e., all samples were sorted entirely, without magnification) were used. In practice, preserved samples generally take more time to process because they are sorted more thoroughly under magnification. Without this difference, the greater ease of finding moving invertebrates is offset by the added difficulty of collecting them while they are moving (Keizer-Vlek *et al.* 2011). Growns *et al.* (2006) tested the use of magnification during live-sorting and found that it did not significantly alter results; however, adjusting live sorting protocols in other ways may help reduce bias. Processing randomly selected portions of a sample for a minimum length of time could reduce the bias associated with live-sorting. Concentration of efforts on a smaller portion of the sample could increase the

chances of observing smaller, more cryptic taxa during processing; however, this practice could also result in cryptic taxa being missed entirely (i.e., if they were present in the unsorted portion of the sample).

### *1.6.3 Subsampling*

Subsampling reduces processing time because only a portion of the sample is processed (e.g., Growns *et al.* 1997). There are three main ways of subsampling benthic macroinvertebrate samples: collecting invertebrates as they are seen, sorting only specific taxa from the sample, and physically splitting the sample material into portions to be sorted. Within each of these subsampling methods, fixed counts or fixed times are often used to set the limit on the number of organisms that are collected or the length of time spent sorting, respectively. Some form of subsampling was used in nearly all RBAs where processing methods were described; however, the specific methods used were often not reported (Figure 1.9). Subsampling will result in a loss of information (Courtemanch 1996; Doberstein *et al.* 2000), making it more difficult to detect impacts if biased methods are used.

First-seen subsampling is simply picking invertebrates as they are seen in the sample material until a fixed count or time has been reached. This is a simple method that likely has the lowest processing times and least equipment needed. First-seen subsampling is a method that is biased toward the collection of large or brightly-coloured macroinvertebrates and it is likely that more small cryptic species will be missed. The proportion of the sample that is processed is not determined, leaving estimation of macroinvertebrate density of the entire sample impossible (e.g., Moulton

*et al.* 2000), and reducing the value of metrics, such as richness, that should be standardized by level of effort (Courtemanch 1996; Vinson and Hawkins 1996). This method has not been commonly incorporated into RBA protocols (Figure 1.9).

Collecting specific taxa from samples can also reduce sample processing times; however, a lot of community composition information is lost. Processing specific taxa will take less time to sort samples as well as to complete identifications. Identifying benthic invertebrates to genus or species is less time consuming when concentrating on a subset of the community because there are fewer individuals to process. This method would also reduce the breadth of taxonomic expertise required to complete identifications by excluding groups that are more difficult to identify (e.g., Rabeni and Wang 2001), or focusing solely on indicator taxa (e.g., Eaton and Lenat 1991; Törnblom *et al.* 2011). The main drawback of using this subsampling method is that information on other macroinvertebrate taxa is lost. Estimates of overall richness and density as well as relative proportion metrics cannot be calculated. Unless specific metrics such as EPT richness are known to detect the impact of interest reliably (e.g., Eaton and Lenat 1991), the loss of information contributing to relative abundances and other taxa will not offset the savings in time.

Randomly selecting portions of the sample for processing (e.g., Barbour and Gerritsen 1996; Nichols *et al.* 2006; Riva-Murray *et al.* 2002) can overcome the biases associated with first-seen subsampling. In general, the sample is first mixed in an effort to distribute organisms randomly; however, the sample is still likely to have aggregations of invertebrates (Moulton *et al.* 2000; Wrona *et al.* 1982). Portions of the sample are then selected randomly using a set volume (Wrona *et al.* 1982), weight

(Sebastien *et al.* 1988) or area (Moulton *et al.* 2000). Based on the number of individuals processed in a known subsample size, estimates can be made for the total volume, weight or area of the sample (Moulton *et al.* 2000). This can allow a rough estimate of density to be calculated for the sample, which has greater informative value than qualitative data alone. This method of random subsampling can also benefit bioassessment protocols by reducing the effect of sorters processing more conspicuous invertebrates. It probably requires more time and equipment than other subsampling methods; however, the reduction of the influence of sorter biases on community estimates and the ability to estimate density make the added costs worthwhile.

Physical subsampling is often accompanied by fixed-count or fixed-time processing limits. In RBAs, fixed count (i.e., a fixed number of invertebrates are sorted) subsampling is more common than using fixed times (i.e., sorting continues until a set time has been reached; Figure 1.10). This may be because fixed counts are expected to be more consistent than those set by time. The time required to process samples thoroughly with different amounts of macrophytes, filamentous algae or coarse material can be highly variable.

Sorters can also vary in their ability to process samples quickly. By setting a time limit, the number of individuals processed may vary widely between different sorters. Because the number of individuals processed affects estimates of richness (Magurran 2004a; Vinson and Hawkins 1996), this variability may make the detection of impacts more difficult. Using fixed count methods, samples may not always be processed quickly; however, estimates of community composition are likely to be more precise than those obtained using timed count methods.

Fixed count limits in RBA studies have been variable (Figure 1.10) and there have been disagreements over what number is the best compromise between information loss and savings in time (e.g., Somers *et al.* 1998; Sovell and Vondracek 1999; Vinson and Hawkins 1996). A fixed count of 100 invertebrates is the single most common choice in RBA; however, when fixed counts of 200 or more are combined, they are more common than counts of 100 (Figure 1.10). Taxa richness generally increases as we process a greater number of individuals (Magurran 2004a; Vinson and Hawkins 1996) and richness is underestimated using a fixed count of 100 individuals (Sovell and Vondracek 1999; Vinson and Hawkins 1996). Doberstein *et al*. (2000) compared the variability of a multimetric index and its component metrics by creating 500 synthetic replicates for fixed counts of 100, 200, 300, 500, 700 and 1000 using bootstrapping methods. They found that variability decreased as more macroinvertebrates were processed and that metric and index values calculated using fixed counts of 100 were too variable to distinguish impact gradients. While in some RBAs, fixed counts of 100 are sufficient for the detection of impacts (e.g., Chessman *et al.* 2007; Riva-Murray *et al.* 2002; Somers *et al.* 1998), the use of higher fixed counts in recent studies (Figure 1.11) may be an indication that fixed counts of 100 are not always reliable. To minimize variability and obtain more accurate richness estimates, using fixed counts of 200 or more is recommended.

### *1.6.4 Taxonomic resolution*

Taxonomic resolution is an important consideration for RBA programs because it affects the scale and resolution of community measures. Community measures

expected to be most affected by taxonomic resolution are richness, diversity and biotic indices. Species in the same family can have variable responses to impact and without identification to species, some changes will not be observable (Resh and Unzicker 1975). Using coarser taxonomy (e.g., family, order), richness estimates will be lower (Marshall *et al.* 2006) and the reduced resolution may make community changes associated with impact more difficult to detect. Richness is an important component of diversity and as a result these effects may also influence our ability to assess impacts with diversity indices. When using diversity indices, errors increase and diversity decreases as taxonomic resolution is reduced (Wu 1982). Biotic indices are affected by coarser taxonomy because they are based on the tolerance of species (Hilsenhoff 1982; Lenat 1993). When a biotic index is used for macroinvertebrate samples identified to family, the mean species tolerance within a family will likely be used (e.g., Hilsenhoff 1988). This means that at severely impacted sites (i.e., more likely to have taxa with high tolerance values), water quality will be interpreted as better than its real condition, and at pristine sites (i.e., more likely to have taxa with low tolerance values), water quality will be interpreted as worse than its actual condition (Hilsenhoff 1988). Despite the effects taxonomic resolution can have on community estimates, RBAs most commonly use family level taxonomic resolution because it can save a lot of time and money (Figure 1.12) (Jones 2008). There is no clear trend towards higher or lower levels of taxonomic resolution through time (Figure 1.12); however, family level taxonomy is considered by many to be sufficient for rapid protocols (e.g., Chessman *et al.* 2007; Metzeling and Miller 2001; O' Leary *et al.* 2004; Wright *et al.* 1995).

The main benefits of resolving macroinvertebrates to family (*versus* genus or species) are that processing times will be lower and less taxonomic expertise will be needed to complete assessments. Processing times are variable by individual skill (Ciborowski 1991; Ferraro and Cole 1995); however, reducing taxonomic resolution from species to family lowers invertebrate processing times by 40 to 55% for marine samples (Ferraro and Cole 1995; Thompson *et al.* 2003). Similar savings in time are expected for freshwater invertebrates. By increasing taxonomic resolution from family to genus, sample processing took approximately seven times longer and costs were approximately four times higher for Australian stream samples (Jones 2008). Lower identification of chironomid larvae can be particularly time consuming because they are usually dominant, extremely diverse and more difficult to identify, and their identification usually requires slide mounting (Coffman and Ferrington 1996). The widespread use of RBA in government biomonitoring programs may also mean that staff with taxonomic expertise are not always present within a study area (i.e., taxonomic expertise is rare) (Ellis 1985; Jones 2008). In less than one quarter of quantitative studies, taxonomists are consulted (Resh and McElravy 1993) and it is likely that even fewer are consulted for studies using rapid bioassessment methods. Our ability to identify to a specified level (i.e., difficulty caused by damaged or missing body parts) as well as identify individuals correctly both decrease as taxonomic resolution increases (Hewlett 2000). While we gain more information on the benthic community using species level taxonomy, in large scale surveys, time and money may be better spent by sampling more lakes.

## 1.7 Data analysis

RBA protocols have included both univariate and multivariate analysis for the assessment of impacts. In general, protocols developed in the United States have relied on univariate metrics and indices (e.g., Barbour *et al.* 1999), whereas Australian and European RBA protocols have used predictive, multivariate models (e.g., Turak *et al.* 2004; Wright *et al.* 1984) to assess impact. In Canada, bioassessments using both multivariate (e.g., Reynoldson *et al.* 1995) and univariate statistical approaches have been conducted (e.g., David *et al.* 1998; Hynes 1998; Jones *et al.* 2004).

Using either univariate or multivariate approaches, it has become common to compare test sites to a group of reference (unimpacted) sites to assess degree of impact and determine whether or not further investigations should be made (Reynoldson *et al.* 1997; Stoddard *et al.* 2006). A group of reference sites is used to define the unimpacted, or more realistically minimally and least disturbed, condition observed in undeveloped areas; the compositional range observed in this group of reference sites is generally referred to as the reference condition (Stoddard *et al.* 2006). If the composition of the benthic community at a test location falls outside of a chosen benchmark for the range of reference sites, the test site is considered impacted (Reynoldson *et al.* 1997; Stoddard *et al.* 2006). For a group of reference sites to be effective for the detection of impacts, it is important that enough sites have been sampled to characterize the full range of communities present in undeveloped sites (Hughes *et al.* 1986; Reynoldson and Wright 2000). When the range of reference communities has not been adequately characterized, assessment accuracy is expected to drop, particularly for sites with uncommon environmental characteristics.

Analytical methods commonly used in freshwater RBA include metrics (34% of RBAs), biotic indices (31% of RBAs), multimetric indices (14% of RBAs) and various multivariate methods (21% of RBAs; Figure 1.13). The detection of specific impact types is not restricted to one RBA method (Figure 1.14).

### *1.7.1 Metrics*

Metrics are simple measures of the benthic macroinvertebrate community used to assess water quality. Metrics include macroinvertebrate abundance, density, richness, evenness, diversity and relative proportions of different taxa. Metrics are simple to calculate and scores can be compared between reference and test sites with minimal statistical expertise. Metrics are chosen based on their ability to detect impacts within a given region and they are the most common way to assess impact in RBA (Figure 1.13).

Richness metrics are influenced by sampling and processing methods; however, when the protocols used are consistent, richness metrics are less variable (Johnson 1998) and more consistently relied upon for RBA than other metrics (e.g., Fore *et al.* 1996; Growns *et al.* 1997; Sovell and Vondracek 1999). Richness estimates will be affected by sampling larger areas and processing more organisms, both of which increase sample richness (Magurran 2004a, b). Richness estimates can also be variable depending on the taxonomic resolution used and the ability of an investigator to identify macroinvertebrates correctly. At species-level taxonomic resolution, accurate identification becomes more difficult (Jones 2008) leading to lumping or splitting taxa by perceived morphotype (Hammond 1994; Magurran 2004b). Macroinvertebrate

richness is expected to decrease under impacted conditions (Barbour *et al.* 1999); however, there are exceptions to this response, including when nutrient poor waters receive an influx of nutrients that provide more food for benthos. Despite these issues, richness estimates have been used in RBAs to distinguish reference sites from those impacted by nutrient enrichment (e.g., Rabeni and Wang 2001; Thorne and Williams 1997; Winger *et al.* 2005), urbanization (e.g., Bonada *et al.* 2006; Schiff *et al.* 2011), agriculture (e.g., Törnblom *et al.* 2011), salinity (e.g., Kefford *et al.* 2006; Piscart *et al.* 2006), metals (e.g., Hoiland and Rabe 1992; Winger *et al.* 2005) and acidification (e.g., Wesolek *et al.* 2010).

Diversity is a measure of species richness and evenness of a community that has been used in some form to describe communities since the 1920s (Washington 1984). Diversity increases when either the number of species in a sample (richness) is higher or the abundances of the species present are more evenly distributed (evenness) (DeJong 1975). Many different indices have been devised, allowing diversity to be calculated in different ways by focusing more or less on the richness or evenness component. For example, the Shannon index is more strongly influenced by species richness than the Simpson index, and the opposite relationship is true for species evenness (DeJong 1975). Diversity indices can be biased by the amount of area sampled; as area sampled increases, so will species richness (Magurran 2004b). Because of the influence of sample area on richness, standardization of sample area is important for unbiased sample comparison using diversity. A diversity index that is not dependent on sample size can also be chosen to avoid this problem (e.g., MacIntosh index) (DeJong 1975). The selection of a diversity index should be based on what

aspects of community composition are of interest; however, index popularity does not appear to be based on merit (Magurran 2004c).

The most popular diversity index in biomonitoring (DeJong 1975; Godfrey 1978), the Shannon index (Shannon and Weaver 1949), is highly criticized (Hurlbert 1971; Magurran 2004c; Washington 1984). The Shannon index is influenced by sampling effort; however, the main argument against the use of this and other diversity indices based on information theory is that they are not biologically meaningful (Hurlbert 1971; Magurran 2004c; Washington 1984). The Shannon index measures the uncertainty of collecting a species from a sample; *H*' (uncertainty) is higher when diversity is higher (Pielou 1969). The relevance of the Shannon index is likely further confused by confounding statements made about the reduction of uncertainty being correlated with higher diversity (Washington 1984). The lack of knowledge regarding what diversity indices such as the Shannon index are actually measuring does not necessarily mean that they cannot be valuable in assessment; however, some researchers have favoured the use of Simpson's index because it is less influenced by rare species (e.g., Peet 1974) and it can be used to measure diversity at different scales (e.g., Routledge 1979). It is not clear why diversity indices that are considered more biologically meaningful (e.g., Simpson's index, Hurlbert's PIE) have not achieved the popularity of the Shannon index (Magurran 2004c; Washington 1984).

Other criticisms have been made of the use of diversity indices in general and their use is not recommended as a stand-alone measure of water quality (Boyle *et al.* 1990; Olsgard and Gray 1995). Diversity should decrease with increased impact or be influenced by community stability – both concepts have yet to be proven unequivocally

(Washington 1984). The utility of diversity as an indicator of water quality is based on the assumption that communities will be less diverse when impacted; however, by the Intermediate Disturbance Hypothesis, diversity will be highest at moderate levels of impact (Connell 1978; Grime 1973). Diversity indices have incorrectly assessed ecological condition (e.g., Lydy *et al.* 2000; Olsgard and Gray 1995) and are less useful than other community measures for biomonitoring (e.g., Lydy *et al.* 2000).

The relative proportions of taxa are commonly used in benthic macroinvertebrate biomonitoring and are very simple to calculate. Relative proportion metrics are typically expressed as percentage of a distinct group of macroinvertebrates (e.g., % Trichoptera, % shredders). Grouping taxa by family and order are common (e.g., % Chironomidae, % Ephemeroptera), as is the lumping of taxa into larger groups (e.g., % EPT, % Insecta). Proportion metrics are also often based on the per cent of dominant and tolerant / intolerant taxa as well as functional feeding groups (e.g., % predators, % collector-filterers). Proportion metrics based on habit (e.g., % clingers) are less common.

The use of proportion metrics is based on predictable biological responses that occur with specific impacts. For example, in eutrophic conditions, dissolved oxygen drops when it is consumed in large quantities by bacterial decomposition of organic matter, as well as by the plants and animals that are present in greater numbers because of additional food resources (Wetzel 2001a). Nutrient enrichment is consequently assessed using metrics such as % Chironomidae, % Ephemeroptera or % EPT because of their predictable responses to low oxygen concentration (e.g., Camargo *et al.* 2004; Gafner and Robinson 2007; Wang *et al.* 2007). Chironomids, such as *Chironomus*, are

generally tolerant of low oxygen conditions in larval form because their bodies contain haemoglobin – a pigment that transports oxygen, even when present in low concentrations (Jónasson 1969). Mayfly larvae have greater difficulty obtaining oxygen from their environment, causing their presence to be reduced in low oxygen conditions (Wiederholm 1984). In low oxygen environments, it is expected that % Chironomidae would increase. The perceived intolerance of Ephemeroptera, Trichoptera and Plecoptera has led to the common use of % EPT for the bioassessment of streams (Lenat and Penrose 1996), while % ETO (Ephemeroptera, Trichoptera and Odonata) is becoming popular for the bioassessment of lakes (e.g., Gerritsen *et al.* 2000; Solimini *et al.* 2008). The response of macroinvertebrate metrics is variable by impact (e.g., Yuan and Norton 2003); however, in general the proportions of tolerant and dominant taxa increase with impact (Barbour *et al.* 1999).

### *1.7.2 Biotic indices*

Biotic indices are calculated using the number of individuals present from a taxonomic group and their respective tolerances to a specific impact (Hellawell 1986). Biotic index scores provide a relative measure of impairment based on proportions of tolerant and intolerant taxa. This is similar to the use of relative proportion metrics (i.e., % Ephemeroptera which are considered intolerant) for impact assessment; however, biotic indices rely on tolerance values, likely providing a more reliable assessment than metrics that often lump multiple taxa into one group (e.g., % EPT). Examples of biotic indices include the FBI (Family Biotic Index) (Hilsenhoff 1988), BBMWP (British Biological Monitoring Working Party) score system (Armitage *et al.* 1983) and

SIGNAL (Stream Invertebrate Grade Number – Average Level) (Chessman 1995). The FBI and SIGNAL have been used as stand alone measures in RBAs (e.g., Chessman 1995; Growns *et al.* 1995; Hilsenhoff 1988) and can be calculated with relative ease. For example, the FBI is calculated using the following formula:

$$\text{FBI} = ( \sum n_i \cdot t_i ) / N ,$$

where $n_i$ is the number of individuals collected from family $i$, $t_i$ is the tolerance value of the $i$th family and $N$ is the total number of individuals processed. The FBI provides a score from zero to ten to indicate the degree of organic pollution in Wisconsin streams (Hilsenhoff 1988). Taxa that have a narrower range of tolerance to environmental conditions are more valuable in freshwater assessment because their presence represents more focused relevance than a species with wide environmental tolerance does (Abel 1989).

Because biotic indices are based on the tolerance of species (Lenat 1993), taxonomic resolution can influence impact detection. The mean tolerance value of all species within a family can be used for family-level biotic indices; however, family-level will not be as precise as species-level biotic indices at describing water quality (Hilsenhoff 1988). In spite of this reduction in precision, most of the RBAs in which biotic indices have been used to assess impacts (~ 61%) resolved taxa to family.

Tolerance values are impact-specific and consequently biotic indices should not be used interchangeably across stressors that affect ecosystems in different ways. The majority of biotic indices have been created for the assessment of organic pollution (e.g., sewage effluent) in streams and are based on the knowledge that this impact reduces dissolved oxygen along with the species that have higher oxygen demands

(Friedrich *et al.* 1996). Because tolerance values are specific to impact, biotic indices created to assess organic pollution should not be used to assess other environmental stressors without caution or modification (Clements 1994). Hilsenhoff's Biotic Index (Hilsenhoff 1982) has been used with success to assess agricultural impacts with minimal modification (i.e., adding tolerance values for non-insect taxa) (Barton and Metcalfe-Smith 1992); however, this success may be specific to impacts that cause similar environmental changes. Biotic indices have also been created for the assessment of acidification (e.g., Fjellheim and Raddum 1990) and heavy metals (Clements *et al.* 1992), but biotic indices have not been developed for the assessment of other impacts.

Biotic indices are designed to be used in a specific region because macroinvertebrate tolerances can be variable across regions due to temperature differences and other factors (Lenat 1993). Using biotic indices outside of their intended study area (e.g., Graça and Coimbra 1998) or modifying them using regional benthic data (e.g., Alba-Tercedor and Sánchez-Ortega 1988; Willsie 1992) is somewhat common; however, for RBAs where assessment sensitivity is maximized so that effort can be minimized, this practice must be avoided. The lack of tolerance values specific to lake impacts in Canada's boreal shield limits the use of biotic indices in this area.

### *1.7.3 Multimetric indices*

Multimetric indices are a combination of metrics and/or indices that are used together to assess water quality. For multimetric calculation, an expected range of metric scores at varying levels of impairment must first be determined. Test sites receive a score for each metric depending on their value that are then summed to

provide a multimetric index score. The first multimetric index was Karr's Index of Biotic Integrity (IBI) which was designed for biomonitoring small streams using fish community structure (Karr 1981). Since then, multimetric indices have become a common assessment tool for benthic invertebrate biomonitoring. While the use of multimetric indices has been common in stream bioassessment (e.g., they are the main method used by the US EPA for RBA impact detection) (Barbour *et al.* 1999), multimetric lake indices are not yet in extensive use (Beck and Hatch 2009). Nijboer *et al.* (2005) highlighted the importance of looking at more than one taxonomic group as an indicator of impairment; using one indicator taxon resulted in large error when attempting to classify Netherlands surface waters. The use of multiple metrics or using measures for more than one taxonomic group is important for correct characterization of complex communities. Using a multimetric index can also be less variable than its component metrics when examined alone (e.g., Kimberling *et al.* 2001), and using more than one metric may be important in detecting community changes from multiple impacts.

Metrics or indices are chosen for a multimetric index based on the power of individual metrics to detect impact and whether or not they provide unique community information (Trigal *et al.* 2009). Removing metrics that are redundant with other metrics (e.g., % Ephemeroptera and % EPT can incorporate a lot of the same data) reduces classification error (Reynoldson *et al.* 1997). In the 17 multimetric indices reported in the RBA literature search, the number of component metrics ranged from 3 to 22, with richness as the most common type of metric incorporated (76%; Table 1.2). This was followed by incorporation of metrics based on relative proportions of taxa

(53%), biotic indices (47%), and metrics based on functional feeding groups (47%; Table 1.2). The least commonly used component metrics were based on habitat characteristics, biomass, and evenness, all used in only one of the 17 multimetric indices (6%; Table 1.2). The choice of different component metrics is likely based on the impacts under investigation or regional differences in macroinvertebrate responses.

Criticisms have been made of multimetric indices including claims that they provide no real information about water quality, community information is not used effectively, metric selection is haphazard, their responses are unpredictable and the responses they do provide are not properly understood (e.g., Green and Chapman 2011; Suter 1993). Karr and Chu (1999) replied to these criticisms and others by highlighting a number of studies in which statistically sound methods were used to monitor streams using multimetric indices. In cases where indices have not been able to reliably detect impact, it is likely that the wrong biological responses were examined (Karr and Chu 1999). Karr and Chu (1999) also say that a greater understanding of biological processes can be required for metric selection (in contrast to multivariate statistics) because each one is chosen based on its predictable and consistent biological response to impact. The combination of metrics into a multimetric index is simply a way of condensing information and finding the desired response to impact (Karr and Chu 1999).

### *1.7.4 Multivariate analysis*

Using multivariate analysis methods, multiple predictor (e.g., water chemistry, lake morphometry, habitat characteristics) and response (e.g., numbers of different

taxa) variables can be combined into a single assessment. Multivariate analyses can be used to explain which factors are influencing community changes, and account for some sources of environmental variability. In RBAs, multivariate methods have often been used to create predictive models for the assessment of sites (e.g., Turak *et al.* 2004; Wright 1995). Multivariate predictive models are now commonly used in regional RBA programs in Europe (e.g., RIVPACS (River Invertebrate Prediction and Classification System)) and Australia (e.g., AUSRIVAS (Australian River Assessment System)) (Freshwater Biological Monitoring and Department of Natural Resources and Mines 2001; Turak *et al.* 2004; Wright 1995) and require a similar level of effort to the American multimetric system once the models have been created; however, they do require a greater initial investment in time and statistical expertise (Norris 1995).

Multivariate predictive models require a large initial time investment to sample reference sites and to determine which environmental characteristics will be used to predict the presence of benthic invertebrates at test sites. Benthic invertebrates are first sampled across a large number of relatively unimpacted sites, along with measurements of the habitat and environmental characteristics of each site (Norris 1995). For the initial design of the RIVPACS predictive model used in the United Kingdom, benthos were sampled at 270 reference sites, and at each site 28 environmental characteristics were measured (Wright *et al.* 1984). Reference sites are then separated into groups based on the composition of benthic invertebrate communities using multivariate classification techniques (e.g., clustering methods such as TWINSPAN (two-way indicator species analysis)), before a correlation and discriminant function analyses (DFA) are performed to determine which environmental variables explain most of the

variation associated with benthic groupings (Norris 1995; Reynoldson *et al.* 1995). In general, 10 to 15 environmental variables are selected for the predictive model and these should not be influenced by human impacts (Norris 1995).

Once a predictive model has been designed, test sites can be assessed based on the benthic and environmental data collected. For all test sites, a standardized benthic sample must be collected and all of the environmental variables selected for the model must be measured. Using the environmental characteristics of the test site, it is assigned to a reference group. The benthic community collected at the test site is then compared to the benthic community predicted for unimpacted condition using the predictive model (Norris 1995; Van Sickle *et al.* 2005). Sites with significant deviations from the predicted benthic invertebrate community would be assessed as impacted using these models.

Predictive models have the potential to increase the precision and accuracy of impact assessment (Hawkins *et al.* 2010); however, few comparisons have been made between univariate and multivariate methods, and those that have been performed have often observed similar levels of accuracy and precision. The accuracy of multivariate assessments was higher than for metrics (e.g., Lücke and Johnson 2009), but equivalent to the accuracy of biotic indices (e.g., Herbst and Silldorff 2006) and multimetric indices (e.g., Lücke and Johnson 2009). Assessment precision using multivariate methods has been reported as both superior (e.g., Milner and Oswood 2000; Reynoldson *et al.* 1997) or equivalent to the precision of multimetric indices (e.g., Lücke and Johnson 2009). Conclusions may be difficult to make from comparisons of different analysis methods because they generally use different components of

community structure to assess impacts (Hawkins *et al.* 2010), which could be variably effective by region or the stressors present.

Multivariate analysis methods have been used less often in RBAs (21%), perhaps because of the large initial time investment required and the perception that they will be too complicated for resource managers to interpret results. The large initial sampling survey of reference sites is a valid concern for programs with limited staff and funding, or in areas where impacts are widespread and few unimpacted sites remain. It is also possible that an employee with sufficient statistical expertise to design the predictive model is not available, making a less complicated analysis method more desirable. When the time, money and expertise are available, predictive models can be designed with a user-friendly interface that allows those with limited statistical backgrounds to enter and interpret data with ease (Norris 1995); however, it is likely that in most cases univariate assessment methods may be a more practical choice for a RBA program.

## 1.8 Conclusions and recommendations

Designing an RBA protocol for lakes requires choosing sampling, processing and analytical methods from a wide range of options. Because there are so many different methods, the design of RBA protocols for this study began with a literature survey of methods used effectively for RBA. This literature survey was supplemented with other pertinent literature in the field of benthic invertebrate biomonitoring when applicable. Based on this literature review, methods that could reduce effort without

compromising our ability to detect impacts were identified and selected for testing in Manitoba's boreal shield.

I recommend a stratified sampling design for the collection of benthos from the littoral zone of lakes. To maximize the cost-efficiency of protocols, I recommend that sampling is stratified by season, sample area and habitat type. This will reduce the temporal and spatial variability of the benthic communities collected (Johnson 1998; Resh and McElravy 1993; White and Irvine 2003), and should improve the precision of assessments based on the collection of one or two samples per site. Fall is recommended as a sampling season for this study because benthic communities have high richness at this time and the length of sampling season could allow more lakes to be sampled than possible in spring (Jones *et al.* 2004). Richness is potentially the most useful metric for freshwater bioassessment (e.g., Kerans and Karr 1994; Resh and Jackson 1993), making the richness of benthic samples an important consideration in the design of this RBA protocol. Thus, benthic macroinvertebrates should be collected from the littoral zone, where the richness of this assemblage is highest (Brinkhurst 1974). By collecting more taxa, the indicator value of benthic macroinvertebrate communities is maximized, potentially allowing a greater range of impacts to be detected. To minimize sources of variability on invertebrate richness, sample area should be standardized because larger samples result in the collection of more taxa (Colwell and Coddington 1995; Magurran 2004b). A hand-held net (e.g., a D-net) should be used to collect samples because it is portable and versatile among habitat types in the littoral zone. Hand-held nets have been used in the majority of stream RBAs and have been recommended for (e.g., David *et al.* 1998; Jones *et al.* 2004; Reid

*et al.* 1994a) and used in the RBA of lakes (e.g., Somers *et al.* 1998; Wesolek *et al.* 2010).

In the lab, washing samples in a 500 µm sieve, subsampling and using coarser taxonomic resolution are recommended to reduce the time to process samples. Washing samples in larger mesh sizes reduces the time and costs associated with sorting benthic samples (e.g., Farara and Burt 1999; Thompson *et al.* 2003; Wildish 1978), and a 500 µm mesh size provides similar estimates of richness, biotic index scores and biomass as finer mesh sizes (e.g., Buss and Borges 2008; Morin *et al.* 2004; Rosenberg *et al.* 1999b). Samples should then be subsampled to reduce processing time (e.g., Growns *et al.* 1997). Subsampling should be random to reduce the bias of collecting invertebrates that are more conspicuous (e.g., Hilsenhoff 1982; Nichols and Norris 2006) and tend to have lower tolerances to impacts such as organic enrichment (Hilsenhoff 1988). This type of sorting bias could reduce our ability to detect impacts by missing a large proportion of the benthic community. At least 200 individuals should be processed to ensure that our ability to detect impacts is not compromised by the more variable metric and index scores associated with fixed counts of 100 (e.g., Doberstein *et al.* 2000). Using a fixed count of at least 200 is also important because it can keep the number of individuals processed relatively consistent; processing more or fewer individuals can affect estimates of richness or community composition and should be avoided (Magurran 2004a; Vinson and Hawkins 1996). Benthic invertebrates should be identified to family instead of genus or species for the RBA of lakes. Family-level taxonomic resolution offers large savings in the time and costs to process samples (e.g., Ferraro and Cole 1995; Jones 2008; Thompson *et al.* 2003) and has been shown to be

effective for RBA (e.g., Chessman *et al.* 2007; Metzeling and Miller 2001; O' Leary *et al.* 2004; Wright *et al.* 1995).

For the assessment of sites, simple univariate metrics or combinations of metrics (multimetric indices) are recommended for the assessment of impacts because they are based on biological responses and are easily calculated and interpreted by non-specialists. Multivariate analysis methods have been successfully used for the RBA of European and Australian streams (e.g., Turak *et al.* 2004; Wright *et al.* 1984); however, the large initial investment in time and the greater statistical expertise required limited the application of these methods to this protocol (Norris 1995). Metrics have been used extensively in RBAs (Resh and Jackson 1993), and the potential to reduce their variability by combining them into a multimetric index should be investigated for lakes (e.g., Kimberling *et al.* 2001).

Field testing was required to address other protocol considerations. These included determining what habitat type to sample in the littoral zone, how large an area should be sampled, and which metrics or indices are the most effective for impact assessment. These remaining issues were addressed in Chapter 2.

## Tables and Figures

Table 1.1 The relative proportion of sampler use reported in benthic invertebrate RBA's (N = 77). Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

| | **Sampler** | **% of RBAs** |
|---|---|---|
| Quantitative | Corer | 2.6 |
| | Artificial substrate | 2.6 |
| | Surber | 11.7 |
| | Hess | 5.2 |
| Qualitative | Hand held net | 72.7 |
| Multiple samplers | Multiple (quantitative and qualitative) | 3.9 |
| | Multiple (qualitative) | 1.3 |

Table 1.2 The number and types of metrics used in multimetric indices (N = 17). Multimetric indices combine different metric values into a unitless score of relative water quality. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1). Korte *et al.* (2010) is not listed in Appendix 1 because it was not returned in the database search of literature. It is listed here because methods used by Stubauer *et al*. (2010) are described.

| | | Number of component metrics | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study** | **Total number of metrics** | **Habitat characteristics** | **Biological / ecological traits** | **Abundance / density** | **Mass** | **% taxa** | **% tolerant / intolerant** | **% dominant** | **Ratios of taxa** | **Functional feeding groups** | **Richness** | **Evenness** | **Diversity** | **Biotic index** | **Similarity index** |
| Fore *et al*. 1996 | 11 | | | 1 | | | 2 | 1 | | | 7 | | | | |
| Thorne and Williams 1997 | 4 | | | | | | | | | | 2 | | | 1 | 1 |
| Whiles *et al*. 2000 | 7 | | | | | | | 1 | 1 | 1 | 2 | | | 1 | 1 |
| Merritt *et al*. 2002 | 13 | 8 | 2 | 1 | 1 | | | | | | 1 | | | | |
| Morley and Karr 2002 | 9 | | 1 | | | | 1 | 1 | | 2 | 4 | | | | |
| Weigel *et al*. 2002 | 8 | | 1 | 1 | | 2 | | | | 2 | 1 | | | 1 | |
| Blocksom and Johnson 2009 | 9 | | | | | 1 | 1 | | | 3 | 4 | | | | |
| Boonsoong *et al*. 2009 | 9 | | | | | 1 | 2 | | | 2 | 3 | | | 1 | |
| Masese *et al*. 2009 | 10 | | | | | 1 | 1 | 1 | | 3 | 4 | | | | |
| Stein *et al*. 2009 | 7 | | | | | 1 | 2 | | | 2 | 2 | | | | |
| Archaimbault *et al*. 2010 | 22 | | 22 | | | | | | | | | | | | |
| Korte *et al*. 2010 | 4 | | | | | 1 | | | | | | 1 | 1 | 1 | |
| | 6 | | | 3 | | | | | | | 1 | | 1 | 1 | |
| | 5 | | | 1 | | 2 | | | | | | | 1 | 1 | |
| | 4 | | | 1 | | 1 | | | | | | | 1 | 1 | |
| Menetrey *et al*. 2011 | 3 | | | | | | | | | | 3 | | | | |
| Oliveira *et al*. 2011 | 9 | | | | | 3 | | | 2 | 1 | 2 | | 1 | | |

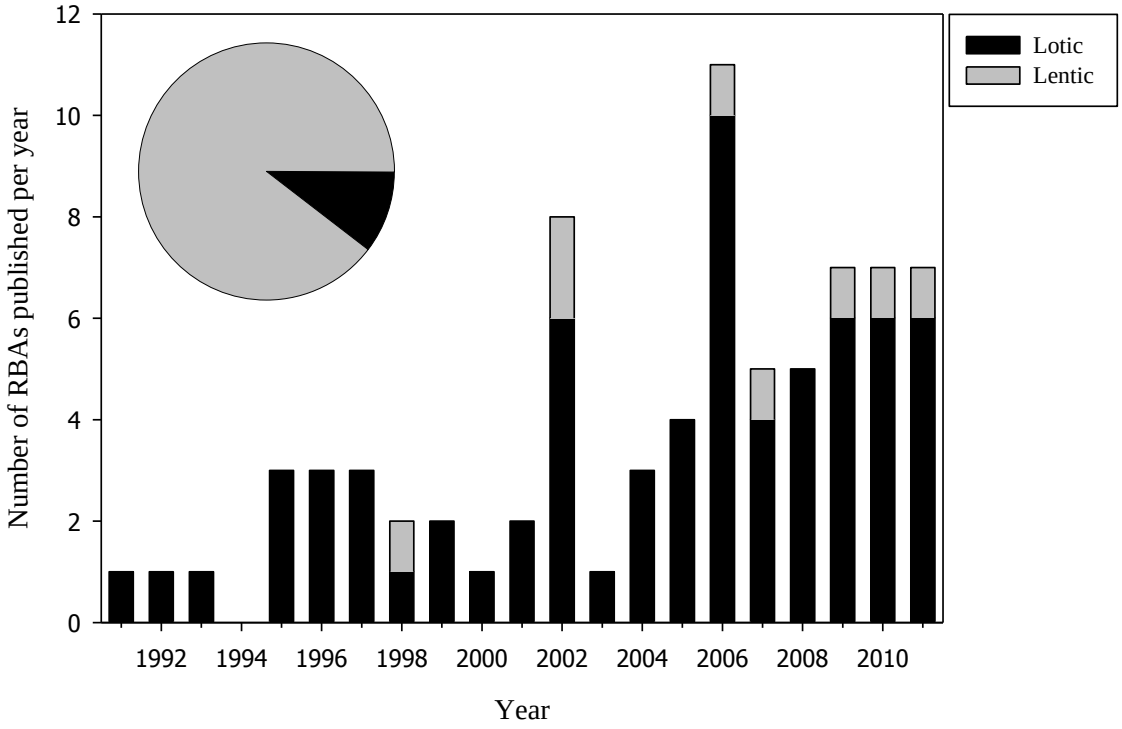


Figure 1.1 Number of lotic (black) and lentic (grey) rapid or qualitative benthic invertebrate assessments published annually (total number of studies (N) = 77). Inset pie chart displays the prevalence of lotic (black) and lentic (grey) studies published overall. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

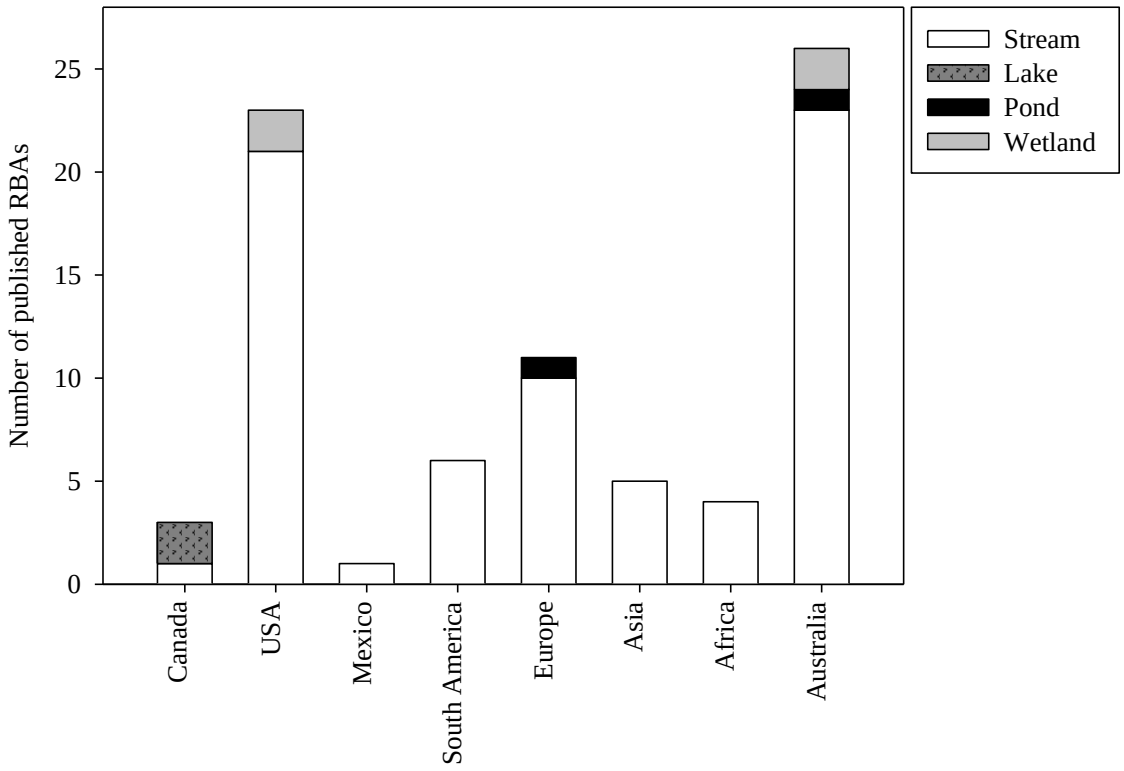


Figure 1.2 Number of rapid or qualitative benthic invertebrate assessments performed in streams, lakes, ponds and wetlands of different regions (N = 77). Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

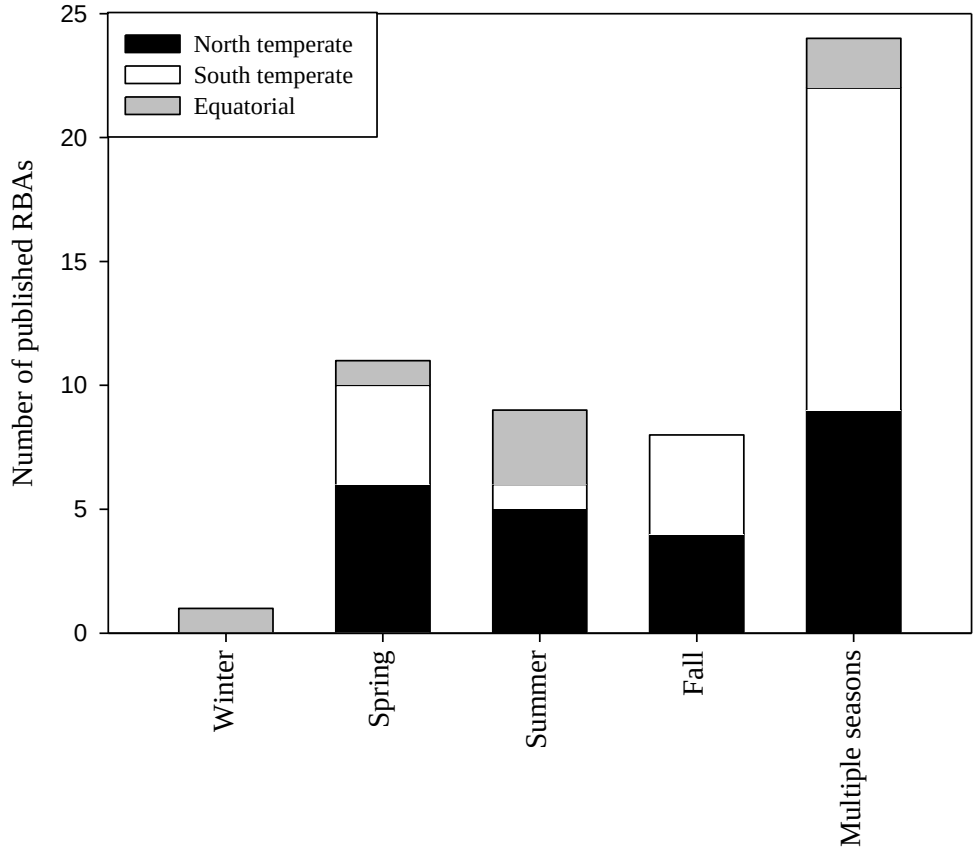


Figure 1.3 Number of north temperate (black), south temperate (white) and equatorial (grey) RBA's reporting one or more seasons for benthic invertebrate sampling (N = 53). Winter samples were collected between November and March in the northern hemisphere, and between June and August in the southern hemisphere. Spring samples were collected between April and May in the northern hemisphere, and between September and October in the southern hemisphere. Summer samples were collected between June and August in the northern hemisphere, and between November and March in the southern hemisphere. Fall samples were collected between September and October in the northern hemisphere, and between April and May in the southern hemisphere. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

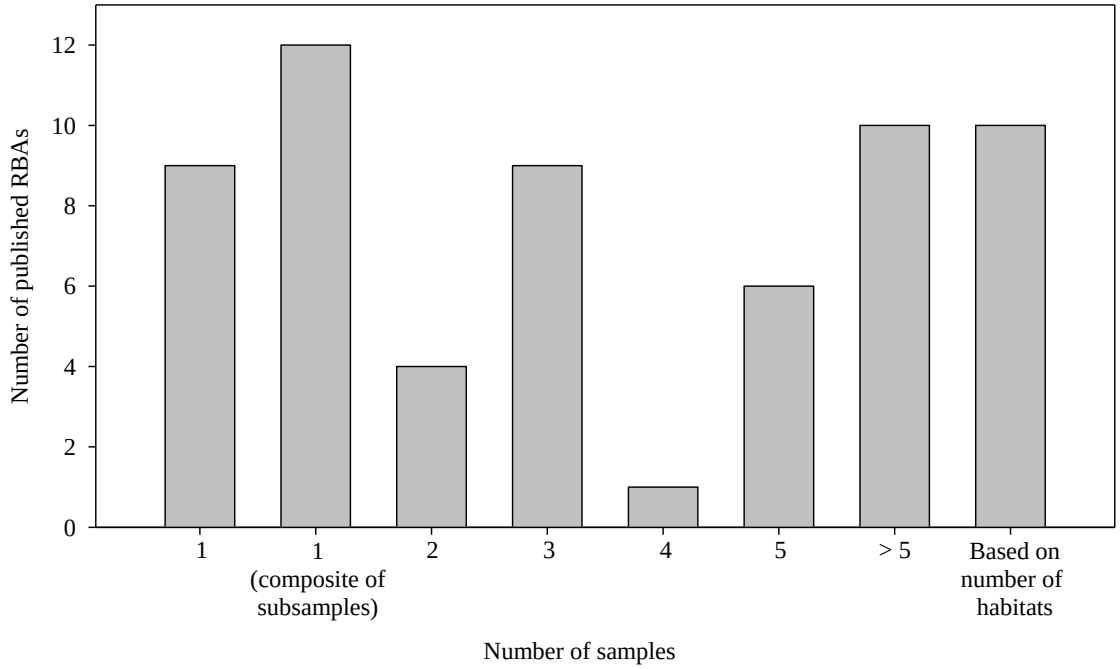


Figure 1.4 Number of benthic invertebrate samples collected per site in published RBA's (N = 61). For studies reporting the use of one composite sample, multiple samples (often collected from different habitat types) were collected per site and combined before they were treated as a single sample. When sampling design was based on number of habitats, the number of samples collected was equivalent to the number of distinct habitats present. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

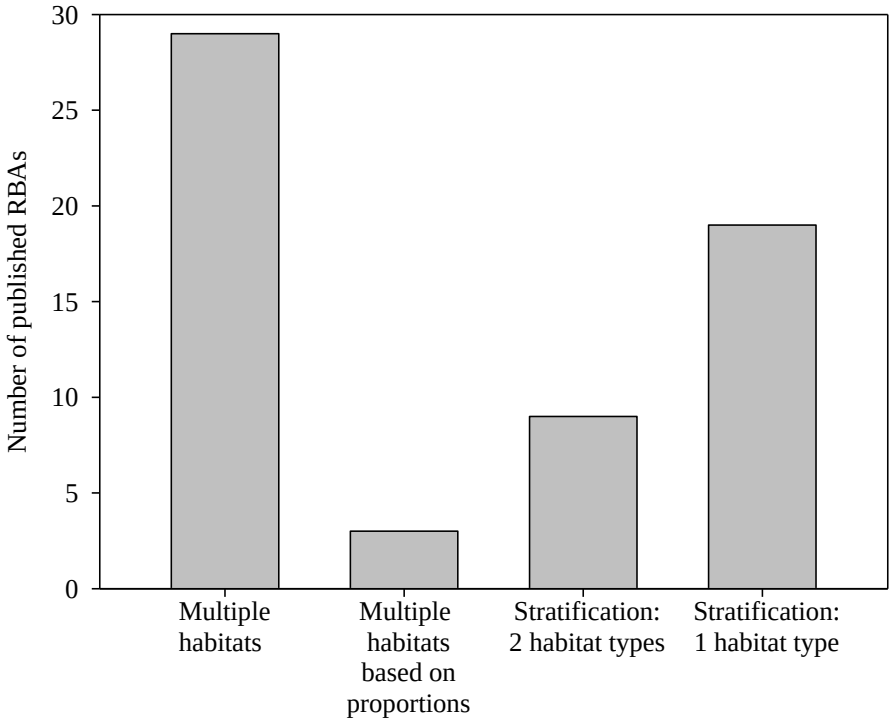


Figure 1.5 Number of RBA's reporting benthic invertebrate sampling from multiple habitats (haphazardly placed or based on proportions of habitats present) or stratified sampling (N = 61). Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

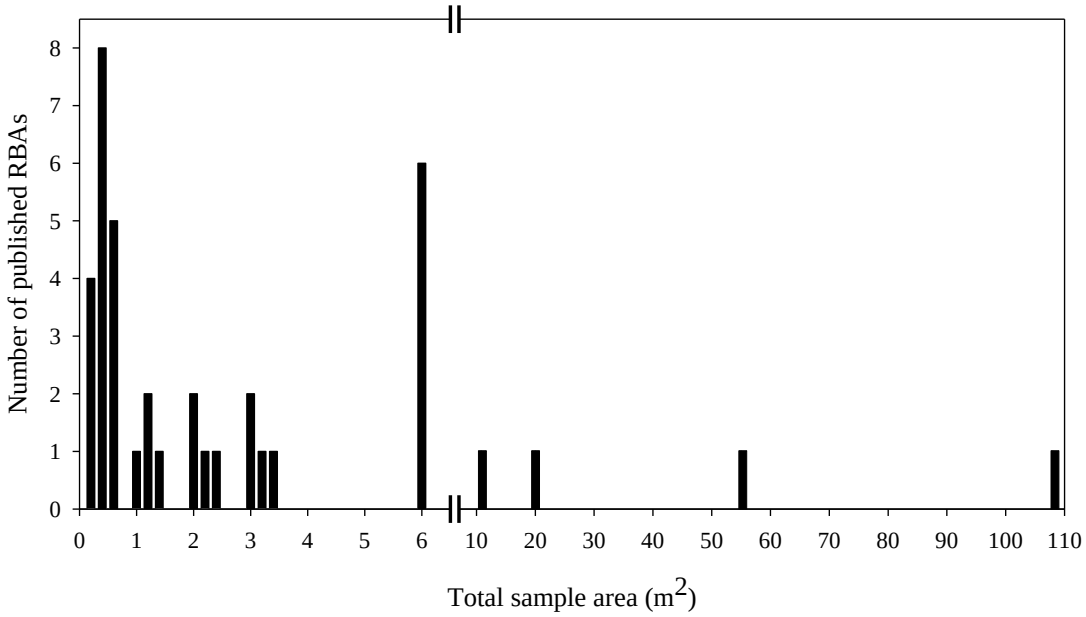


Figure 1.6 Histogram of the total area sampled per site in rapid or qualitative benthic invertebrate assessments (N = 39). Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

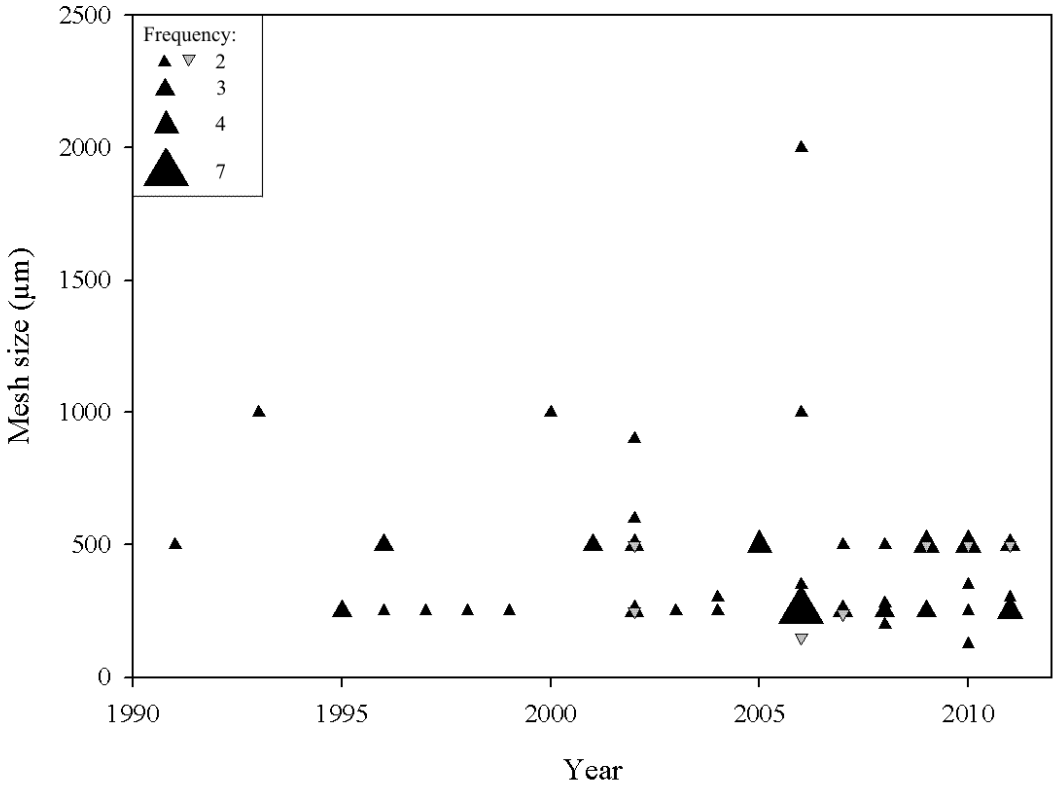


Figure 1.7 Mesh size reported in lotic (black) and lentic (grey) benthic invertebrate RBAs by publication year (N = 68). The frequency of mesh size use each year is represented by the relative size of each data point. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

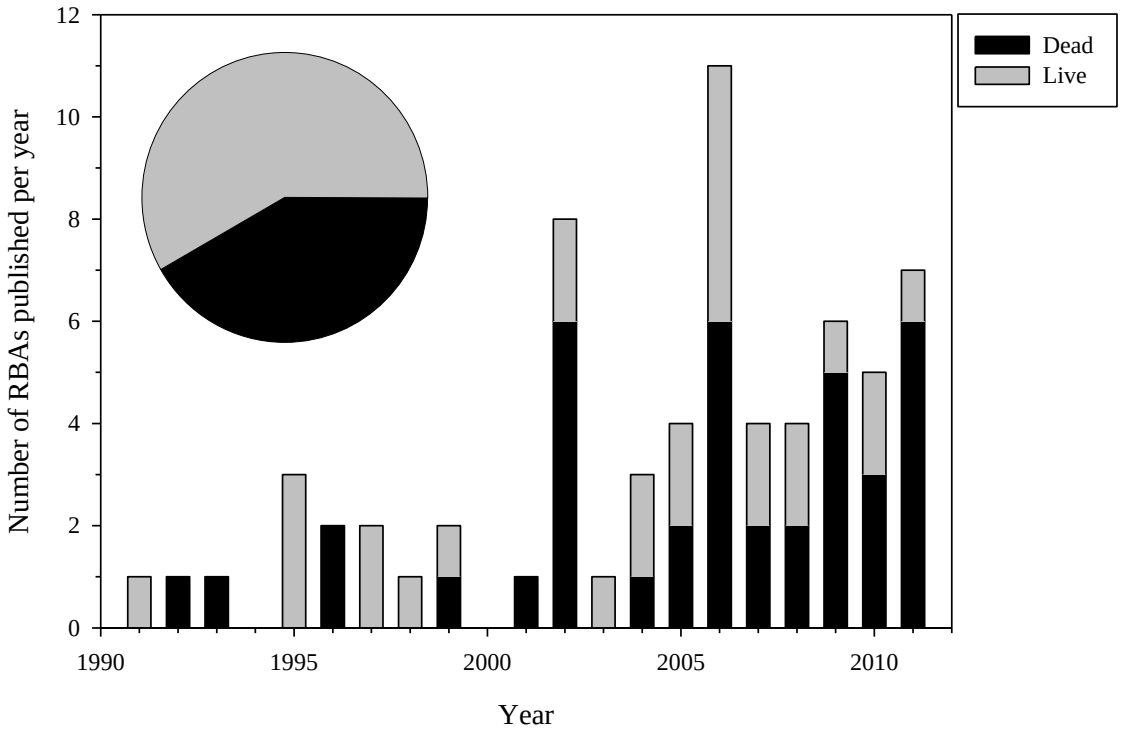


Figure 1.8 Number of RBAs reporting dead (black) or live (grey) sorting of benthic invertebrate by year (N = 67). Inset pie chart displays the prevalence of dead (black) or live (grey) sorting benthic invertebrates in RBA's overall. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

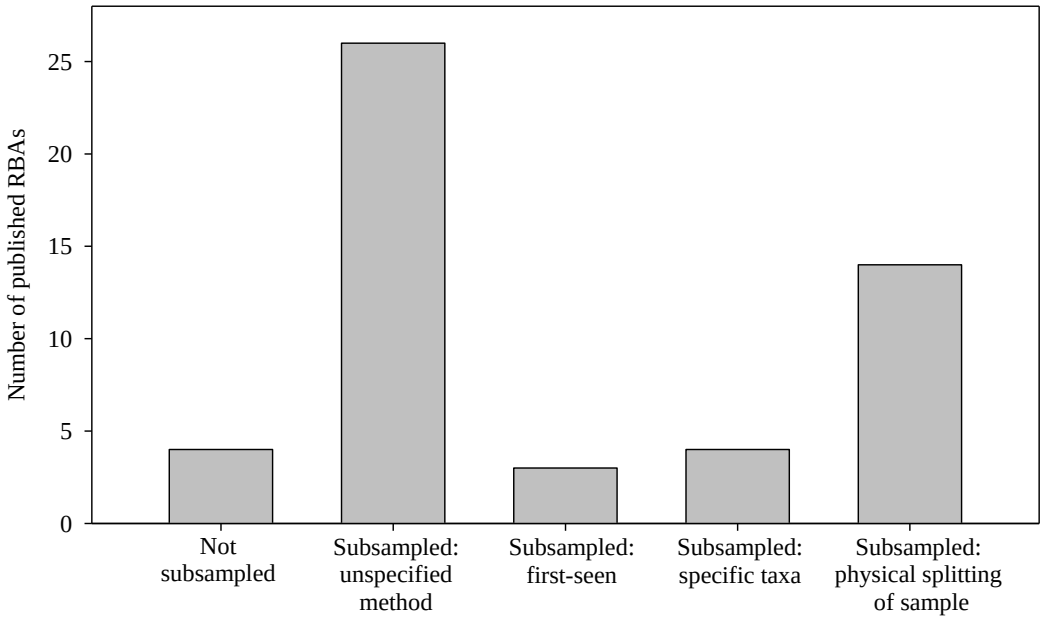


Figure 1.9 Subsampling protocols reported in published benthic invertebrate RBA's (N = 77). First-seen subsampling was characterized as sorting invertebrates from sample material as they are seen until a fixed time had elapsed or a specified number of individuals were collected. When subsampling focused on specific taxa, only certain taxa were collected from sample material. Samples that were physically split were sorted randomly by a known area, volume or weight. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

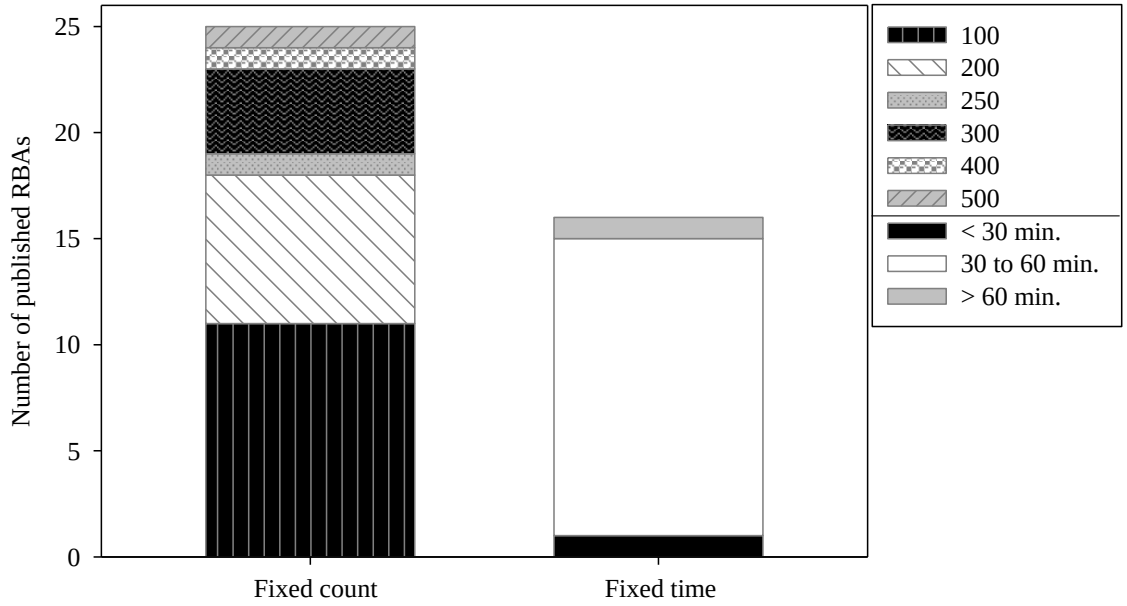


Figure 1.10 Number of RBA's sorting benthic invertebrates using a fixed count or fixed time (N = 41). Fixed-count sorting requires that invertebrates are picked from sample material until a specified count has been reached (e.g., 100, 200, 250, 300, 400, 500). Fixed-time sorting continues until a specified amount of time has elapsed. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

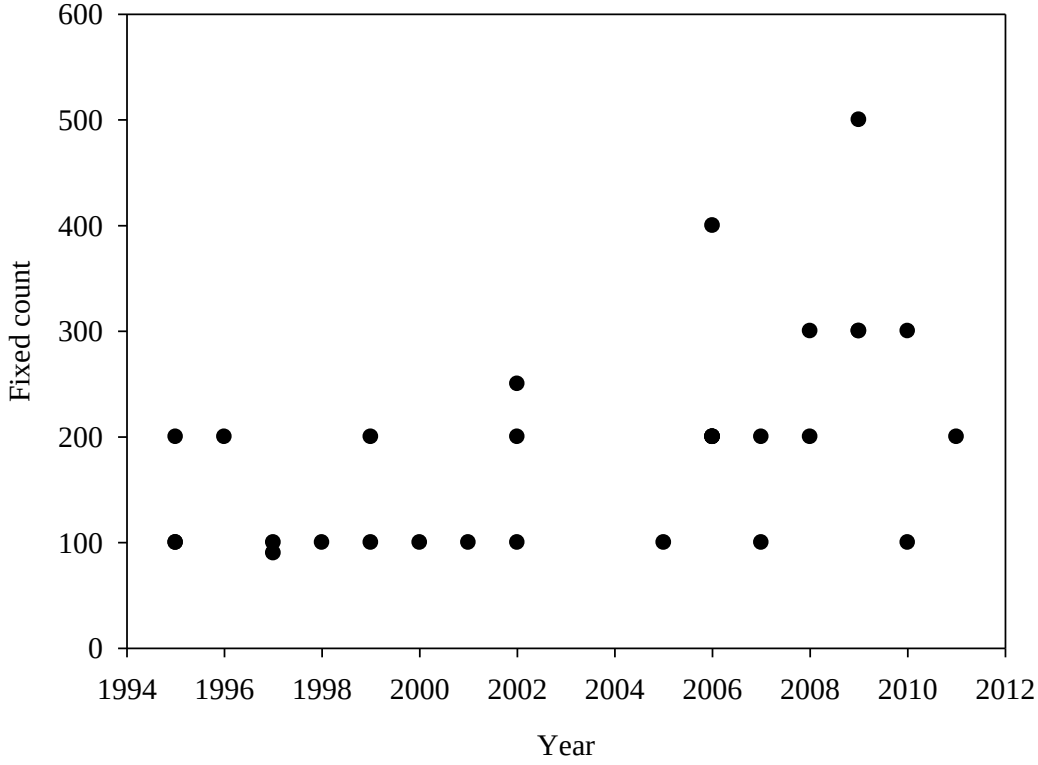


Figure 1.11 Fixed counts reported in benthic invertebrate RBA's by year (N = 30). Fixed-count sorting continues until a specified number (fixed count) of individuals have been collected. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

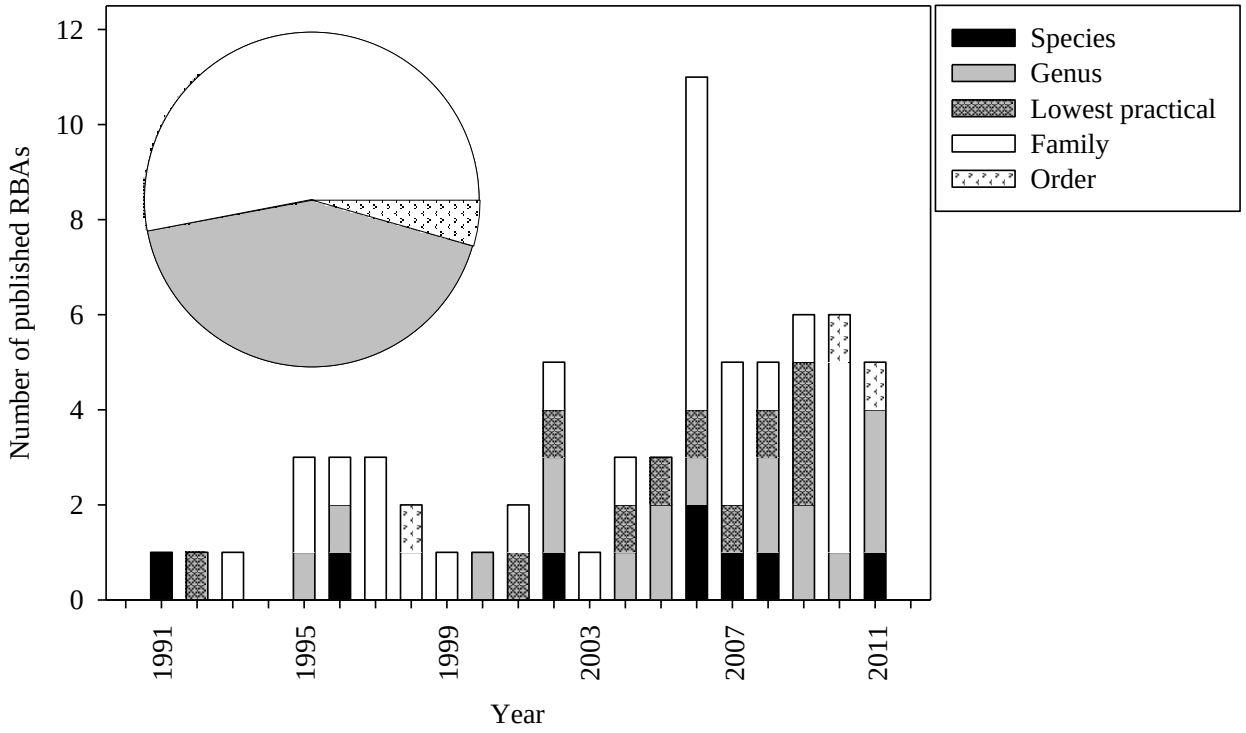


Figure 1.12 Taxonomic resolution in published RBA's by year (N = 68). Inset pie chart displays the overall prevalence of species, genus, lowest practical, family or order taxonomic resolution of benthic invertebrates in RBA's. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

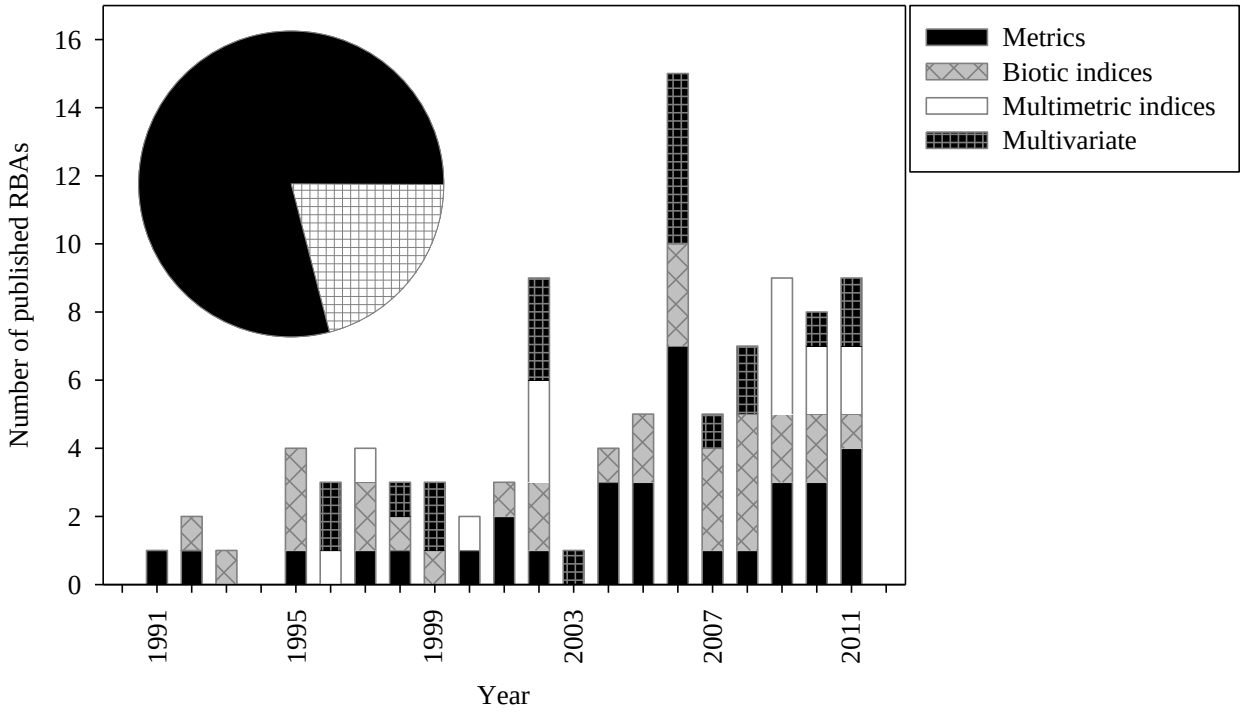


Figure 1.13 Number of RBA's reporting the assessment of impacts using metrics, biotic indices, multimetric indices or multivariate analysis methods (N = 77). Inset pie chart displays the overall prevalence of using metrics, biotic indices, multimetric indices or multivariate analysis methods in RBA's. Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

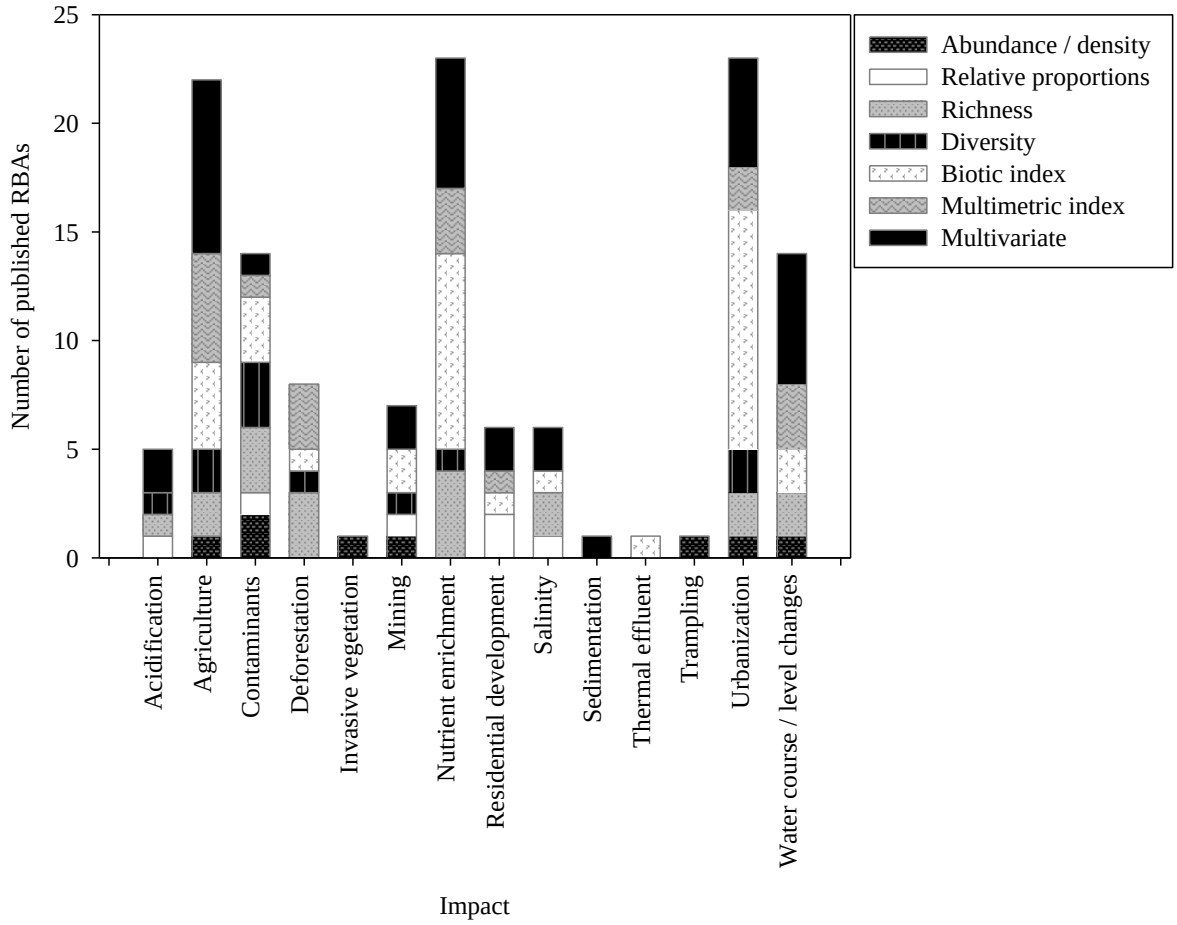


Figure 1.14 Number of RBA's reporting assessment of various impacts using benthic invertebrate community measures (abundance, density, richness, diversity), biotic indices, multimetric indices or multivariate analysis methods (N = 66). Data obtained from a review of freshwater RBA's published prior to July 2011 (Appendix 1).

# CHAPTER 2. Kick-sampling in the littoral zone of a boreal shield lake: effects of habitat, depth and sampled area on estimates of macroinvertebrate community composition

## 2.1 Introduction

Designing biomonitoring programs that are large in geographic scale require protocols that are cost-efficient and accurate. This requires consideration of how samples should be collected and where they should be collected, along with consideration of how environmental variability will affect the samples collected. The use of rapid bioassessment (RBA) protocols has allowed large-scale stream biomonitoring programs to be developed in the United States, Europe and Australia (e.g., Barbour *et al.* 1999; Turak *et al.* 2004; Wright 1995) with qualitative and semi-quantitative sampling strategies; however, the development of similar programs for lakes is far less common (e.g., David *et al.* 1998; Jones *et al.* 2004). To design rapid sampling protocols for lakes, sampling methods require further investigation in the field.

To improve the cost-efficiency of a sampling program, emphasis has been placed on the collection of a broad range of macroinvertebrates using qualitative or semi-quantitative techniques (e.g., Humphries *et al.* 1998; Mackey *et al.* 1984; Metzeling and Miller 2001). Macroinvertebrates are widely used to assess impacts, largely because of the variable responses of species to different environmental conditions (Abel 1989; Hellawell 1986). Thus, sampling communities that have more taxa could facilitate the detection of impacts. In lakes, the littoral zone supports a broad

community of macroinvertebrates expected to show a greater response to impacts in comparison with those in the profundal zone (Donohue *et al.* 2009).

The littoral zone of lakes is a heterogeneous environment that supports a diverse benthic community with great potential to be used in bioassessment. The variable tolerances of macroinvertebrates to different environmental conditions make this community particularly valuable for the purposes of detecting impacts; however, the heterogeneity of the littoral zone and the variability this causes in the benthic community can make this area difficult to sample effectively in a biomonitoring program (Brinkhurst 1974; Kratz *et al.* 2005; Stoffels *et al.* 2005; White and Irvine 2003). Littoral zone heterogeneity is caused by climate, catchment properties, stream and groundwater inflows, thermal regimes within the lake, and the morphometry of the lake basin (Kratz *et al.* 2005). For the littoral zone to be sampled cost-efficiently, sources of environmental variability that are likely to influence communities of benthic macroinvertebrates should be investigated.

Benthic environments are variable within lakes mostly because of differences in exposure to waves, both as depth increases and along the shoreline. Wind speed and fetch affect the size of waves that form and their velocity (Brown *et al.* 1989b; Carper and Bachmann 1984; Smith and Sinclair 1972). In areas exposed to high wave energy, larger rocks predominate because waves suspend and transport sediments composed of smaller particle size (e.g., mud, sand, gravel) which will eventually settle in areas of lower energy (Brown *et al.* 1989a; Likens and Davis 1975). Wave-swept shores not only have larger average grain sizes, but sediment size is also more variable, creating a more complex habitat (Håkanson and Jansson 1983; Minshall 1984). In areas with low

wave energy (i.e., sheltered bays, hypolimnion), only very fine particles are transported by currents (Bloesch 2004; Brown *et al.* 1989a). The sediments in these environments become covered with a layer of fine-grained particles and seston; seston settles in lakes in a similar manner to silt and clay particles (Rasmussen and Rowan 1997). As water depth increases, sediment grain size decreases (Håkanson and Jansson 1983; Wetzel 2001d), organic content of sediment increases (Ali *et al.* 2002; Ostrovsky *et al.* 1997) and plant communities change composition (Pokorný and Květ 2004; Wetzel 2001e). These effects of wave exposure affect the distribution and composition of benthic communities (Beaty *et al.* 2006; Jónasson 2004; Minshall 1984).

Sediment grain size directly affects the distribution and community composition of benthic macroinvertebrates. Macroinvertebrates spend time within interstitial spaces searching for food (Lopez and Holopainen 1987; Williams and Hynes 1974; Zanetell and Peckarsky 1996), hiding from predators (Brusven and Rose 1981) and protecting themselves from environmental disturbances (Dole-Olivier *et al.* 1997; Fenoglio *et al.* 2006; Palmer *et al.* 1992). Macroinvertebrate movement through interstitial spaces is limited by their body size and shape as well as their ability to squeeze themselves through tight spaces (Gayraud and Philippe 2001; Williams and Hynes 1974). The varying ability of taxa to move through sediments by displacing or excavating fine mud particles or by moving through interstitial spaces will affect community composition, and many authors have described invertebrate preferences for differing grain sizes (e.g., Doeg *et al.* 1989; Minshall 1984; Wieser 1959; Williams and Mundie 1978). Because differences in community composition are one of the primary means for detecting

impacts, the habitat preferences of benthos may be a factor complicating assessment when samples are collected from variable environments.

Communities of benthic invertebrates are also affected by the distribution and composition of plants in the littoral zone. The types of plants present (e.g., Biggs and Malthus 1982; Pinel-Alloul *et al.* 1996), their abundance (e.g., James *et al.* 1998) and biomass (e.g., Rasmussen 1988; van de Berg *et al.* 1997; Weatherhead and James 2001) can all influence benthic macroinvertebrate community structure. Plant leaves provide surface area for the growth of algae (Klugh 1926; Pokorný and Květ 2004; Wetzel and Allen 1970) and both the leaves and their epiphytic algae can be food for benthos (Glowacka *et al.* 1976). Macroinvertebrates live on the surface of plant leaves, on the epiphyton covering their leaves, as well as within plants stems (Glowacka *et al.* 1976) and around roots (Prejs and Wiktorzak 1976). Densely distributed plants can serve as a refuge for macroinvertebrates from predators (Beckett *et al.* 1992; Crowder and Cooper 1982; Hershey 1985), reduce wave energy (Barko and James 1998; Fonseca and Cahalan 1992; McComb and Chambers 2003) and stabilize sediments (Klugh 1926; Mackie 2004; McComb and Chambers 2003). This sheltered environment is able to support more generalist invertebrates than wave-swept shores (Burton *et al.* 2004) where species adapted to the more turbulent environment are found (i.e., insects adapted to cling to objects as are observed in fast moving streams) (Ward 1992). Compositional changes in plant communities with increased water depth (Jónasson 1969; Pokorný and Květ 2004; Wetzel 2001e) can consequently influence the distribution of benthic macroinvertebrates at different depths in the littoral zone.

Detritus is a food source for some benthos (France 1995; Muto *et al.* 2011; Reid *et al.* 2008) and thus also influences the distribution and composition of benthic assemblages (Cole and Weigmann 1983; Petridis 1993). Feeding, excretion and turnover by aquatic communities can all contribute to the amount of detritus falling to the bottom of the lake and the organic content of sediments (Jónasson 1969; Steele and Baird 1972; Wetzel *et al.* 1972). The accumulation of detritus therefore tends to increase towards the deeper regions of a lake where there are more planktonic organisms (e.g., phytoplankton, zooplankton) in the water column and where rates of decomposition are longer. These depth related changes in the availability of food can also influence what macroinvertebrate communities are present at increasing distance from shore.

For littoral zone benthos to be used in bioassessment programs, sampling protocols must be designed to allow samples representative of the community to be collected while minimizing sample variability associated with natural lake heterogeneity. Littoral zone communities have been used less often than those of streams and the profundal zone (Wiederholm 1984), perhaps because of the high variability of these communities (Barton and Hynes 1978; Harrison and Hildrew 1998; Rasmussen 1988) and the lack of appropriate methods. To reduce the problem of variability in community composition, sampling methods can be used that allow more consistent results to be obtained, such as stratifying sampling to a specific habitat type or by collecting more samples per site (Prepas 1984). The main drawback of increasing the number of samples collected is the large increases in time and costs this will add to a sampling program. For sampling programs designed for bioassessment of large

geographic areas, reducing metric variability by collecting samples from similar habitats (Johnson 1998; Resh and McElravy 1993; White and Irvine 2003) may be the best way of ensuring a large number of lakes can be monitored; however, the choice of habitat to sample is not obvious. Deciding where to collect samples in the littoral zone and how variability can be minimized are critical to the design of an effective biomonitoring program and there are relatively few published studies in which these issues have been discussed.

An additional consideration for the design of a sampling program is the size of area sampled. This is because macroinvertebrate richness is an important metric for the assessment of freshwater ecosystems (Beck and Hatch 2009) and estimates of richness are dependent on the area sampled. As a larger area is sampled, rare species and microhabitat differences with their associated species are more likely to be encountered (Brinkhurst 1974; Vinson and Hawkins 1996). A characteristic species-area relationship arises in which, as sample area increases, richness rises steeply at first and then gradually levels off to an asymptote (Colwell and Coddington 1995; Magurran 2004b). Species-area curves have been used by ecologists to estimate the area that should be sampled to collect a specific proportion or all of the species present (Magurran 2004b). Sampling areas at or beyond the asymptote of the curve should allow the collection of all species for a given habitat. Collecting smaller samples reduces processing time; however, if a sample area does not allow most species present to be collected, richness estimates will be lower and potentially more variable. Populations that have clumped distributions, such as macroinvertebrates (Elliott 1977b; Resh 1979), may require larger sample areas for the collection of consistent samples

(Elliott 1977b). The sample area required for assessment will differ by habitat type (e.g., Schreiber and Brauns 2010) and region, and needs to be investigated in a preliminary sampling survey before a sampling program is designed.

In preparation for a larger survey of boreal shield lakes (Chapter 3), the objectives of this study were to examine the contribution of sediment type, water depth and area sampled on the composition and variability of benthic macroinvertebrates in kick samples collected in the littoral zone. Macroinvertebrates were collected from boulder, cobble and mud sediments to examine the effects of habitat type on the samples collected. Within each habitat type, samples were collected from three different depths using a standard sample area. The effect of sample area on richness was investigated by combining sample data collected from the same habitat type and depth to form larger, synthetic samples. Data collected were used to investigate the effects of habitat, depth and sample area on benthic community composition, richness and metric variability. These investigations were made to aid in design choices for a new rapid bioassessment protocol for lakes.

## 2.2 Methods

Macroinvertebrate sampling was conducted in Malloy Lake, Whiteshell Provincial Park, Manitoba (lat 50˚01'13"N, long 95˚26'41"W) in May 2007. Malloy Lake has a surface area of 515 ha, a maximum depth of 4.41 m, a mean depth of 2.43 m and is considered mesotrophic based on Secchi depth and the concentrations of phosphorus, nitrogen and chlorophyll *a* (Wetzel 2001b). Malloy Lake was chosen because it is relatively unimpacted and easy to access. The littoral zone of Malloy Lake

also appeared typical of boreal shield lakes in the area, which are dominated by rocky, wave-swept shores and sheltered bays with macrophytes in mud sediments.

A sampling layout of thirty, 2 m² plots was created in three sediment types common in the study area (Figure 2.1). Three sections of shoreline were selected where boulder (> 256 mm), cobble (64 – 256 mm) and mud (< 0.05 mm) were the proportionally dominant grain size in the first 50 cm of water depth from the land-water interface.

Sampling beyond 1 m in depth is difficult using a D-net, so the first metre of water depth was evenly divided into three sections for this investigation. The depth ranges used were 0 to 0.33 m (shallow), 0.33 to 0.66 m (medium) and 0.66 to 0.99 m (deep). The slope of the lake bottom resulted in different plot shapes (i.e., the plots closest to shore were generally long and thin compared to the plots furthest from shore where slope increased; Figure 2.1). Each of the 2 m² plots was measured along the surface of the water for each depth range. For example, within the shallow depth range, 2 m² was divided by the surface distance required to span from 0 to 0.33 m in depth (measured perpendicular to the shoreline) to obtain the required plot width. Plot corners were marked with flags and separated by at least 1 m to reduce the disturbance to macroinvertebrates between plots during sampling. Ten, 2 m² plots were sampled in each depth range.

### *2.2.1 Macroinvertebrate sampling*

Samples were collected using a D-net with a 500 μm mesh size and a traveling kick and sweep method (e.g., David *et al.* 1998; Jones *et al.* 2004). In mud-dominated

sediment, the collection method was slightly modified to minimize the ratio of mud to invertebrates within the samples; instead of disturbing sediment by kicking, the net was tapped along the sediment, disturbing only the first few cm of sediment depth. When macrophyte stands were present in the sampling area, the D-net was first swept quickly through the plants before walking through them using the traveling kick and sweep sampling method. In macrophyte beds, there are often many invertebrates that are associated with the stems and leaves of macrophytes (Cyr and Downing 1988; Gerking 1957; Krecker 1939) and these often fast-moving animals would not otherwise have been effectively collected. Sampling continued in a plot until it was believed that all areas were thoroughly covered (i.e., more time was spent in areas that were difficult to sample, such as those with large logs).

Samples were washed using the 500 μm mesh net in the field and preserved in a 10% formalin solution before they were transported back to the lab for processing.

### *2.2.2 Sample processing*

In the laboratory, samples were washed in 500 μm sieves until all or nearly all of the fine sediment was flushed out. Samples were then transferred into 95% ethanol, which was replaced with 70% ethanol approximately one week later. The samples were transferred to ethanol because formalin is an excellent fixative but it damages specimens over prolonged storage (i.e., makes them brittle) (Giere 2009). The two concentrations of ethanol were used because the high water content of plant, animal and detrital material dilutes the first addition of ethanol.

Boulder and cobble samples were then processed entirely, whereas mud samples were subsampled because of their large size and resulting high processing time. All samples were sorted by placing a small portion of the sample in a gridded Petri dish and searching under incident light for invertebrates using a minimum stereomicroscope magnification of 8×. Each grid was searched twice, using forceps to move or break up material and collect invertebrates; material in each dish was mixed using forceps between the two searches. The subsampling procedures used for mud samples followed those recommended by the United States Geological Survey (USGS) for the collection of 300 organisms (Moulton *et al.* 2000). Macroinvertebrates were identified to family with the exceptions of Nematoda, Acari, Ostracoda and Copepoda, which were identified to phylum, superorder, class and order, respectively. Taxonomic keys used to identify macroinvertebrates were in Edmunds *et al*. (1976) for Ephemeroptera, Wiggins (1996a) for Trichoptera, Kathman and Brinkhurst (1998) for Oligochaeta, Clarke (1981) for Mollusca and Merritt and Cummins (1996) and Thorp and Covich (2001) for remaining insects and non-insect invertebrates, respectively. Voucher specimens are stored at the Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, MB.

To ensure samples were sorted properly, samples were subjected to a Quality Assurance (QA) procedure that required reprocessing by someone other than the original sorter. One of every 10 samples was randomly chosen and reprocessed to determine the sorting efficiency of the original sorter. Sorting efficiencies that fell beneath 95% were considered a fail and required the original sorter to reprocess the remaining 9 samples in that set of 10.

### *2.2.3 Data analysis*

*Habitat type and depth*

Taxa richness and mean abundance of chironomids, oligochaetes, Ephemeroptera and Trichoptera (ETs), amphipods and all invertebrates were compared using a two-way ANOVA. All mean abundance data were $\log_{10} + 1$ transformed to validate ANOVA assumptions concerning variance. Habitat (boulder, cobble, mud) and depth (shallow, medium, deep) were the two factors used; habitat was considered a fixed factor whereas depth was considered a random factor. Depth was considered a random factor for this analysis because if this study was repeated I would not necessarily use the same depth ranges. The two factors were crossed to assess if there was a significant interaction between them. An adjusted error rate of $P < 0.008513$ was used to assess the significance of main effects and interaction effect for all ANOVAs to account for multiple comparisons being made. This adjusted error was calculated using the Dunn-Sidak correction (Gotelli and Ellison 2004) with the number of comparisons being represented by the total number of response variables in the experiment ($N = 6$). Because of the loss of one sample in the deep-mud treatment, one sample was randomly selected and removed from each of the remaining treatments to create a balanced experimental design for the investigation of habitat type and depth. This balanced design allowed means of abundance and richness to be compared with greater ease.

Non-orthogonal contrasts were used to determine the origin of significant interaction terms (Grafen and Hails 2002). The contrasts were chosen *a posteriori* based on a visual assessment of data among treatments (i.e., means with the largest

differences were compared). When the interaction between habitat and depth was insignificant, differences in treatment means were tested for significance using Tukey's "honestly significant difference" (HSD) test (Gotelli and Ellison 2004). Because of the large number of comparisons performed in this experiment (N = 42), the experiment-wide error rate was adjusted using the Dunn-Sidak correction ($\alpha \leq 0.001221$).

*Metrics*

The variability of metrics commonly used for detection of impacts was examined in relation to habitat and depth. Relative proportion, richness and diversity metrics were calculated using synthetic plot data (see Table 2.1 for a list of metrics calculated).

In order to complete this study it was not practical to collect all of the samples needed for comparison. Thus, synthetic plots were created to maximize the use of data collected. Data from each plot sampled (Figure 2.1) were combined in different ways to allow multiple comparisons to be made. Without the use of synthetic plots, more samples would be needed, and most of these would have been larger in size and required more time to process.

Synthetic plot data were created by randomly selecting individual plots, with replacement, using Microsoft Access, and combining these data to form a 12 $m^2$ synthetic plot (i.e., six individual plots were randomly selected and combined). Synthetic 12 $m^2$ plots were created for each combination of habitat type and depth (e.g., shallow-mud, medium-cobble, etc.) as well as by combining samples from different depths and habitats (Table 2.2). Data from boulder habitat were not used in the creation

of synthetic multi-habitat samples because the samples collected from boulders had lower richness and abundance than samples collected in the other habitats and an examination of the species area curve suggested that an impractical sample area of at least 20 $m^2$ would have been required for this habitat. For cobble and mud habitats, two samples were combined from each depth (i.e., two shallow, two medium and two deep samples combined). A set of synthetic multi-habitat samples was created by combining one shallow, one medium and one deep sample from both cobble and mud habitat. Fifteen hundred synthetic plots were created for each synthetic sample type (for each combination of sediment type and depth, by habitat type spanning all depths, multi-habitat). Fifteen hundred was the largest number of iterations that could be made for this analysis using Microsoft Access.

Mean proportions and richness of taxa and metric values were calculated and the variability of each metric was assessed using the coefficient of variation (CV), which is calculated by dividing standard deviation by the mean and then multiplying the result by 100 to express the value as a per cent (Elliott 1977a). The coefficient of variation was calculated for each set of 1500 synthetic plots.

*Required sample area*

To examine the effect of area sampled on taxa richness, richness-area curves were created using data from randomly selected individual plots, with replacement, to form 4, 6, 8, 10, 12, 14, 16, 18 and 20 $m^2$ synthetic plots for each treatment using Microsoft Access. Plots were combined within each combination of habitat type and depth (e.g., shallow-mud, medium-cobble, etc.) until each desired sample area was

reached (i.e., two plots were combined to form one synthetic 4 m² plot, three plots were combined to form one synthetic 6 m² plot). This process was repeated until 3000 synthetic plots were created for each sample area and treatment (i.e., 3000 synthetic 4 m² plots were created for shallow-mud). Three thousand was the largest number of iterations that could be made for this analysis using Microsoft Access. Mean richness was used to plot richness-area curves for each treatment. Mean richness at 2 m² was calculated using non-resampled data because no combinations were required for this sample area. Richness-area curves were created for all taxa as well as for taxa groups based on rarity within each treatment; taxa present in > 80% of samples, 50 – 80% of samples, or < 50% of samples were considered commonly, intermediately and rarely occurring, respectively (e.g., Schreiber and Brauns 2010).

## 2.3 Results

### *2.3.1 Habitat type and depth*

Significant effects of habitat type were observed on the mean abundance of chironomids and oligochaetes (Table 2.3); chironomid and oligochaete abundances in boulder habitat were significantly lower than in mud habitat (Figure 2.2). There were no significant effects of depth on mean richness or mean abundance of chironomids and oligochaetes.

A significant ($P \leq 0.008513$) interaction between habitat and depth was observed for total invertebrate abundance and the abundances of ETs and amphipods (Table 2.3). This means that mean abundance (all invertebrates, ETs, amphipods) was affected by depth in different ways depending on which habitat type was sampled. In

shallow water, mean abundance of all invertebrates and amphipods was significantly lower in boulder habitat (Figure 2.2) and mean abundance of ETs was significantly lower in boulder and mud habitat than in cobble. In medium depths, mean abundance of all invertebrates, ETs and amphipods was lowest in boulder habitat and mean abundance of all invertebrates and amphipods was highest in mud habitat. In deep water, mean abundance of all invertebrates and amphipods was highest in mud and the mean abundance of ETs was lowest in boulders. In boulder habitat, mean abundance of all invertebrates and amphipods was significantly higher in deep water. Mean amphipod abundance was significantly higher in shallow water for samples collected from cobble habitat. In mud habitat, mean abundance of all invertebrates and ETs was lowest in shallow water.

Invertebrate abundance was highest in mud and lowest in boulder habitat. The highest mean abundance of invertebrates was observed in mud habitat in deep water (Figure 2.2). This was primarily because a large number of chironomids was collected in deep, mud samples.

### *2.3.2 Metrics*

The relative proportions (Figure 2.3) and richness (Figure 2.4) of macroinvertebrate taxa were variable by habitat type. Chironomids were the most abundant taxon in all habitats, followed by Acari in boulder, mud and multi-habitat samples. Amphipoda was the second most common taxon in the synthetic cobble samples. Other common taxa included Ostracoda in boulders, mayflies (Ephemeroptera) and caddisflies (Trichoptera) in cobble, and beetles (Coleoptera),

mayflies, amphipods and clams (Bivalvia) in mud and multi-habitat samples. Taxa richness was highest in multi-habitat samples, followed by mud, cobble and boulder samples.

The coefficient of variation of three types of metrics: diversity, richness and relative proportions, are shown in Figures 2.5, 2.6, and 2.7, respectively. Of the three types of metrics, diversity metrics had the lowest CVs overall, with no values exceeding 18% (Figure 2.5). Richness metrics also had relatively low variability with the majority of metric CVs at < 20% and higher CVs mostly restricted to Gastropoda and Diptera (Figure 2.6). The CVs of relative proportion metrics were higher than richness and diversity metrics, particular for the % Gastropoda and % Oligochaeta metrics (Figure 2.7).

The lowest CV, for each of the synthetic sample habitat types (boulder, cobble, mud, multi-habitat) regardless of water depth was for the metric % 5 dominant taxa, where all CVs were below 5.2%. The lowest of these % 5 dominant taxa CVs was present in the mud samples (CV = 3.0%). For richness metrics, the lowest CVs for each habitat type were observed in the all taxa richness metric (CV range of 8.1 – 11.6%). The lowest CVs of the relative proportion metrics were observed in % Insecta for each habitat type (CV range of 8.7 – 11.6%).

Without considering water depth, there was no habitat type (boulder, cobble, mud, multi-habitat) that consistently had either the highest or lowest CVs for all metrics. Overall, boulder was the habitat type that most often had the highest metric CVs (highest in 59% of metrics). Boulder samples most often had the highest metric CVs for diversity (highest in 60% of metrics) and relative proportion metrics (highest

in 70% of metrics), and, along with mud samples, had the highest metric CVs most often for richness metrics (highest in 43% of metrics). Overall, mud was the habitat type that most often had the lowest metric CVs (lowest in 41% of metrics). Mud samples had the lowest CV by habitat type for all diversity metrics. The habitat type that most often had the lowest CVs for richness metrics was multi-habitat (lowest in 43% of metrics) and for relative proportion metrics, cobble habitat most often had the lowest CV (lowest in 60% of metrics).

### *2.3.3 Required sample area*

Richness-area curves (all, commonly, intermediately and rarely collected taxa) by each combination of habitat type and depth are presented in Figure 2.8. For all taxa and rare taxa curves, no clear asymptotes were reached by a sample area of 20 $m^2$, regardless of habitat type or depth. At sample areas of 20 $m^2$, richness (all taxa) appeared to be close to reaching an asymptote in medium-boulder, shallow-boulder and medium-cobble samples. To collect all intermediately occurring taxa, a smaller sample size was required. Using an 8 $m^2$ sample area, all intermediately occurring taxa should be collected, regardless of habitat or depth. Samples collected from medium-mud sites only needed an area of 4 $m^2$ to collect all intermediately occurring taxa, while samples collected from medium-cobble required sample areas of 6 $m^2$. For commonly collected taxa, the sample area needed to maximize richness dropped substantially. In all habitats and depths, the richness of commonly collected taxa reached an asymptote by 4 $m^2$.

Taxa richness was relatively similar between treatments with the exception of samples collected from medium- and shallow-boulder sites. In medium- and shallow-

boulder sites, taxa richness was lower than in other treatments. In general, more rare taxa were present in each treatment than common or intermediately occurring taxa, with the exceptions of medium- and deep-cobble, where more common taxa were present.

## 2.4 Discussion

To aid in the development of cost-efficient sampling methods for lakes, the influence of habitat type, depth and sample area were investigated in a boreal shield lake. I have shown estimates of benthic macroinvertebrate communities and their variability are affected by these factors. Depending on how and where benthic macroinvertebrate samples are collected in a lake, different estimates of community composition can result, potentially confounding our ability to detect impacts in a biomonitoring program. I have also shown that some metrics are more variable than others in general, as well as being more variable in specific habitat types. To maximize the effectiveness and cost-efficiency of a bioassessment program using kick-sampling, the habitat type, water depth and area of benthic macroinvertebrate samples should be standardized and assessments should be made using community metrics with low within-lake variability.

### *2.4.1 Habitat type and depth*

There were differences in communities in benthic macroinvertebrate samples collected from different habitat types, particularly those collected from shallow and medium boulder habitats. Shallow and medium boulder samples contained fewer

macroinvertebrates than other samples. This was most likely caused by difficulty sampling this habitat effectively and the simplicity of the habitat. Boulders are often too large to disturb and therefore invertebrates on the surface of boulders would have been collected while those inhabiting the interstitial spaces between would have been missed. Other investigators have sampled boulders by scraping their surfaces (e.g., Weatherhead and James 2001); however, the only way to sample effectively within the interstitial spaces of boulders would be using suction samplers, which are impractical for large scale biomonitoring programs. Boulder habitat is simpler (i.e., fewer interstitial spaces than cobble habitat, less variability in grain size, and no macrophytes), providing less niche space for macroinvertebrates. Tolonen and Hämäläinen (2010) similarly reported lower density of macroinvertebrates along wave swept shores as compared with sheltered areas.

Samples collected from mud habitat, specifically deep mud plots, contained the greatest number of benthic macroinvertebrates. The low wave energy and high sediment stability in this environment favour the colonization of more invertebrates than in wave swept shores (Tolonen and Hämäläinen 2010; Tolonen *et al.* 2001). The high abundance of benthos observed in deep mud samples were mainly because of the large number of chironomids in these samples. Within the first metre of water depth in the littoral zone, there is a larger volume of water available for macrophyte growth as we move further from shore. Macrophytes that grow to a greater height have more surface area as habitat by benthos (Soszka 1975). Chironomids use macrophytes as habitat and also favour the organic-rich sediment that accumulates around them (Brodersen *et al.* 2001; Nyman *et al.* 2005; Waters and San Giovanni 2002).

Macrophytes can also shelter macroinvertebrates from predators such as fish (Beckett *et al.* 1992; Crowder and Cooper 1982; Hershey 1985) or birds, further contributing to prevalence of benthic macroinvertebrates in deep mud sites.

Differences in the community composition of benthic macroinvertebrates were also observed over a very small depth range of 0 to 1 m. Donahue *et al*. (2003) and Macan and Maudsley (1969) similarly reported differences in community composition over depth in the shallow littoral. Thus, even if stratification by habitat is used to reduce variability, community changes with depth could reduce our ability to detect impacts because this can also affect metric scores commonly used for assessment. For example, if we used more effort sampling in the shallow water of a mud shoreline in one pristine lake *versus* another, that lake would have a lower % ET score, incorrectly indicating impact. Along cobble shorelines, if more time was spent sampling near water depths of 1 m *versus* 0.5 m or less, a greater proportion of chironomids would be collected and the site could be incorrectly assessed as impacted. The relationship between benthic macroinvertebrate community structure and water depth can be further complicated by slope (James *et al.* 1998), wave exposure (James *et al.* 1998; Weatherhead and James 2001) and water level-fluctuations (Baumgärtner *et al.* 2008); however, controlling for all of these characteristics would be logistically difficult and more complicated to measure in comparison with water depth.

### *2.4.2 Metrics*

It was expected that multi-habitat samples would have the most variable benthic macroinvertebrate metric scores when compared with samples stratified by habitat type

(Johnson 1998; Resh and Rosenberg 1989); however, in my study, the metric scores of samples collected from boulder habitat were the most variable overall. The high variability of metrics in boulder samples was unlikely the result of inefficient sampling of this habitat. The CVs of metrics calculated from deep boulder samples (i.e., a depth where sampling was efficient) were not consistently lower than those from shallow and medium boulder samples. Consequently, the variability was most likely caused by greater environmental differences (e.g., wave exposure, slope, organic content of sediment, periphyton) and patchiness along the length of the boulder shoreline in comparison with the cobble and mud-dominated shorelines. It was expected that mud-dominated shorelines would have had the most environmental variation within the first metre of water depth in the littoral zone (i.e., because of differences in the types and densities of macrophytes present) as observed by Tolonen *et al*. (2001), but this is not supported by the variability of metrics; the least variable metrics overall were those calculated using mud samples. Multi-habitat samples provided the least variable richness metrics overall. Many taxa were present in both cobble and mud habitats (60% of taxa found in cobble or mud were found in both of these habitat types; 14% of taxa were exclusive to cobble; 26% of taxa were exclusive to mud). However, the increased variability expected using multi-habitat samples in comparison with samples stratified by habitat type may not have been observed because this study used family level taxonomic resolution.

In general, CVs were lower for metrics that use data from a larger proportion of the community (e.g., % 5 dominant taxa, % Insecta, % Chironomidae, taxa richness). For relative proportion metrics, a difference of the same number of organisms will

cause a larger change in metric values for rare taxa than for dominant taxa. This may be why metrics based on taxa that make up a small mean proportion of the sample community (e.g., % Gastropoda, Gastropoda richness, % Oligochaeta) had the highest CVs.

### *2.4.3 Sample area*

To collect all of the taxa present in a given habitat, large kick sample areas of more than 20 m² are required. This sample area is larger than the ≤ 2 m² sample areas used in most stream (Figure 2.6) and wetland (e.g., Chessman *et al.* 2002; King and Richardson 2002; Stein *et al.* 2009) RBAs, as well as most sampling areas used in the littoral zone of lakes (≤ 1 m²) (e.g., Aroviita and Hämäläinen 2008; Brodersen *et al.* 1998; Čiamporová-Zat'ovičová *et al.* 2010; De Sousa *et al.* 2008; James *et al.* 1998; Scheifhacken *et al.* 2007; Stoffels *et al.* 2005; Weatherhead and James 2001; White and Irvine 2003). With the exception of the sample area used by White and Irvine (2003), it is unclear whether or not these studies were designed using species-area curves. The kick sampling area of 0.25 m² used by White and Irvine (2003) was chosen because it allowed the collection of most taxa present (White 2001). Species-area curves have also been used to determine adequate sample size in the littoral zone of German lakes (e.g., Brauns *et al.* 2007b; Schreiber and Brauns 2010). Brauns *et al.* (2007b) found that sample areas of approximately 2 m² were sufficient for the collection of most taxa along natural shorelines, while Schreiber and Brauns (2010) recommended a sampling area of 6.4 m² to collect all littoral zone taxa. The larger sample area required in this study is perhaps because of more taxa being present. Using the taxonomic resolution of

this study, Schreiber and Brauns (2010) collected 29 taxa from two lakes, while 74 taxa were collected from Malloy Lake. This is likely influenced by differences in sampling effort (i.e., a total area of 180 $m^2$ was kick sampled in Malloy Lake and within each German lake a total of only 5 $m^2$ was sampled). Sorting samples by eye (e.g., White 2001) would also reduce the sample area needed to collect all taxa because small, cryptic taxa are more likely to be missed. Processing kick samples 20 $m^2$ in size would be very time consuming making it difficult to monitor many lakes because assessment costs would be high; therefore, we need to consider if it is necessary to collect all taxa.

In general, there were more rare taxa than common and intermediately occurring taxa, as observed by Schreiber and Brauns (2010). The presence of so many rare taxa is the reason why sample areas over 20 $m^2$ are required to collect all taxa; without them, much lower sample areas ($\leq$ 8 $m^2$) could be used. Because rare taxa can obscure community composition patterns by increasing variability (Boulton *et al.* 1992; Marchant 2002), their removal may improve our ability to detect impacts as well as reducing the time needed to process samples, allowing the geographic scope of a biomonitoring program to be increased.

### *2.4.4 Recommendations*

Based on the results of this survey, it is recommended that RBA samples in boreal shield lakes be collected from cobble, mud or both of these habitats. Fewer taxa and less of them were collected in boulder habitat, reducing the indicator value of benthic macroinvertebrates by only collecting a small portion of those present in the

littoral zone. The metrics calculated using boulder sample data were also more variable, making them less useful in distinguishing impacted from unimpacted condition.

If an investigator decides to collect samples from either cobble or mud, it is recommended that a sample area of 10 $m^2$ be used, covering all depths from 0 to 1 m with equivalent sampling effort. To collect all commonly and intermediately occurring taxa, a sample area of 8 $m^2$ is needed for each habitat type, but because there is a large chance that samples will need to be subsampled to keep assessment time as low as possible, an additional 2 $m^2$ sample area is recommended to increase the likelihood that intermediately occurring taxa are not missed. Using the same level of effort from 0 to 1 m in depth is important because macroinvertebrate community composition is affected by depth even within the first metre of depth in the littoral zone (this study; Macan and Maudsley 1969). To reduce the variability of metric scores, sampling effort at different depths must be consistent.

Choosing which metrics (richness, diversity, relative proportions) to use for bioassessment is based on the response of the metric to impact as well as the spatial and temporal variability of metrics. Richness is one of the most consistently used metrics in freshwater bioassessment using benthic macroinvertebrates (Resh and Jackson 1993). Richness is less variable than abundances, relative proportions, or ratios of taxa (e.g., Barbour *et al.* 1992; Johnson 1998; Stephens *et al.* 2008), making it a reliable choice for the bioassessment of lakes. Relative proportion metrics are potentially useful if impacts affect abundances of intolerant taxa (e.g., Blocksom *et al.* 2002). If using relative proportion metrics, it would be best to collect samples from cobble habitat because of their reduced variability in samples from this habitat type. Metrics such as

% Insecta, % Chironomidae or % Ephemeroptera had lower CVs and should be investigated for response to impact before other relative proportion metrics. In my study, the within-lake spatial variability of some relative proportion metrics (e.g., % Gastropoda, % Oligochaeta) was so high that even if these metrics do change with impact, it is unlikely that this could be detected with any certainty.

A combination of metrics (multimetric index) is often recommended for bioassessment. Impacts can alter freshwater ecosystems in multiple ways (e.g., urbanization can increase littoral sedimentation reducing the complexity of stony sediments by filling in interstitial spaces, while at the same time nutrient enrichment can increase the growth of periphyton providing more food for benthos). Using a single metric may not be as reliable as using a combination because ecosystems are complex and we should not expect them to respond in simple ways. The selection of metrics will ultimately depend on an identification of which metrics are sensitive to the suspected impacts; however, for biomonitoring boreal shield lakes, I emphasize that a consideration of variability should be included in the choice of metrics. In cobble habitat, taxa richness, ETO richness, ET richness, Simpson's index, % 5 dominant taxa, % Insecta, % Chironomidae or % Ephemeroptera are recommended. In mud habitat, taxa richness, ETO richness, Simpson's index, % 5 dominant taxa, % Insecta, % Chironomidae, % ETO and % Amphipoda are recommended. If using multi-habitat samples (from cobble and mud sediments), taxa richness, ETO richness, Simpson's index, % 5 dominant taxa, % Insecta, % Chironomidae, % Ephemeroptera and % Acari are recommended.

Based on the results of this survey, we now have a greater understanding of how sediment type, water depth and area sampled affect the composition and variability of benthic macroinvertebrate kick samples collected in boreal shield lakes; however, some limitations remain. This survey of Malloy Lake was performed to determine how data from unimpacted boreal shield lakes are affected by different sampling choices. Unfortunately, the unimpacted boreal shield lakes surrounding Malloy Lake differ morphologically, chemically and biologically, and thus sampling methods may affect the data collected from these lakes in different ways. The sampling recommendations made here need to be tested in a wide range of unimpacted lakes before any firm conclusions can be made. Recommended sampling methods should also be tested in lakes impacted by a specific stressor to determine if the kick-samples collected have the potential to be used in regional biomonitoring programs.

## Tables and Figures

Table 2.1 Macroinvertebrate metrics calculated using synthetic plot data for the assessment of metric variability from samples collected from Malloy Lake, MB. Abbreviations used for metrics are ETO = Ephemeroptera, Trichoptera and Odonata, and ET = Ephemeroptera and Trichoptera. For diversity metrics based on dominant taxa, the cumulative per cent of the 3 or 5 most dominant taxa was calculated.

| Relative proportions | Richness | Diversity |
|---|---|---|
| % Insecta | All taxa | Shannon index |
| % ETO | ETO | Simpson's *D* |
| % ET | ET | % 1 dominant taxon |
| % Ephemeroptera | Ephemeroptera | % 3 dominant taxa |
| % Trichoptera | Trichoptera | % 5 dominant taxa |
| % Chironomidae | Diptera | |
| % Oligochaeta | Gastropoda | |
| % Gastropoda | | |
| % Amphipoda | | |
| % Acari | | |

Table 2.2 The combinations of 2 m² plots by habitat type and depth that were selected randomly, with replacement to form synthetic plots of 12 m². The combination of plot data to form synthetic plots of 12 m² was repeated until 1500 synthetic plots were created for each synthetic sample type. Abbreviations used for depth range are S = shallow, M = medium and D = deep. Data obtained from kick samples collected in Malloy Lake, MB.

| Synthetic | Boulder | | | Cobble | | | Mud | | |
|---|---|---|---|---|---|---|---|---|---|
| sample type | S | M | D | S | M | D | S | M | D |
| Boulder | 2 | 2 | 2 | | | | | | |
| BS | 6 | | | | | | | | |
| BM | | 6 | | | | | | | |
| BD | | | 6 | | | | | | |
| Cobble | | | | 2 | 2 | 2 | | | |
| CS | | | | 6 | | | | | |
| CM | | | | | 6 | | | | |
| CD | | | | | | 6 | | | |
| Mud | | | | | | | 2 | 2 | 2 |
| MS | | | | | | | 6 | | |
| MM | | | | | | | | 6 | |
| MD | | | | | | | | | 6 |
| Multi-habitat | | | | 1 | 1 | 1 | 1 | 1 | 1 |

Table 2.3 Community composition of benthic macroinvertebrates collected from different habitats and depths in Malloy Lake, MB. Mean richness and abundances were calculated for 2 m² samples. Results from two-way ANOVA of each community measure are presented under 'Effects'. Statistically significant effects of habitat type, depth or their interaction are followed by an asterisk (*). The critical value corrected for experiment-wide error rate is 0.008513.

| | | | | | Effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean (standard error) | | | Habitat | | Depth | | Interaction | |
| **Community measure** | **Depth** | **Boulder** | **Cobble** | **Mud** | ***F*** | ***P*** | ***F*** | ***P*** | ***F*** | ***P*** |
| Taxa richness | Shallow | 12.6 (1.1) | 20.5 (1.2) | 18.8 (0.9) | 24.58 | 0.006* | 2.48 | 0.199 | 2.51 | 0.049 |
| | Medium | 11.8 (1.0) | 20.1 (1.0) | 21.3 (0.9) | | | | | | |
| | Deep | 16.8 (1.3) | 21.3 (0.5) | 22.9 (0.9) | | | | | | |
| Invertebrate abundance | Shallow | 71.9 (15.8) | 399.3 (42.3) | 596.6 (167.2) | 24.50 | 0.006* | 2.77 | 0.176 | 5.96 | < 0.001* |
| | Medium | 87.1 (9.6) | 195.4 (17.6) | 1013.6 (123.7) | | | | | | |
| | Deep | 176 (20.5) | 443.3 (88.8) | 1596.3 (223.0) | | | | | | |
| ET abundance | Shallow | 7.8 (1.3) | 58.8 (8.2) | 10.3 (2.9) | 4.63 | 0.091 | 0.04 | 0.965 | 11.54 | < 0.001* |
| | Medium | 3.7 (0.8) | 31.3 (3.4) | 55.1 (9.0) | | | | | | |
| | Deep | 8.0 (1.3) | 27.1 (4.0) | 94.8 (23.5) | | | | | | |
| Chironomidae abundance | Shallow | 23.2 (5.3) | 144.3 (28.6) | 172.9 (70.3) | 34.83 | 0.003* | 10.95 | 0.024 | 2.35 | 0.062 |
| | Medium | 20.2 (8.1) | 69.8 (8.7) | 224.2 (22.5) | | | | | | |
| | Deep | 71.6 (13.7) | 312.9 (82.8) | 613.7 (130.7) | | | | | | |
| Oligochaeta abundance | Shallow | 11.4 (3.9) | 36.9 (6.5) | 195.7 (60.7) | 32.39 | 0.003* | 2.65 | 0.185 | 2.02 | 0.100 |
| | Medium | 14.2 (3.5) | 34.6 (5.8) | 216.1 (23.0) | | | | | | |
| | Deep | 36.2 (5.8) | 21.3 (5.2) | 330.2 (57.9) | | | | | | |
| Amphipoda abundance | Shallow | 1.2 (0.5) | 87.6 (17.3) | 82.1 (21.1) | 10.39 | 0.026 | 0.09 | 0.917 | 13.61 | < 0.001* |
| | Medium | 2.5 (0.6) | 19.7 (3.5) | 89.5 (21.9) | | | | | | |
| | Deep | 10.9 (2.9) | 18.4 (3.2) | 74.2 (12.1) | | | | | | |

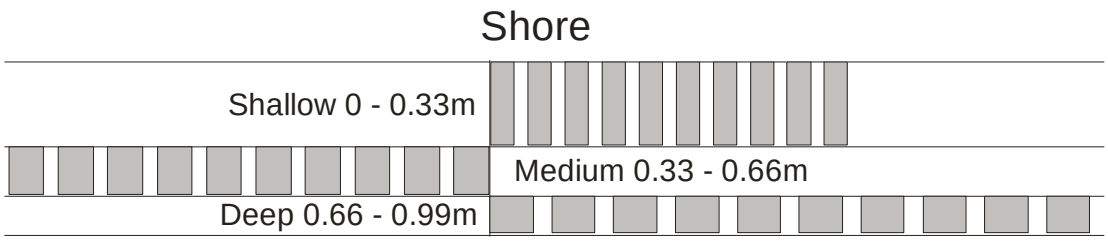


Figure 2.1 Sampling layout used in boulder, cobble and mud-dominated sections of shoreline in Malloy Lake, MB. Thirty, 2 m² plots were sampled from 0 – 0.33 m, 0.33 - 0.66 m, and 0.66 – 0.99 m depth ranges; 10 plots were sampled per depth range.

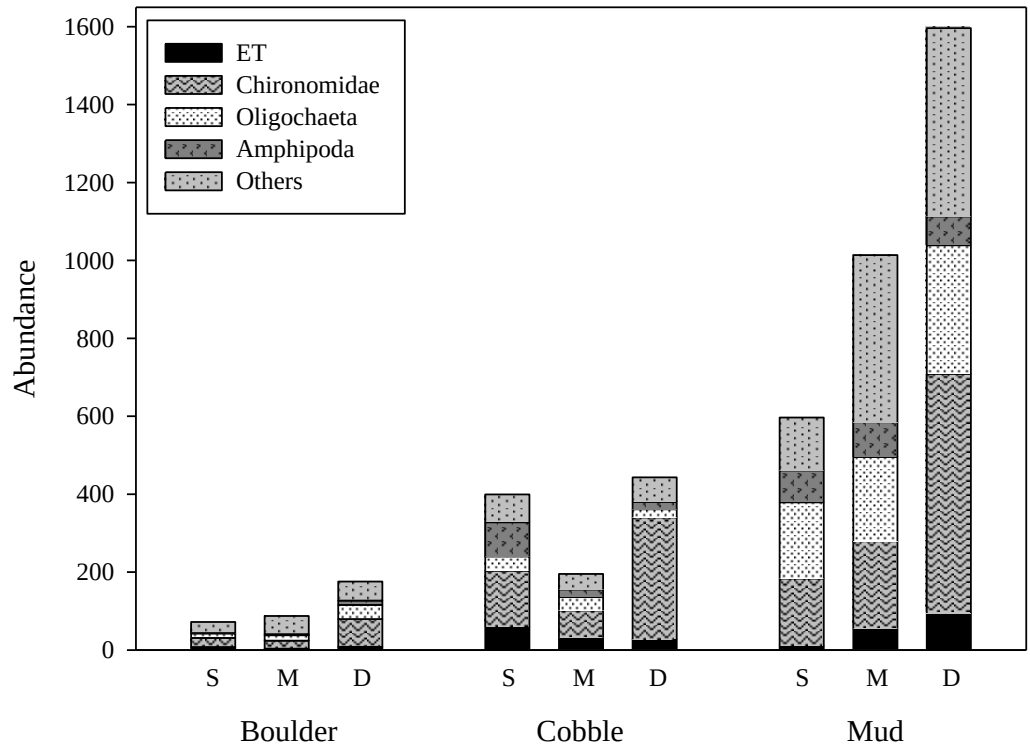


Figure 2.2 Mean abundance of invertebrate taxa by treatment. Mean values were calculated using data from 2 m² samples. Abbreviations used for depth range are S = shallow, M = medium and D = deep. Data obtained from kick samples collected in Malloy Lake, MB.

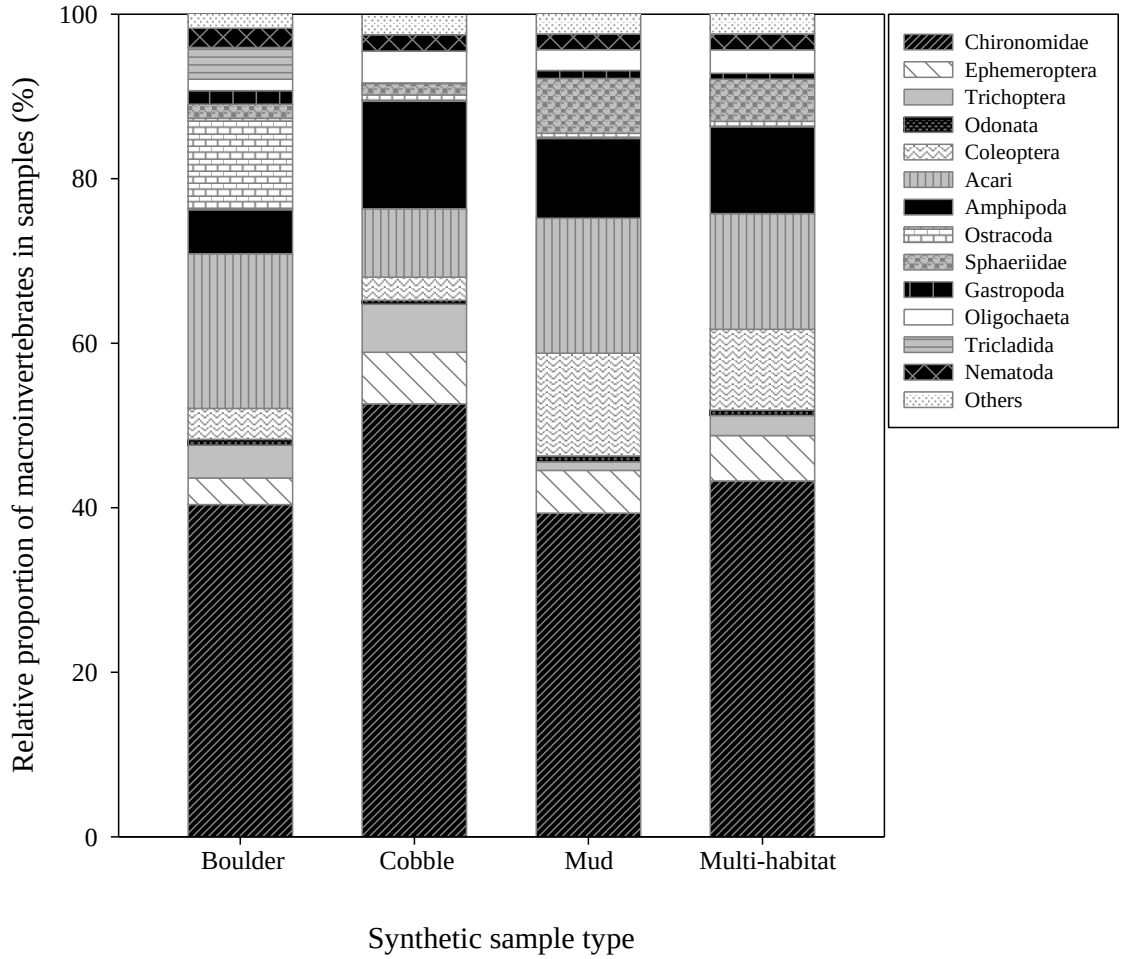


Figure 2.3 Relative proportions of taxa in 12 m² samples by habitat type. Relative proportions were calculated using synthetic sample data created by combining data from individual plots using Microsoft Access. Data obtained from kick samples collected in Malloy Lake, MB.

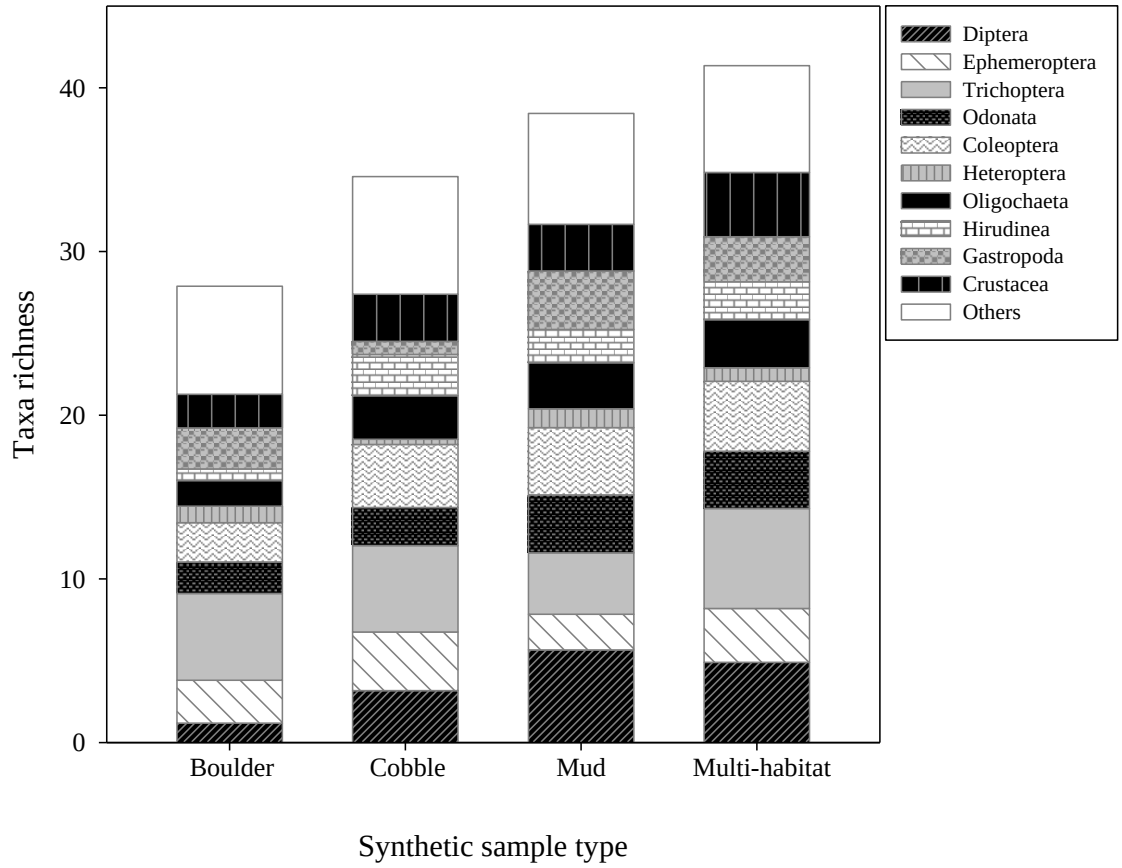


Figure 2.4 Taxa richness in 12 m² samples by habitat type. The richness of macroinvertebrate taxa were calculated using synthetic sample data created by combining data from individual plots using Microsoft Access. Data obtained from kick samples collected in Malloy Lake, MB. Taxa identified primarily to family.

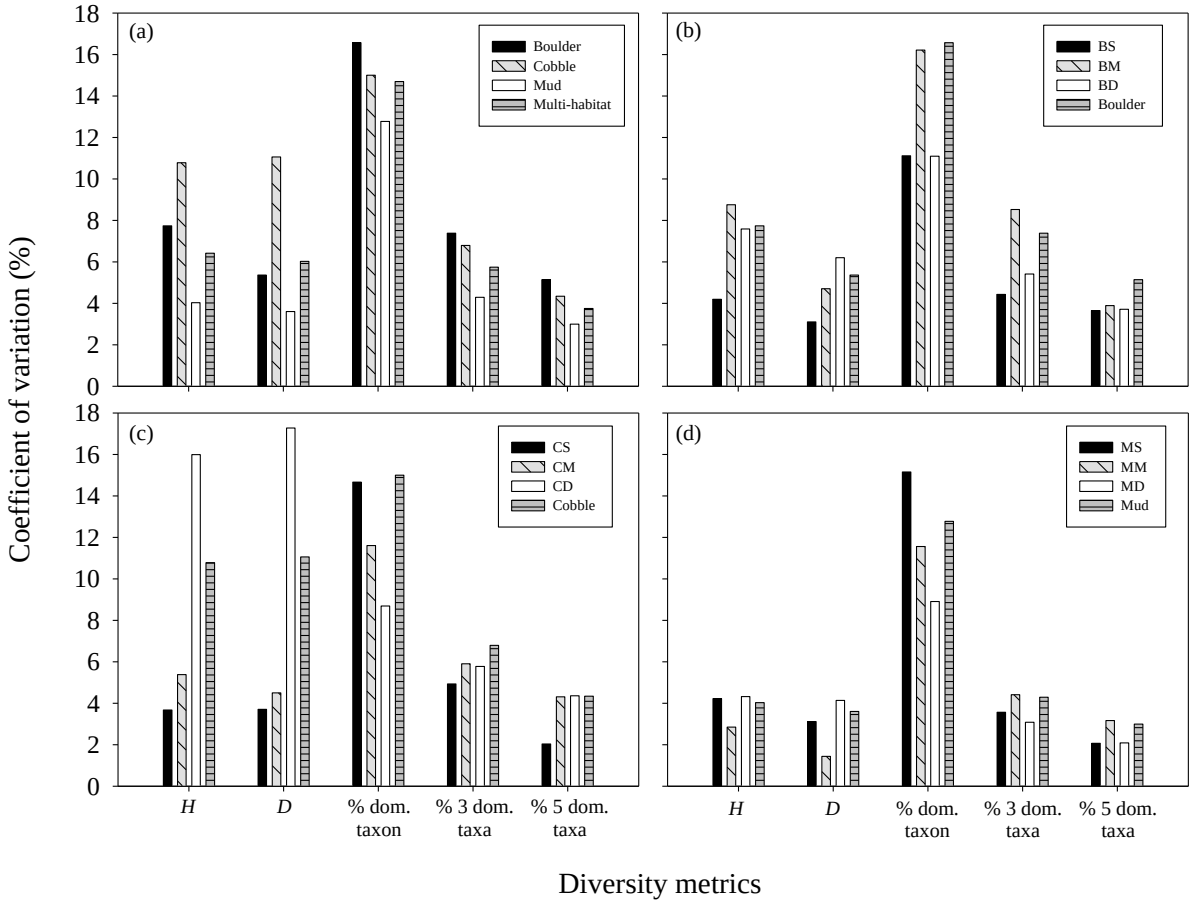


Figure 2.5 CV of diversity metrics using synthetic plot data by treatment (BS = boulder-shallow, BM = boulder-medium, BD = boulder-deep, CS = cobble-shallow, CM = cobble-medium, CD = cobble-deep, MS = mud-shallow, MM = mud-medium, MD = mud-deep), by habitat type spanning all depths ('Boulder', 'Cobble', 'Mud') and composite samples of cobble and mud ('Multi-habitat'). Data obtained from kick samples collected in Malloy Lake, MB. Metric abbreviations *H*, *D*, % dom. Taxon, % 3 dom. Taxa and % 5 dom. taxa represent the Shannon index, Simpson's index, % dominant taxon, % 3 dominant taxa and % 5 dominant taxa, respectively.

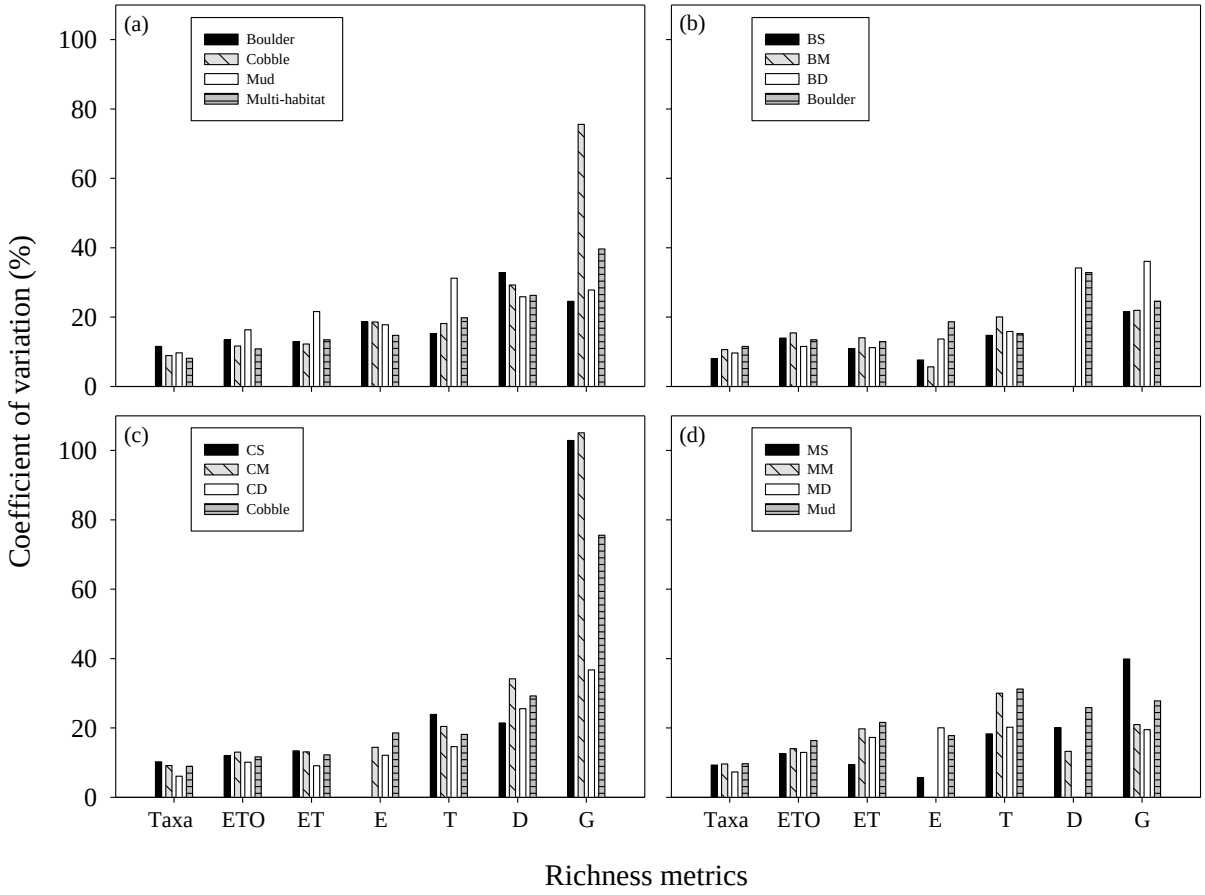


Figure 2.6 CV of richness metrics using synthetic plot data by treatment (BS = boulder-shallow, BM = boulder-medium, BD = boulder-deep, CS = cobble-shallow, CM = cobble-medium, CD = cobble-deep, MS = mud-shallow, MM = mud-medium, MD = mud-deep), by habitat type spanning all depths ('Boulder', 'Cobble', 'Mud') and composite samples of cobble and mud ('Multi-habitat'). Data obtained from kick samples collected in Malloy Lake, MB. Metric abbreviations ETO, ET, E, T, D and G represent the richness of Ephemeroptera, Trichoptera and Odonata; Ephemeroptera and Trichoptera; Ephemeroptera; Trichoptera; Diptera and Gastropoda, respectively.

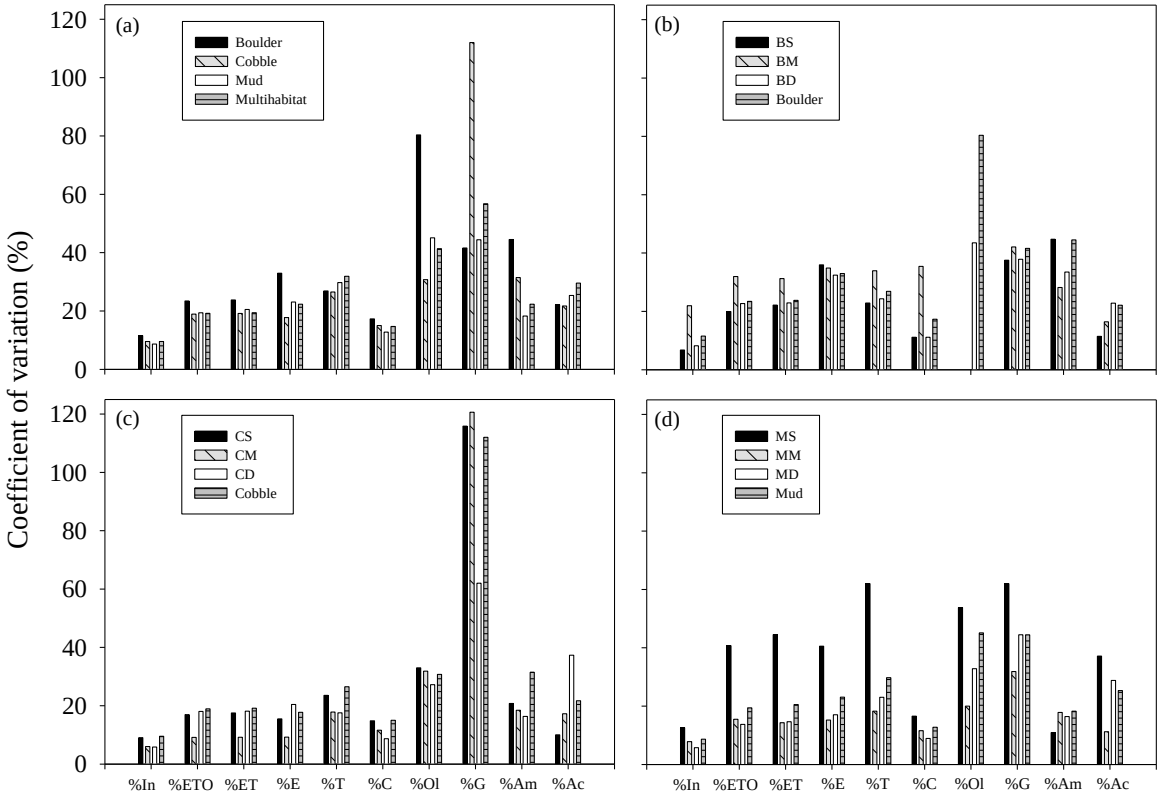


Figure 2.7 CV of relative proportion metrics using synthetic plot data by treatment (BS = boulder-shallow, BM = boulder-medium, BD = boulder-deep, CS = cobble-shallow, CM = cobble-medium, CD = cobble-deep, MS = mud-shallow, MM = mud-medium, MD = mud-deep), by habitat type spanning all depths ('Boulder', 'Cobble', 'Mud') and composite samples of cobble and mud ('Multi-habitat'). Data obtained from kick samples collected in Malloy Lake, MB. Metric abbreviations % In, %ETO, %ET, % E, % T, % C, % Ol, % G, % Am and % Ac represent % Insecta; % Ephemeroptera, Trichoptera and Odonata; % Ephemeroptera and Trichoptera; % Ephemeroptera; %Trichoptera; % Chironomidae; % Oligochaeta; % Gastropoda; % Amphipoda and % Acari, respectively.

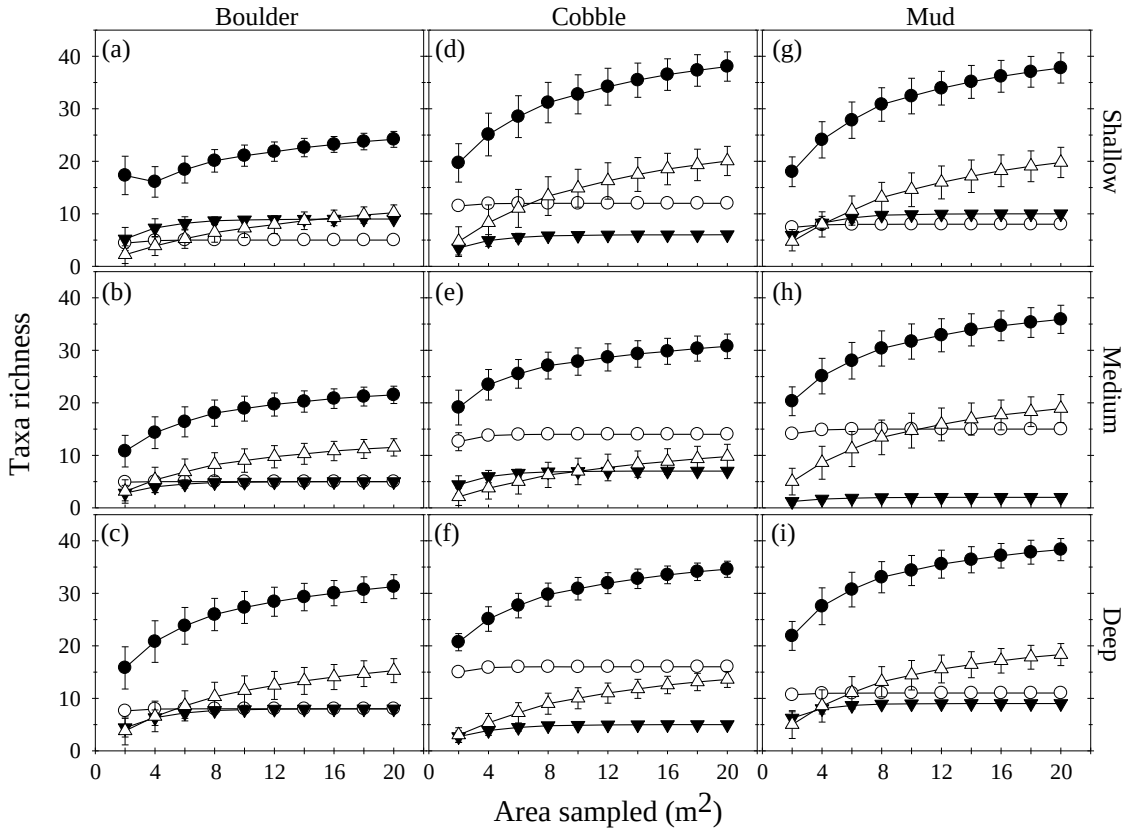


Figure 2.8 Taxa richness-area curves for all (●), commonly (○), intermediately (▼) and rarely (Δ) collected taxa in Malloy Lake, MB treatments. Curves were plot using data from synthetic plots created using Microsoft Access, with the exception of sample areas of 2 m² where values were calculated used non-resampled data. Taxa were considered commonly occurring if they were collected in > 80% of samples, intermediately occurring if they were collected in 50 – 80% of samples and rarely occurring if they were collected in < 50% of samples (e.g., Schreiber and Brauns 2010). Error bars represent standard deviation.

# CHAPTER 3. Testing rapid bioassessment protocols in lakes impacted by cottage development

## 3.1 Introduction

Progress in the field of rapid bioassessment (RBA) has led to a number of government agencies increasing the geographic scope of stream biomonitoring programs (e.g., Barbour *et al.* 1999; Turak *et al.* 2004; Wright 2000); however, there is a lack of protocol development for the RBA of lakes. Traditional lake bioassessment methods using benthic macroinvertebrate communities are time- and cost-intensive, limiting the number of lakes that can be monitored. If RBA methods could be used in lakes to assess impacts, biomonitoring programs could be expanded to include a number of lakes impacted by a variety of stressors. Because of the importance of freshwater resources, the development of RBA protocols for lakes could help ensure that biological changes are identified early before impacts become severe.

Previous work in Manitoba's Whiteshell Provincial Park (Chapter 2) was performed to aid in the design of RBA sampling protocols for boreal shield lakes. The particle size of sediments (e.g., Doeg *et al.* 1989; Minshall 1984; Williams and Mundie 1978) and water depth (e.g., James *et al.* 1998; Weatherhead and James 2001) influence the distribution of benthic macroinvertebrates. In Chapter 2, I examined how these factors affected benthic macroinvertebrate samples, and based on my results, recommended that sediments are sampled that allow the collection of diverse communities (e.g., cobble and mud), and that equal sampling effort be used at all depths between the shoreline and 1 m in water depth. Sample area was also investigated because it influences the proportion of taxa that are collected (Brinkhurst

1974; Vinson and Hawkins 1996); richness-area curves rise steeply until gradually levelling out at an area where all taxa present are collected (Colwell and Coddington 1995; Magurran 2004b). Based on the richness-area curves created using Malloy Lake data, I recommended that a kick-sample area of 10 $m^2$ be used for lake assessments because this area allowed for the majority of taxa present to be collected. However, the Chapter 2 analysis was performed on one lake and protocols required further testing in a group of lakes.

In this study, RBA protocols were tested in a group of boreal shield lakes exposed to a gradient of cottage development. RBA methods were used to sample, process and analyze data collected from 69 lakes that varied chemically and morphometrically. Protocols were tested to determine how well the resulting benthic data could be used to distinguish between lakes with and without cottages and whether or not sampling different habitat types or using different analyses could improve assessment accuracy or precision.

Cottages have long been considered a wilderness escape for humans; however, the increased demand for cottages in Canada's boreal shield has resulted in this once pristine environment being increasingly impacted by a number of different stressors (Urquizo *et al.* 2000). In Manitoba, the number of cottages has grown from approximately 3,815 in 1941 (Wolfe 1951) to 45,540 in 2009 (Statistics Canada 2010), with most of this development occurring on the boreal shield. Building cottages often requires clearing trees, building access roads and corridors for power lines into formerly remote areas (Clark *et al.* 1984), and managing the disposal of human sewage. Lakefront cottages are also associated with the removal of aquatic (Jennings *et al.*

2003; Urquizo *et al.* 2000; Werner *et al.* 2005) and riparian plants (Clark and Euler 1984; Racey and Euler 1983), adding sand or gravel to shorelines (Hicks and Frost 2011), building docks (Taillon and Fox 2004) and man-made erosion-control structures such as riprap and retaining walls, resulting in natural shorelines becoming heavily modified. Recreational use of lakes brings motorized watercraft that emit pollutants (Gabele and Pyle 2000), fish stocking (Lintermans 2004; St. Jacques *et al.* 2005), increased fishing (Kaufman *et al.* 2009; Salmi *et al.* 2006; Schindler *et al.* 2000) and the introduction of invasive species (Lintermans 2004; Schindler *et al.* 2000; Weisz and Yan 2010). Lakes with cottages are consequently affected by multiple stressors. The severity of these stressors is difficult to predict because the activities of cottagers (Henning and Remsburg 2009; Racey and Euler 1983) and the characteristics of lakes are variable, and cumulative impacts to lakes are poorly understood.

For lakes impacted by cottages, eutrophication is a major concern (Gibbs 1977; Gilliom and Patmont 1983; Robertson *et al.* 1998). In nutrient-poor lakes, such as those commonly found in the boreal shield, primary production is generally limited by phosphorus concentrations (Wetzel 2001b). When lakes receive an influx of a limiting nutrient, primary production increases, water clarity decreases and increased decomposition in the hypolimnion can lead to anoxic conditions (Carpenter *et al.* 1998; Dillon and Rigler 1974). In Quebec's boreal shield, residential development resulted in the nutrient enrichment of lakes and consequent increases in the biomass of phytoplankton (Lambert *et al.* 2008) and zooplankton (Gélinas and Pinel-Alloul 2008).

Human waste from remote, lakeside dwellings is commonly disposed in septic systems that treat sewage using a settling tank and a septic field (Brandes 1977;

Gilliom and Patmont 1983; Postma *et al.* 1992); however, these systems do not always prevent nutrients and bacteria from reaching lakes. When septic effluent enters lakes or streams, phosphorus is often absent, or present in concentrations below detection limits, because it is readily adsorbed by sediments (e.g., Jones and Lee 1979; Postma *et al.* 1992; Robertson *et al.* 1991); however, in areas with thin soils or in older septic fields that have become saturated with sewage, the levels of phosphate that sediment can adsorb may be exceeded (Gilliom and Patmont 1983; Jones and Lee 1979; Robertson *et al.* 1998). When this occurs, phosphorus can enter lakes via groundwater or runoff (e.g., Gilliom and Patmont 1983; Moore *et al.* 2003). Phosphorus can also enter lakes via groundwater or runoff in regions where the water table is near the sediment surface; periods of heavy rain can cause effluent to overflow from septic fields under these conditions (Arnade 1999). Septic systems only used in summer may also be more likely to contaminate lakes; Postma *et al.* (1992) reported that nitrate and bacteria (faecal coliform and *Clostridium perfringens*) were not retained by seasonally-used septic systems and suggested that 8 to 15 months of regular septic system use is required to develop a clogging mat that increases the removal of pollutants and bacteria.

The prevalence of thin soils in Canada's boreal shield could cause septic systems to be less effective. On Ontario's boreal shield, phosphorus concentrations were generally higher in lakes with shoreline development and septic systems than in those without (Dillon *et al.* 1994). The only exception to this was observed in a lake with thicker soils that most likely allowed greater adsorption of phosphorus from sewage effluent (Dillon *et al.* 1994).

Other impacts to the littoral zone associated with cottage development reduce the diversity of habitats available to biological communities. Clearing trees, shrubs, vegetation and felled logs from the riparian zone increases watershed erosion and results in the loss of a natural buffer that would have reduced the amount of sediment entering the lake (Bannister 1979; Lowrance *et al.* 1985). This can lead to increased sedimentation rates (e.g., Bookman *et al.* 2010; Jennings *et al.* 2003), reductions in the amount of coarse woody debris (e.g., Christensen *et al.* 1996; Francis and Schindler 2006; Jennings *et al.* 2003; Marburg *et al.* 2006), and the loss of sediment organic matter that is no longer retained by coarse wood in the littoral zone (e.g., Francis *et al.* 2007). Fewer emergent and floating-leafed plants have also been observed in lakes with cottages (e.g., Hicks and Frost 2011; Jennings *et al.* 2003; Radomski and Goeman 2001). Aquatic plants are often removed by cottagers to enhance swimming and boating conditions (Hicks and Frost 2011) or are damaged by motorized boats (Asplund 2000; Asplund and Cook 1997). Reduced complexity of habitats from shoreline development has resulted in shifts in the densities of macroinvertebrates (e.g., Francis *et al.* 2007), and reduced aggregation (e.g., Scheuerell and Schindler 2004) and growth rates of fish (e.g., Schindler *et al.* 2000).

Based on the number of changes in lakes with cottages, it was expected that littoral zone communities such as benthic macroinvertebrates could be used to assess impacts in boreal shield lakes. Benthic macroinvertebrates respond to nutrient enrichment (e.g., Donohue *et al.* 2009; Tolonen and Hämäläinen 2010; Tolonen *et al.* 2001) and the complexity of habitat in the littoral zone of lakes (e.g., Brauns *et al.* 2007b; Rennie and Jackson 2005; Sloey *et al.* 1997), and are widely used in the

assessment of freshwater impacts (Abel 1989; Rosenberg and Resh 1993). It was expected that benthic macroinvertebrate community composition would differ between lakes with and without cottages; however, it was unclear how benthos would respond to multiple stressors (i.e., the amounts and types of macroinvertebrates present could be influenced positively by nutrient enrichment or negatively by habitat simplification).

My main goal was to test newly designed RBA protocols using cottage development as a test impact. This was done by examining whether or not the composition of benthic macroinvertebrate communities collected from lakes with cottages differed from those collected from unimpacted lakes. Cottage development was simply the impact used to test protocols; however, the results of this study were also used to discuss how shoreline development affects littoral zone benthos.

The secondary goal of refining rapid methods was accomplished by evaluating assessment accuracy using samples collected from different habitats and using different analysis methods. Collecting benthic samples from different habitat types can influence impact detection (e.g., Donohue *et al.* 2009; Tolonen and Hämäläinen 2010; Tolonen *et al.* 2001) as well as processing time, and is thus an important consideration in the design of bioassessment protocols. Samples were collected from two sediment types to determine if one habitat was more cost-efficient and accurate than the other for the detection of impacts. Similarly, the choice of statistical method can also influence impact detection (e.g., Hawkins *et al.* 2010; Lydy *et al.* 2000; Reynoldson *et al.* 1997). Metrics, regression-based metrics and multimetric indices were compared to determine which were the most sensitive for detecting impacts to lakes.

Lastly, I investigated the influence of temporal and spatial variability on the samples collected. The temporal and spatial variability of benthic macroinvertebrate communities can make the detection of impacts more difficult (Johnson 1998). To investigate the influence of temporal and spatial variability on the benthic macroinvertebrate samples collected, some lakes were sampled in 2007 and 2008 and others were sampled more extensively in 2007.

In summary, the objectives of this study were:

1. to test a new rapid bioassessment sampling protocol for boreal littoral zones using cottage development as a test impact;
2. to test how the collection of kick-samples from different habitat types (cobble and mud sediments) affects impact assessment;
3. to compare the use of community metrics, regression-based metrics (i.e., community metrics that incorporate environmental data) and multimetric indices for analysis of data collected using the new RBA protocol; and,
4. to investigate the influence of temporal and spatial variability on the benthic macroinvertebrate samples collected.

## 3.2 Methods

### *3.2.1 Sampling sites*

During the fall (early September to early October) of 2007 and 2008, 40 lakes were sampled in Whiteshell and Nopiming Provincial Parks, Manitoba. Thirty of the lakes were unimpacted by cottage development (i.e., zero or one cottage along their

lakeshores; Table 3.1) and 10 were chosen to represent a gradient of cottage development (Table 3.2). Lakes known to be impacted additionally by other human activities (e.g., mining, wild rice harvest, forestry) were avoided for this study. Numerous reference lakes were sampled so the natural range of community variability could be assessed; 30 lakes was the greatest number of lakes that could be sampled because of time constraints. There was some overlap in the set of lakes sampled each year to allow temporal variation to be examined. For lakes with cottages along their shorelines, development intensity ranged from 8 to 71% shoreline alteration by cottages, or 0.8 to 18 cottages per km of shoreline. The percentage of developed shoreline was estimated from park subdivision maps of cottage lots when available, aerial photos, or by using a typical lot size of 30 × 46 m and the number of cottages.

In each lake, two littoral habitats were sampled; one sample was collected from a cobble site and the other from a mud site. Sites were characterized by the dominant grain size observed between the land-water interface to a water depth of approximately 50 cm. Beyond this depth, grain size at all shorelines decreased to sand or silt. Cobble sites had a dominant grain size of 20-256 mm (includes larger gravel) and mud sites were dominated by fine grained sediments such as clay, mud or silt. These sediment types were chosen because they are common within area lakes and they allow the collection of diverse assemblages using kick sampling methods.

After arriving at a lake, the shoreline was circled in either direction until a stretch of shore of the appropriate substrate type was encountered. Sites were sampled in this way because surveying each lakeshore ahead of time and randomly selecting sites from those available would have taken too much time (i.e., poor access into most

of the lakes generally required the use of canoes for sampling) and it was determined that the resolution of available air photos did not allow littoral habitat to be determined accurately prior to entering the field. At lakes with cottages along their shorelines, the shoreline areas adjacent to cottages were neither avoided nor preferentially sampled; sites were sampled as they were found, as they were for undeveloped lakes. However, it is likely that cottages were closer to access points than would have been expected by random.

The surface area sampled was standardized to 10 $m^2$ in both cobble and mud sites. Sample plots were defined by measuring the perpendicular distance, or length of the plot ($l$), from the shoreline to a depth of 1 m using a tape measure held along the lake bottom. This distance was used to determine the plot's width ($w$): $w = 10\ m^2 / l$. Plot edges were marked using flags and the entire 10 $m^2$ area was sampled.

During 2007, an additional three cobble and three mud plots were sampled in eight lakes to allow within-lake variability of samples to be assessed. Of these eight lakes, four had cottages (Barren, Caddy, Red Rock, Star) and four had none (Bedford, Cabin, Euclid, Ritchey). These lakes were selected based on relative surface area of lakes (i.e., larger lakes were avoided) and logistical considerations (i.e., additional sampling was avoided in lakes that were more difficult to access).

### *3.2.2 Sampling methods*

Water samples were collected from the epilimnion of each lake to assess the concentrations of chlorophyll *a*, nutrients and major ions. After rinsing sample bottles twice in lake water, water was collected in high-density polyethylene bottles held

approximately 30 cm below the lake surface above the deepest area of the lake (when bathymetric maps were available) or near the center of the lake. Sample bottles were kept cool and brought back to the lab as soon as possible for analysis. Water chemistry was analyzed at the Freshwater Institute, Winnipeg, MB using the methods described by Stainton *et al.* (1977).

The percentages of bedrock, boulder, cobble, pebble, sand and silt were recorded at each cobble site before it was disturbed by sampling. The size distribution of rocks was estimated by eye and recorded along with any other notable site characteristics. Benthic macroinvertebrates were then collected using traveling kick-sampling methods until all areas of the plot were thoroughly covered (David *et al.* 1998; Jones *et al.* 2004).

At mud sites, the net was tapped along the substrate instead of disturbing the substrate by kicking. Because most infauna are found in the first few centimetres of sediment (Beckett *et al.* 1992; Kajak and Dusoge 1971), sampling only the surface layer reduces the ratio of mud to invertebrates. This can reduce the time needed to sort invertebrates from sediment.

At both cobble and mud sites, precautions were taken to ensure unbiased collection and to allow collection of a greater diversity of benthic invertebrates. Care was taken to expend an equal amount of effort in the shallow, middle and deep regions of each plot. This was important to avoid a sampling bias in certain invertebrate taxa, distribution and density of which vary by depth (Chapter 2). Care was also taken to ensure microhabitats such as crevices, reeds, logs, etc. were sampled thoroughly in spite of their added difficulty. Constant movement is required for kick sampling lentic

habitats to ensure that the invertebrates collected in the net could not swim out. When macrophyte stands were present in a plot, the D-net was swept through them before walking over them, to maximize the collection of fast swimming invertebrates.

Samples were washed and preserved using the same methods used in the survey of Malloy Lake (Chapter 2).

### *3.2.3 Sample processing*

All samples were brought back to the lab for processing. This process began with transferring samples to 95% ethanol and then waiting a week before transferring them to 70% ethanol. The samples were transferred to ethanol because formalin is an excellent fixative, but damages specimens during prolonged storage (i.e., makes them brittle). Samples are transferred to the second concentration (70%) of ethanol because the high water content of organic sample material dilutes the first addition. Samples were subsampled using a fixed count of 300 benthic invertebrates per sample, recommended by the USGS (Moulton *et al.* 2000).

Samples were elutriated to reduce processing time when a large amount of inorganic material (e.g., sand, gravel) was present in samples. Sample material was placed in a white pan with water, and the contents were gently circulated until a vortex was created. This allowed the lighter organic material to be raised into the water column, whereas the heavier inorganic material remained on the bottom of the pan. Organic material was then poured into a 500 µm sieve. This process was repeated until only the heavier-inorganic material remained in the pan. Approximately 20% of the inorganic material was then randomly selected and sorted within gridded sorting trays

using a stereomicroscope. If any organisms were found, with the exception of molluscs and encased caddisfly larvae, the sample was re-elutriated. If no organisms were found, a quick scan of the remaining inorganic material was performed without magnification for any remaining molluscs or caddisflies in the sediment. All of the invertebrates collected during this process were placed into the organic fraction of the sample prior to subsampling.

To determine the proportion of sample to be sorted, an estimate of invertebrate density was made based on a small proportion of the sample (Moulton *et al.* 2000). Sample material was stirred and evenly distributed in a gridded tray before randomly selecting portions to estimate the density of macroinvertebrates within each sample. This estimate was used to determine how much of the sample should be processed to collect a minimum of 300 organisms. To ensure that estimates of density could be made for the entire sample, the proportion of sample processed was always recorded.

After subsamples were sorted, a search for large-rare specimens was performed on the unprocessed portion of the sample by scanning the remaining sample by eye (Moulton *et al.* 2000). Any large macroinvertebrates that were uncommon or absent in the sorted subsample were collected. This search provided a more accurate measure of richness, as some taxa will be missed when only sorting a portion of a sample. Because this search was performed by eye, rare taxa that were small were likely missed during sorting.

Processing time was recorded for all of the macroinvertebrate samples collected in 2008. The time needed to process a sample after it had been transferred into 70%

ethanol and until the large-rare search was completed was divided into components to determine which parts of subsampling took the most time to complete.

A total of 94 taxa was found in the lake samples, and these were identified to phylum, class, order, family, subfamily or genus (see Table 3.3 for a list of taxa present and their corresponding identification level). The majority of invertebrates were identified to family level (e.g., annelids, insects and molluscs). When identification was more difficult and time consuming for a particular group, taxa were identified at a lower resolution. Family was considered an adequate identification level for this rapid protocol because it is sufficient for the rapid assessment of streams (e.g., Chessman *et al.* 2007; Hilsenhoff 1988; Metzeling *et al.* 2006) and it can greatly reduce the time and costs associated with macroinvertebrate assessments (Jones 2008). Taxonomic keys used to identify macroinvertebrates were Merritt and Cummins (1996) for insects in general, Edmunds *et al*. (1976) for Ephemeroptera, Wiggins (1996a) for Trichoptera, Kathman and Brinkhurst (1998) for Oligochaeta, Clarke (1981) for Mollusca, and Thorp and Covich (2001) for other non-insect invertebrates. Voucher specimens are stored at the Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, MB.

To ensure samples were sorted and identified properly, processed portions of samples were reprocessed and identifications were verified by someone other than the original sorter. The first ten samples processed by new sorters were verified, followed by a randomly-chosen 10% of subsequent samples. This was done by recording each sample that was processed by an individual sorter in the order that they were processed and then dividing these into blocks of ten. One sample was randomly selected from each block of ten and was then resorted to see how many organisms had been missed.

Sorting efficiencies that fell beneath 95% were considered a fail, and required the original sorter to reprocess the remaining nine samples within that block of ten. The identification of representatives of each taxon was verified by an expert.

In all cases where only a portion of the sample was processed, an estimate was made of the abundance of invertebrates that would have been collected if the entire sample was sorted. The number of invertebrates collected during subsampling was divided by the proportion of sample that was processed for all taxa prior to data analysis.

### *3.2.4 Data analysis*

A few lakes and samples within lakes were removed from the dataset prior to analysis because they did not meet criteria desired for this investigation. Lakes were removed if impacted by wild rice harvest (Euclid, Stormy, Manigotagan) and forestry (Meditation). Specific samples were omitted from the data set after an assessment of the substrate particle size data if they did not meet the defined habitat type (i.e., dominated by either cobble or mud sediments). Five samples from cobble sites and one sample from mud sites were omitted because they were collected from the wrong type of habitat.

#### *Benthic communities at unimpacted sites*

Estimates of community composition were qualitatively assessed to investigate the benthic communities typical of undeveloped boreal shield lakes. Mean density, relative proportion and richness of macroinvertebrate taxa were calculated for reference

lake data, separated by sampling year and habitat type. Mean values were plotted to allow a qualitative assessment of any compositional differences among habitat types and sampling years. The number of taxa across different proportions of reference lakes was graphed as a histogram to determine how many rare and common taxa were present.

*Sample processing time by habitat type*

The processing times recorded for all benthic samples collected in 2008 were compared by habitat type to see if assessment time could be reduced by restricting sampling to either cobble or mud shorelines. A one-way ANOVA was performed using habitat type as the fixed factor of interest and processing time as the response variable.

*Effects of cottage development on pelagic water chemistry*

To help identify the most prominent stressors among lakes with cottages, Spearman's correlation coefficients were calculated between water chemistry and development intensity measured as per cent developed shoreline, cottages per km of shoreline and number of cottages.

*Selection of benthic macroinvertebrate metrics for use in RBA protocol*

To determine which metrics are most effective for the assessment of cottage impacts, 42 metrics (Table 3.4) were evaluated. The choice of metrics was based on dominant taxa, anticipated response to shoreline development (e.g., scraper metrics, % Oligochaeta), or their recommendation in published literature. Scrapers were

distinguished from other taxa based on the feeding groups described by Merritt and Cummins (1996). Metrics were divided into four categories: abundance, relative proportions of taxa, richness and diversity indices. The effectiveness of metrics for the assessment of cottage impacts was determined based on their correlation with measures of shoreline development, their variability, and how accurately they could be used to distinguish between lakes with and without cottages.

Metrics were first evaluated based on their relationship with measures of shoreline development and open-water chemistry. Spearman's correlation coefficients were calculated between each metric and the per cent developed shoreline, number of cottages per km of shoreline and the number of cottages per lake. Three measures of shoreline development were evaluated because it was unclear from existing literature which would be the most accurate for the quantification of cottage impacts. Spearman's correlation coefficients were also calculated between open-water total phosphorus (TP), total nitrogen (TN), chlorophyll *a*, Secchi depth levels and macroinvertebrate metrics. This was to determine if metrics also respond to variables that are used to quantify trophic status and eutrophication. Because eutrophication is a major concern for lakes impacted by cottages, determining if macroinvertebrate metrics were similarly correlated with measures of shoreline development and trophic status was of interest.

Spatial and temporal variability of metrics among reference lakes were assessed using the coefficient of variation (CV). Spatial variability was calculated for two components: among-lake variability and within-lake variability. Among-lake CV was calculated using metric scores from all reference lakes. Within-lake CV was calculated using metric scores from the four benthic samples collected in Bedford, Cabin and

Ritchey (i.e., within-lake CV was the mean metric CV from these three lakes). Temporal variability was calculated using sample data from reference lakes that were sampled in both 2007 and 2008 (e.g., Elbow, McGregor and Tulabi). Because natural variability can make community changes caused by impacts difficult to detect, choosing metrics less influenced by spatial and temporal variability is an important consideration (Johnson 1998).

Lastly, metrics were evaluated based on how well they could be used to distinguish between lakes with and without cottages. This was accomplished by first bootstrapping the metric scores from reference sites 10,000X (i.e., sampled with replacement 10,000X) using StatTools 5.5 (Palisade Corporation), and then calculating the $5^{th}$ and $95^{th}$ percentiles of the bootstrapped distribution using @RISK 5.5 (Palisade Corporation). Lower ($5^{th}$ percentile) and upper ($95^{th}$ percentile) confidence limits were calculated for each metric to define the 'normal' range of benthic macroinvertebrate communities observed in unimpacted lakes. A lower or upper boundary was selected for each metric depending on whether metric scores were negatively or positively correlated with per cent developed shoreline (i.e., lower confidence boundaries were selected for metrics negatively correlated with shoreline development and upper confidence boundaries were selected for metrics positively correlated with shoreline development). Sites were assessed as impacted if their metric scores fell outside of the designated confidence boundary, whereas scores falling within boundaries were considered unimpacted (i.e., 95% of the observations from reference sites define the normal or unimpacted range) (e.g., David *et al.* 1998; Kilgour *et al.* 1998). This method was chosen because it limits the number of unimpacted sites that are misclassified by

allowing a wide range of community estimates to be considered normal (i.e., small community differences are unlikely to be assessed as impacted and there is more confidence that sites have been accurately assessed as impacted). The percentage of lakes with cottages that fell outside of each metric's reference boundary was calculated to allow for comparison.

From the 42 metrics evaluated, 10 metrics were selected for further investigation for both cobble and mud habitat types. Metrics were chosen based on strength of correlation with development, low variability or preferentially, their ability to be used to distinguish between lakes with and without cottages. Metric selection was also based on contribution of unique community information (i.e., both the abundance and % Ephemeroptera would not have been chosen for one habitat type and the selection of both ETO and Ephemeroptera, Trichoptera or Odonata metrics was avoided).

*Comparison of metrics, regression-based metrics and multimetric indices*

The three bioassessment methods examined here used metric data in different ways for the assessment of lake sites. The 10 metrics were first evaluated individually based on their correlation with shoreline development and accuracy. They were then converted into regression-based metrics using regression equations that accounted for sources of variability unrelated to shoreline development. Lastly, the selected metrics were combined into a single measure as a multimetric index.

The three bioassessment methods were compared by their accuracy (i.e., ability to identify lakes with cottages correctly as impacted and lakes without cottages as in

reference condition) and their precision (i.e., the consistency of assessments between sites sampled within the same lake). Because lakes with low to moderate cottage development may not be adversely affected, the accuracy of assessment was also investigated for lakes with over 25% developed shoreline. Precision was quantified as the per cent agreement between each possible pair of samples, from a single habitat type, that was collected per lake (e.g., Stribling *et al.* 2008). The analysis of precision was thus confined to Barren, Bedford, Cabin, Caddy, Red Rock, Ritchey and Star lakes where additional samples were collected in 2007.

The scores from the most effective metric, regression-based metric and multimetric index for each habitat type were also plotted *versus* per cent developed shoreline to investigate if certain measures were capable of detecting cottage development along a gradient.

*Creating regression-based metrics*

Regression-based metrics were investigated to determine if accounting for some of the variability in metrics associated with physicochemical characteristics could lead to greater accuracy and precision of assessments. Using biological and physicochemical data from the reference set of lakes, regression analysis was used to create a model to predict unimpacted metric scores from environmental characteristics (e.g., Bailey *et al.* 1998). Using environmental characteristics to refine predictions of community composition should increase the effectiveness of metrics in distinguishing impacted from unimpacted sites (Bailey *et al.* 1998).

Regression-based metrics were created using metric scores and environmental data in multiple regression analyses (e.g., Bailey *et al.* 1998). Using only reference lake scores for a given metric (dependent-response variable), environmental characteristics (independent-predictor variables) and all of their two-way interactions were entered into a regression analysis, and sequentially removed in order of descending P values until only significant predictor variables remained (i.e., backward elimination). Predictor variables were evaluated if they were correlated with the metric of interest but not with cottage development and showed minimal collinearity. Various combinations of significant predictor variables were examined after backward elimination was complete to ensure that the best regression equation (i.e., that retained significance for all included predictor variables and maximized $r^2$) had been selected for each metric. The resulting regression equation calculated for each metric defined the parameters of each regression-based metric.

Regression-based metrics (i.e., regression equations) were then used to calculate residual values for all sites. Residuals are the difference between the predicted metric score based on environmental data and the observed metric score. Reference lake residuals were then bootstrapped with replacement 10,000X, before calculating the $5^{th}$ and $95^{th}$ percentile values to be used as lower and upper boundaries for bioassessment, respectively (as with metric scores). Falling outside of the lower or upper reference boundary was used as an indicator of impact, as was done for metrics (i.e., lower confidence boundaries were used for regression-based metrics negatively correlated with per cent developed shoreline and upper confidence boundaries were used for regression-based metrics positively correlated with development).

Regression-based metrics were evaluated by determining how well they distinguished lakes with cottages from the distribution of reference lakes and the strength of their correlation with per cent developed shoreline.

*Creating multimetric indices*

The ten metrics selected for further investigation were used to design a multimetric index for each habitat type. Metrics were selected for inclusion in the index if they were individually effective at distinguishing between lakes with and without cottages (i.e., multiple test lake scores fell outside of the $5^{th}$ or $95^{th}$ percentile scores from reference sites). Various combinations of the ten metrics were investigated to maximize the differences between lakes with and without cottages.

A simple scoring method was used to combine the values from metrics into a unitless score. First, a 1 was assigned to each metric score per site that fell outside of the set reference lake boundary. When a metric score was within the reference boundary, a score of 0 was assigned. For each site, the scores (of 1 or 0) from each metric were summed and then divided by the total number of metrics that were retained for the multimetric index (e.g., Hering *et al.* 2006).

This value was used as the multimetric index score, and was calculated for all reference lakes to determine the $95^{th}$ percentile value to be used as a bioassessment boundary; multimetric index scores above the $95^{th}$ percentile were considered an indicator that a site was impacted. Higher index scores were considered an indication of greater impact from cottage development.

*Influence of habitat type on the detection of impacts*

The influence of sediment type on the detection of cottage impacts was investigated by comparing the accuracy and precision of the most effective bioassessment methods observed using cobble and mud data. The percentage of test lakes assessed as impacted (accuracy) was the primary consideration for the comparison of habitat types; however, the ranking of test lakes based on divergence from reference lake scores was also examined. The precision of bioassessment in each habitat was then compared by examining the consistency of assessment results (impacted *versus* unimpacted) across the multiple sites that were sampled in Barren, Bedford, Cabin, Caddy, Red Rock, Ritchey and Star lakes.

## 3.3 Results

### *3.3.1 Benthic communities at unimpacted sites*

The composition of benthic macroinvertebrate samples collected from reference lakes was variable by habitat type and year. The largest community differences were visually observed in the densities of a few macroinvertebrate taxa (Figure 3.1a), followed by the relative proportions of different taxa (Figure 3.1b), with only minor differences observed between the richness of different taxa by habitat type (Figure 3.1c). In general, mud samples had higher densities of chironomids, amphipods, nematodes and gastropods than samples collected from cobble habitat. The relative proportion of mayflies was higher in samples collected in cobble habitat than samples collected in mud. The number of mayfly and caddisfly families made up a large proportion of total richness in both habitat types.

Many taxa were rare in reference lakes (i.e., present in less than 10% of reference lakes) in the samples collected from both cobble and mud sites (Figure 3.2). Most taxa were collected from fewer than half of the sites sampled in reference lakes, regardless of habitat type. At least 10 taxa were present in fewer than 5% of reference lakes in cobble and mud habitats. The only taxa found exclusively in cobble or mud samples were rare; nine taxa were exclusive to cobble (Branchiobdellidae, Unionidae, Pontoporeiidae, Hypogastruridae, Hydropsychidae, Psychomyiidae, Hydraenidae, Stratiomyidae and Porifera) and eleven taxa were exclusive to mud (Sminthuridae, Siphloneuridae, Dipseudopsidae, Lestidae, Georyssidae, Veliidae, Tipulidae, Dolichopodidae, Sciomyzidae, Muscidae and Pisauridae). Less than 8% of taxa were present in 90% or more of the reference lakes sampled for both cobble and mud sites; taxa collected from 90% or more of reference lakes, regardless of habitat type, were Naididae, Sphaeriidae, Acari, Hyalellidae and Chironomidae.

### *3.3.2 Sample processing time by habitat type*

The processing of kick-samples from cobble shorelines took significantly ($P \leq 0.05$) less time to process than samples from mud shorelines (Table 3.5). The mean times required to process samples collected from cobble and mud habitats were 539 minutes (approximately 9 hours) and 711 minutes (approximately 12 hours), respectively. Regardless of habitat type, sorting macroinvertebrates from fine substrate material was the most time-consuming component of sample processing (Figure 3.3).

### *3.3.3 Effects of cottage development on pelagic water chemistry*

Correlation of development intensity and open-water chemistry was significant for 11 variables (Table 3.6). The strength of correlation with per cent developed shoreline was highest for chloride, followed by sulfate, sodium, conductivity, potassium, calcium, soluble reactive silica, pH, magnesium, nitrate and alkalinity. With the exception of nitrate, all correlations were positive. The highest values of calcium, chloride, conductivity, potassium and sodium were observed in Hunt Lake. A lake without any cottages, Camp Lake, also had higher levels of calcium and sulfate in comparison with other lakes. The degree of correlation between these chemical variables and the three measures of development (i.e., per cent developed shoreline, cottages per km of shoreline, and cottages per lake) was fairly consistent; however, higher correlations were more often present when quantifying development as the number of cottages per km of shoreline.

### *3.3.4 Selection of benthic macroinvertebrate metrics for use in RBA protocol*

The correlation of invertebrate community metrics with shoreline development and open-water chemistry varied among habitat types (Table 3.7). The number and strength of metric correlations were higher in mud habitat than in cobble. In cobble habitat, there was a set of metrics (primarily relative proportion metrics) significantly correlated with measures of shoreline development, and another set of metrics (primarily abundance metrics) correlated with open-water variables. In mud, nearly all significant correlations were between metrics and measures of shoreline development, not open-water variables. Nearly all correlations observed in cobble habitat were for

abundance or relative proportion metrics, while in mud habitat, significant correlations were present for all metric types.

Similar responses were observed among both habitats for a few of the metrics. Significant positive correlations between shoreline development and % Gastropoda, Gastropoda abundance and scraper abundance, and significant negative correlations between shoreline development and % Insecta, % Diptera and % Chironomidae were observed among cobble and mud sites. Chironomids made up a large proportion of dipterans and insects.

The strength of metric correlations with the different measures of shoreline development was also variable by habitat type. In cobble habitat, the strength of metric correlations was more often higher using per cent developed shoreline in comparison with the number of cottages per lake and the number of cottages per kilometre of shoreline; however, this difference was relatively small. In mud habitat, the strength of metric correlations with shoreline development was usually strongest when the number of cottages per lake was used to quantify shoreline development.

Metric variability was dependent on habitat type; however, certain trends were consistent within cobble and mud samples (Table 3.8). In both habitats, the least variable metrics were those based on diversity. These were followed sequentially by richness, relative proportion and abundance metrics. Among-lake CV was often higher than within-lake or temporal variability for a given metric. Within mud habitat, temporal CV was often lower than among- and within-lake variability.

The accuracy of metrics for the assessment of cottage impacts was also variable by habitat type (Table 3.9). The most accurate metrics were calculated using cobble

data. The highest accuracy overall was observed using % Chironomidae, % Diptera, % Insecta and % Amphipoda metrics calculated with cobble data. The most accurate mud metrics were % 5 dominant taxa and abundance metrics for ETO, ET, Coleoptera, Hirudinea and Ephemeroptera.

The metrics selected for further investigation for both habitats are presented in Table 3.10 along with their directional response to increased shoreline development. Hurlbert's PIE, % 5 dominant taxa, % scrapers and % Insecta were chosen for both cobble and mud habitat types. With the exception of % 5 dominant taxa, these metrics displayed the same direction of response to increased shoreline development. Gastropod and amphipod metrics were also selected for each habitat type; however, these differed by metric type (e.g., abundance *versus* relative proportion). For cobble habitat, most metrics were chosen based on the relative proportions of different taxa, and in mud habitat an equal number of abundance and relative proportion metrics were chosen.

The majority of metrics were selected for their accuracy, followed by relative CV or contribution of unique information. For cobble habitat, % Chironomidae, % Insecta, % Amphipoda, Gastropoda abundance, % 5 dominant taxa and % scrapers were chosen for their accuracy. Using cobble habitat data, % Trichoptera and Odonata abundance metrics were selected instead of the more accurate % ETO metric because these metrics contain redundant community information and % Trichoptera and Odonata abundance are more accurate than % ETO when they are combined (i.e., as would be done for a multimetric index). Hurlbert's PIE and Odonata richness were selected for cobble habitat for their relatively good accuracy, their contribution of

unique community information and in the case of Hurlbert's PIE, its lower variability in comparison with the Shannon index and Simpson's D index. For mud habitat, metrics with the highest accuracy were favoured; however, Amphipoda abundance was chosen instead of Trichoptera abundance, Odonata abundance and % Trichoptera because it was not redundant with ETO abundance. ETO abundance was equally as accurate for the detection of impacts in lakes with over 25 % developed shoreline as Trichoptera abundance, Odonata abundance and % Trichoptera combined.

For mud habitat, nine of the ten metrics selected were significantly correlated with shoreline development (all except % Acari). Four of the metrics selected for cobble habitat were significantly correlated with shoreline development (% Insecta, % Trichoptera, % Chironomidae and Gastropoda abundance).

### *3.3.5 Regression-based metrics*

Regression-based metrics explained 7.9 – 29.2% and 12.6 – 46.2% of the variability associated cobble and mud metrics, respectively (Tables 3.11 and 3.12). Predictor variables that were most often used in regression-based metrics were nitrite ($NO_2$) and year for cobble, and year and ammonia ($NH_4$) for mud.

The accuracy of detecting impacts in lakes with over 25% developed shoreline was highest using the % Insecta and % Chironomidae regression-based metrics in cobble habitat (30% of lakes assessed as impacted), and using the ETO abundance regression-based metric in mud habitat (36% of lakes assessed as impacted).

Significant correlations between regression-based metrics and per cent developed shoreline were not observed using cobble data; however, using mud data,

four of the seven regression-based metrics (Hurlbert's PIE, % Gastropoda, % Acari, ETO abundance) were significantly correlated with per cent developed shoreline (Tables 3.11 and 3.12).

### *3.3.6 Multimetric indices*

Multimetric indices were more effective at distinguishing between lakes with and without cottages using cobble *versus* mud data (Table 3.13). The cobble index combined data from all ten of the metrics that were examined, while the mud index combined six metrics (Table 3.13). Seventy and 64% of lakes with over 25% of their shorelines occupied by cottages, and 57 and 41% of all lakes with cottages were identified as impacted using the cobble and mud indices, respectively. Scores from both indices were significantly correlated with the three measures of shoreline development that were examined (% developed shoreline, cottages per km of shoreline, number of cottages).

The majority of lakes identified as impacted were consistent between the two habitat types (Table 3.14). The lake with the highest percentage of developed shoreline (Brereton) and the lake with the largest number of cottages (Falcon) were both assessed as impacted using cobble and mud indices. The remainder of lakes assessed as impacted using both indices displayed high (Betula, Caddy (2008), Star) and moderate (Barren, Jessica) levels of cottage development.

Differences were observed in the ranking of impacted lakes based on index scores and whether or not a lake was assessed as impacted using cobble and mud multimetric indices. Using the cobble index, the most impacted lake was Hunt Lake.

Using the mud index, Caddy Lake (2008) was assessed as the most impacted, and certain lakes were notably not considered impacted. One of the most heavily developed lakes (West Hawk) and the lake that appeared most affected by development based on water chemistry (Hunt) were considered to be in reference condition using the mud index. Reference lakes assessed as impacted were also variable by habitat type. There was only one cottage on the reference lake identified as impacted using the cobble index, Elbow Lake (2008). Using the mud index, Marion and Camp Lakes were assessed as impacted. Neither of these lakes have cottages present; however, Camp Lake had displayed relatively high concentrations of calcium and sulfate.

### *3.3.7 Comparison of metrics, regression-based metrics and multimetric indices*

Of the three bioassessment methods examined, multimetric indices were the most accurate, regardless of habitat type (Tables 3.15 and 3.16). Cobble and mud indices assessed 70% and 64% of lakes with over 25% of their shorelines developed as impacted, respectively. In contrast, metrics assessed up to 50% and 36% of heavily developed lakes as impacted using cobble and mud data, respectively. Regression-based metrics were the least accurate method using cobble data (36% of lakes with > 25% developed shoreline assessed as impacted), and were equally as accurate as metrics (36%) using mud data. The accuracy of all bioassessment methods was generally higher when considering only test lakes with more than 25% of their shorelines developed with cottages.

Unexpectedly, regression-based metrics were generally less accurate in comparison with metrics for the assessment of lakes with cottages as impacted (Tables

3.15 and 3.16). The only exceptions to this observation were for Odonata richness using cobble data, and % Gastropoda using mud data. A number of the metrics (Hurlbert's PIE, % 5 dominant taxa, % scrapers) were equally accurate between metrics and regression-based metrics using cobble data, while ETO abundance was equally accurate using mud data.

Of the three bioassessment methods examined, regression-based metrics had the highest precision (Tables 3.17 and 3.18). Regression-based metrics had a mean per cent agreement of 88 and 90 in the assessment of sites using cobble and mud data, respectively. Metrics had the next highest precision for site assessment with 85 and 78% agreement observed using cobble and mud data, respectively. Multimetric indices were the least precise, with only 77 and 71% agreement for site assessment in cobble and mud habitats, respectively. The precision of multimetric indices did not appear to be related to proximity to development or sampling location; sampling sites located close to one another yielded variable results within lakes with cottages (Figure 3.4).

Based on plots of the most accurate measure for each bioassessment method, multimetric indices appear the most reliable for the detection of cottage impacts along a gradient (Figure 3.5). This is largely because of the way multimetric indices were scored, causing divergence from the reference distribution to be in one direction. In contrast, lakes with cottages displayed metric and regression-based metric scores above and below the distribution of reference lakes.

### *3.3.8 Influence of habitat type on the detection of impacts*

Based on the scores from multimetric indices, the detection of cottage impacts is more accurate and precise when samples are collected from cobble sediments. Using the cobble index, up to 70% of lakes with more than one quarter of their shoreline occupied by cottages were assessed as impacted. This was a 6% increase in accuracy when compared to the mud multimetric index. Assessment precision was also consistently higher across developed lakes using the cobble multimetric index in comparison with the mud multimetric index.

The level of impact determined using multimetric indices also appeared more accurate using cobble *versus* mud data. Using the cobble index, the three lakes with over 40% of their shorelines occupied by cottages (Brereton, Falcon and West Hawk) were assessed as impacted, whereas using the mud index West Hawk Lake was considered unimpacted. The lakes considered the most impacted based on multimetric index scores (i.e., higher scores indicate greater impairment), were Hunt Lake using cobble data and Caddy Lake (2008) using mud data.

The precision of bioassessment using the three bioassessment methods did not appear consistently better or worse using cobble *versus* mud data. Samples that were collected from nearby sections of shoreline within the same habitat type did not consistently indicate either impacted or unimpacted condition. For example, along one section of shoreline in Barren Lake, cobble and mud samples indicated both unimpacted and impacted condition (Figure 3.4a). This was also observed along a mud shoreline in Red Rock Lake (Figure 3.4c) and a cobble shoreline in Star Lake (Figure 3.4d). In Caddy Lake, multimetric index scores were variable by sampling year but not

habitat type (Figure 3.4b and Table 3.14); in 2007 all sites were characterized as unimpacted and in 2008 they were all characterized as impacted. The collection of samples along shorelines with cottages, in contrast to undeveloped shorelines, did not appear to influence the assessment of sites.

## 3.4 Discussion

Based on the results of this study, it appeared that this newly designed rapid bioassessment protocol could be used effectively in boreal shield lakes. This protocol was designed based on previous RBAs (reviewed in Chapter 1) and a preliminary sampling survey of Malloy Lake (Chapter 2). The protocol was then tested in 69 lakes to determine if it could be used to assess impacts across regions such as provincial parks and if methods could be further refined. With this protocol, one, 10 $m^2$ kick-sample collected from cobble sediments in the littoral zone was used to distinguish lakes with over 25% of their shorelines occupied with cottages from undeveloped lakes with 70% accuracy.

The refinement of protocols in this study focused on selecting one habitat type to sample and choosing an analysis method for the assessment of impacts. Two sediment types (cobble and mud) were sampled to allow processing time and assessment accuracy to be compared. This was followed by a comparison of the accuracy and precision of three bioassessment methods (metrics, regression-based metrics and multimetric indices). Sampling cobble sediments and using a multimetric index to assess impacts are recommended based on the results of this survey.

### *3.4.1 Influence of habitat type on the detection of impacts*

The habitat type sampled affects assessment time, the community estimates that respond to impact, the variability of community estimates and the ability to assess developed lakes as impacted. These findings are important in the design of a biomonitoring protocol because they will affect the cost-efficiency of a biomonitoring program. Shorelines dominated by cobble and mud differ in multiple ways, are inhabited by distinct benthic macroinvertebrate communities and should not be expected to respond in a consistent way to impacts, particularly those associated with multiple stressors such as cottage development.

For littoral zone macroinvertebrates to be used cost-effectively in the assessment of cottage impacts, a habitat to be sampled should be chosen that is common within the study area, that allows a wide range of macroinvertebrates to be collected and that is strongly affected by stressors associated with shoreline development. In Whiteshell and Nopiming Provincial Parks, shorelines dominated by cobble or mud sediments were widespread and allowed the collection of samples with diverse assemblages of macroinvertebrates. In previous studies, a stronger response to nutrient enrichment on rocky shores was observed in comparison with shores dominated with finer sediment (i.e., mud to sand) among periphyton (e.g., Lambert and Cattaneo 2008; Lambert *et al.* 2008) and macroinvertebrate assemblages (e.g., Donohue *et al.* 2009; Tolonen and Hämäläinen 2010; Tolonen *et al.* 2001). The opposite relationship was observed by Brauns *et al*. (2007a) and De Sousa *et al*. (2008) in their studies of littoral zone macroinvertebrates. The findings reported by De Sousa *et al*. (2008) should be the most applicable to this study because they were also

investigating effects of shoreline development on macroinvertebrates. They reported that the only changes in community composition correlated with shoreline development occurred in finer sediments; however, they also reported differences in grain size between the sites they sampled in unimpacted and developed lakes. In undeveloped lakes, finer sediment habitat was mostly medium-sand to small-gravel in size. For lakes with developed shorelines, sediment size was generally clay to fine-sand. This difference was attributed to the increased deposition of fine sediment in lakes with developed shorelines. Different community compositions observed could have been influenced by the preference of macroinvertebrates for different sediment sizes (Doeg *et al.* 1989; Minshall 1984; Wieser 1959); however, it is difficult to be certain. Within Whiteshell and Nopiming Provincial Parks, mud-dominated shorelines were present in lakes with and without cottages and allowed the comparison of macroinvertebrate response to cottage impacts between two standardized habitat types (i.e., consistent sediments were compared between lakes with and without cottages) previously reported to be well suited for bioassessment.

Despite the stronger correlations between shoreline development and mud metrics, samples collected from cobble habitat were more sensitive for the detection of cottage impacts. Whether individually or used in combination as a multimetric index, cobble metrics could be used to distinguish a greater proportion of developed lakes from the distribution of reference lakes. Because periphyton can obtain nutrients from fine sediments, but not rocks, nutrient enrichment of the water column may have a stronger influence along rocky shores (i.e., after enrichment, the difference in nutrient availability will be greater for epilithon *versus* epipelon) (Lambert and Cattaneo 2008).

It is also possible that the benthic communities found along rocky shores are more affected by nutrient enrichment because they are less tolerant of reduced oxygen condition (i.e., rocky shores are often wave swept and more oxygen-rich environments than sheltered bays) (Donohue *et al.* 2009).

Rocky shorelines have fewer plants and coarser sediments and consequently the kick-samples collected from them were smaller and took less time to process than those collected from muddy shorelines. Samples with filamentous algae or a lot of plant material were more time consuming to sort, and this type of material was more common in mud samples. Similar findings were reported by Tolonen and Hämäläinen (2010) in their comparison of samples collected from rocky, sandy and vegetated lakeshores in Finland. This is an important consideration for RBA protocols where savings in time need to be made that do not sacrifice the ability to detect impacts. While either sediment type could be used, the reduced processing time associated with kick-sampling rocky shores is a further benefit for this RBA protocol.

Some benthic community changes associated with cottage development were consistent across cobble and mud shorelines. Regardless of habitat type, there were higher percentages of gastropods, lower percentages of insects and sub-taxa Diptera and Chironomidae, and increased abundances of scrapers and gastropods observed in lakes with cottages. Gastropods and other scrapers likely benefited from more algae (Lambert and Cattaneo 2008) or submerged macrophytes being present in the littoral zone (Gélinas and Pinel-Alloul 2008). The increased abundance of scrapers appears to be why lower proportions of chironomids were recorded in lakes with cottages; decreased proportions of chironomids, flies and insects were not accompanied by

significantly lower abundances of these groups. For RBA programs where more than one habitat type is sampled, these macroinvertebrate metrics would be the most effective for the detection of cottage impacts; however, focusing on the changes that occur within one habitat type would likely be a more cost-efficient use of resources.

Littoral zone macroinvertebrates had higher diversity and richness in lakes with cottages; however, this was only observed along mud shorelines. Nutrient enrichment and consequent increased macrophyte growth (e.g., Gélinas and Pinel-Alloul 2008) are the most likely causes of this difference among habitat types. Macrophytes provide macroinvertebrates with habitat, food, and a greater diversity of niche spaces; consequently, benthic communities along vegetated shorelines are more diverse than along rocky shores (Tolonen and Hämäläinen 2010; Tolonen *et al.* 2001). More macrophytes can also provide greater surface area for the growth of periphyton; however, increased periphyton biomass associated with shoreline development is more pronounced on rocks than on plants (e.g., Lambert and Cattaneo 2008).

With temporal variability as the lowest contributor (in comparison with among- and within-lake spatial variability) to mud metrics, there is some support for sampling this habitat type for long-term RBA programs. The ability to use macroinvertebrate data collected from different years is an important consideration for programs that aim to conserve time and costs of assessments and monitor recovery efforts. If temporal variability is too high, sampling a new set of reference lakes may be required annually. The assumption that the calculation of temporal variability in this study was not heavily influenced by spatial variability may also be incorrect because, within a lake, the same sites were not sampled in 2007 and 2008. While the effectiveness of the cobble

multimetric index did not appear to be biased by sampling year, this is a consideration that requires further investigation through long-term monitoring using RBA methods.

### *3.4.2 Comparison of metrics, regression-based metrics and multimetric indices*

The precision of bioassessment methods was highest for regression-based metrics, perhaps because some sources of variability were accounted for. Because the ability to provide repeatable results is an important consideration in biomonitoring, it is unfortunate that regression-based metrics were not more accurate for the detection of impacts. Multimetric indices were the least precise bioassessment method; however, their accuracy was often twice as high as those observed for metrics and regression-based metrics.

The greater accuracy of multimetric indices may have resulted from the complexity of cottage impacts or the complexity of benthic communities. Because multiple stressors are cooccurring and it is difficult to predict how some changes affect others, a single metric or regression model could be less sensitive for impact assessment. Depending on which stressors (e.g., nutrient enrichment, sedimentation) are dominant within a lake, the metrics that deviate from reference condition may vary. Lakes such as Brereton that have heavily modified shorelines may be more heavily influenced by the reductions in coarse woody debris (e.g., Christensen *et al.* 1996; Jennings *et al.* 2003; Marburg *et al.* 2006), increased sedimentation (e.g., Bookman *et al.* 2010; De Sousa *et al.* 2008; Jennings *et al.* 2003), and reduced organic matter (e.g., Francis *et al.* 2007) that have previously been reported in lakes with developed shorelines. Other lakes that were designated as impacted using the cobble index had

relatively high nutrient (e.g., Betula, Jessica) or chloride (e.g., Caddy, Hunt) concentrations or high proportions of shoreline development along with heavy recreational use (e.g., Caddy, Jessica). Because there is a poor understanding of how cottages and their associated stressors will cumulatively affect macroinvertebrates, the use of a multimetric index may be the only way to detect impacts from complex stressors. Reliance on more than one macroinvertebrate metric may also be warranted because the range of communities present in unimpacted lakes is wide. If the initial composition of macroinvertebrate communities differs between lakes, impacts could cause different community changes to occur.

Site assessment using the cobble multimetric index appears valid based on the scores of most test lakes. The cobble index identified Hunt Lake as the most impacted and also identified lakes with the highest levels of development (Brereton, West Hawk, Falcon) as impacted. Since Hunt Lake displayed the highest levels of chloride, calcium, potassium, sodium and conductivity that were measured in this lake survey, it has likely been affected by impacts to a greater degree than other lakes in the area. The increased number of changes observed in the macroinvertebrate community of Hunt Lake may have been influenced by the small size of the lake (i.e., this is the smallest test lake by surface area, causing less dilution of nutrients and sediments in comparison with larger lakes) and its proximity to the heavily developed West Hawk Lake and the Trans Canada Highway (i.e., larger influence of watershed impacts). It is also possible that Hunt Lake has been impacted to a greater degree because of longer residence times in comparison with other lakes; however, this type of data was not available for this study.

The mud index appeared less effective for the assessment of cottage impacts because fewer lakes with cottages differed from the distribution of reference lakes and the ranking of lakes was more difficult to explain. Using the mud index, Caddy Lake (2008) was assessed as the most impacted. Caddy Lake had 39 % of its shoreline occupied by cottages and heavy recreational use (i.e., motorized watercraft, camping, resorts, fishing); however, these characteristics are also present in West Hawk Lake, which was assessed as unimpacted.

Despite the apparent success of the cobble index in the detection of cottage impacts, it is unclear whether or not these results would be reproducible in different sampling years or lakes. The discrimination between lakes with and without cottages was variable between 2007 and 2008 when samples were collected from the same lakes. This difference could be attributable to weather differences; however, only slight decreases in rainfall (6 mm) and higher mean air temperature (1.5 ºC) occurred during the 2008 field season in comparison with 2007 (The Weather Network 2012). It is also plausible that community changes would be observed at the same sites from year to year when there are no changes in weather. There are many biotic assemblages present in lakes that can influence trophic structure from the top-down or the bottom-up (Blumenshine *et al.* 1997; Gélinas and Pinel-Alloul 2008) and changes that have occurred over the course of the year may alter the community composition of macroinvertebrates at a given sampling site. The observation that sites sampled in close proximity to one another within one lake provided variable assessment results using metrics alone or in combination supports the uncertainty of these results. Multimetric indices were created by selecting metrics that maximized the differences between lakes

with and without cottages, thus if a different set of lakes with cottages was sampled, it is unclear if they would be assessed as impacted with the same accuracy (i.e., if different stressors or environmental variables were prevalent, the index may not be as effective for impact assessment). Creating a multimetric index using regression-based metrics could be an alternative to the indices created here; an index calculated using regression-based metrics could have increased precision because of the incorporation of environmental data.

### *3.4.3 Impacts from cottages*

The RBA protocols used here identified most lakes with > 25% shoreline development as impacted; however, the intensity of impact as indicated by the multimetric index score did not always correspond with either the number of cottages present or the amount of shoreline that they occupied. Multimetric indices were relatively sensitive for the assessment of cottage impacts along a gradient of development in comparison with metrics and regression-based metrics (Figure 3.5). The lakes that were not assessed as predicted, based on level of cottage development, is perhaps a consequence of less rigorous methods used for RBA (e.g., the collection of fewer samples per lake, family-level taxonomic resolution, assessment using a multimetric index), necessary to complete assessments more quickly, an inadequate quantification of impact, or the complexity of stressors associated with cottage development.

To my knowledge, only Metzeling *et al*. (2006) have reported the detection of impact gradients using RBA and discussed how this was influenced by taxonomic

resolution and analytical method. Metzeling *et al*. (2006) sampled riffle and edge habitats at each Australian stream site for the detection of impacts along gradients of salinity and habitat simplification. They reported no difference in the influence of species- *versus* family-level resolution for the detection of impact gradients; however, they did report differences associated with assessment method. Multivariate analysis detected the salinity gradient effectively but not the gradient of habitat simplification, whereas the opposite observations were made using family richness and EPT-family richness for assessment (Metzeling *et al.* 2006). A gradient of cottage development would perhaps be detected more effectively using multivariate analysis methods (e.g., Milner and Oswood 2000); however, multivariate analyses were avoided in my study because of the large initial investment of time and expertise required for these methods to be developed into a predictive model that can be used by non-specialists for RBA.

The adequate quantification of impacts from cottages is difficult because there are multiple stressors and their prevalence and impact can vary widely. This is largely influenced by the attitudes of cottagers, whose activities (i.e., using motorized watercraft, fishing) can differ across and within lakes (Henning and Remsburg 2009; Racey and Euler 1983). Cottagers influence the level of impact to lakes through the ways they develop their property (Henning and Remsburg 2009; Racey and Euler 1983). For example, crib docks will affect littoral communities to a greater extent than floating docks because they remove available habitat, but developments that do not reduce the complexity of littoral zone habitat may have no effect on the biological communities present (e.g., Jennings *et al.* 1999; Taillon and Fox 2004). The prediction of impacts from cottages can also be difficult because the use of cottages can be highly

variable (i.e., some are occupied year round whereas others are used occasionally in summer). As a consequence, certain cottages cause larger impacts to lake ecosystems than others and quantification measures such as per cent developed shoreline and cottages per kilometre are inadequate. For example, based on cobble index scores, Hunt Lake was the most impacted by cottage development despite having only eight cottages. While it is unclear whether the impacts to Hunt Lake were more pronounced because of its small surface area, its proximity to the Trans Canada Highway and other heavily developed lakes, or because less effective septic systems were present, it is clear that this lake has been heavily impacted by development.

There were differences between the open-water chemistry of lakes with and without cottages; septic effluents or road salts may have contaminated some lakes. Increased concentrations of chloride, sodium, calcium and potassium, as was observed in developed lakes within this study, have been reported in water contaminated by septic effluent (e.g., Robertson *et al.* 1991) and road salts (e.g., Mayer *et al.* 1999). The highest correlation between shoreline development and measured open-water variables was observed for chloride, an analyte that is often used to determine the spatial extent of sewage contamination (e.g., Alhajjar *et al.* 1990; Gilliom and Patmont 1983; Jones and Lee 1979; Schmidt 1975). However, chloride is also a major component of road salts (Gillis 2011; Kaushal *et al.* 2005; Mayer *et al.* 1999).

Chloride is useful in the assessment of sewage contamination because it is not removed by septic systems, it is present in human waste in relatively high concentrations (Alhajjar *et al.* 1990) and it is conservative (i.e., it is not readily adsorbed) (Hendry *et al.* 2000). In this study, the highest chloride concentrations were

observed in Hunt, Caddy, Red Rock, Falcon and West Hawk lakes. Concentrations of chloride, sodium, calcium and potassium were particularly high in Hunt Lake. Despite only moderate development being present, Hunt Lake appears to be the most impacted by cottages based on water chemistry. It is possible that septic systems are less effective around this lake because they were set up improperly or soils have become saturated with sewage over time (Gilliom and Patmont 1983); however, there were no corresponding increases in nutrient concentrations to support this hypothesis.

Contrary to the findings of Dillon *et al*. (1994), phosphorus concentrations were not higher in the developed lakes sampled in this study. This could mean that the septic effluent reaching lakes has relatively low phosphate levels, that septic effluent is not contaminating lakes, or that nutrients are assimilated by biota before changes to open-water chemistry can occur (e.g., Havens *et al.* 2004; Kauppila and Valpola 2003; Lambert and Cattaneo 2008). Based on the increased abundance of scrapers observed in lakes with cottages, nutrient enrichment and consequent assimilation by littoral zone communities are suspected. Nutrients entering lakes through the littoral zone can be rapidly consumed by periphyton (e.g., Lambert and Cattaneo 2008). The increased biomass of periphyton could have led to the increased abundance of scrapers by providing them with more food.

It is at least equally probable that changes in water chemistry were related to the use of road salts. Four salts that are applied to roads are NaCl, $CaCl_2$, KCl and $MgCl_2$, which readily dissolve in lakes, increasing the concentrations of chloride, sodium, calcium, potassium and magnesium (Mayer *et al.* 1999). The concentrations of each of these elements displayed elevated concentrations in developed lakes within this study.

Road salts can also contain sulfates as impurities (Mayer *et al.* 1999). The increased concentrations of magnesium and sulfate observed in developed lakes in this study add support to the possibility that road salts are responsible for changes in water chemistry, as their elevated concentrations are not explained by contamination from septic systems. The lakes with the highest concentrations of chloride (Hunt, Caddy, Red Rock, Falcon and West Hawk) are all located in close proximity to a highway.

The lack of correlation between macroinvertebrate metrics and measures of both shoreline development and trophic state could be an indication that nutrient enrichment is not the main driver of community changes associated with cottages. Although some lakes have most likely been affected by nutrient loading from septic effluent, perhaps the majority of lakes are more strongly influenced by habitat simplification. If nutrient enrichment in the littoral zone was the main stressor affecting macroinvertebrates, I would expect greater consistency in correlation between metrics and both shoreline development and pelagic trophic variables; however, without a more detailed description of the complexity of sites (i.e., based on the amounts of coarse woody debris and fine sediment) it is impossible to make any firm conclusions.

### *3.4.4 Conclusions*

Based on results of this survey, it is recommended that boreal shield lakes are monitored using RBA techniques that focus on kick-sampling cobble shorelines and using the multimetric index that was designed here for the bioassessment of sites. These methods allowed most lakes with cottages to be distinguished from reference lakes, despite the complexity of stressors associated with this type of impact. For the

bioassessment of other impacts, particularly those that can affect lakes in similar ways (e.g., urbanization, roads, forestry, agriculture), it is expected these methods would also be applicable.

In addition to the benthic macroinvertebrate sampling that is performed within each lake, the collection of at least one water sample is recommended. In this study, the community composition of Hunt Lake displayed the strongest divergence from reference communities. Without the analysis of water chemistry, it would not have been clear that this lake was influenced more heavily than others by sewage effluent or road salts. The collection of water samples can contribute to the understanding of why some lakes appear more impacted than others. In cases where some type of contamination is suspected (i.e., high chemical concentrations accompanied by biological changes), a more thorough sampling survey should be performed to determine the source of impacts.

The success of this RBA protocol for the detection of impacts allows boreal shield lakes to be monitored in a more affordable and less time-intensive way. The collection of one sample per lake allowed a number of lakes to be sampled in one season and many of these samples can be processed in one work day. This is most likely an improvement on other lake RBA methods that recommend the collection of five samples per lake within dominant habitat types (e.g., Somers *et al.* 1998; Wesolek *et al.* 2010). Sampling dominant habitat types requires a preliminary survey of each lake's shoreline, which could take a long time depending on the size of lake, access and the type of boat used. It could also result in the collection of samples with a lot of macrophytes that can take a long time to process. It is believed that the methods used in

this study are accurate with reduced sampling in part by restricting sampling to one habitat type (i.e., reducing variability). It is a further benefit that the habitat type sampled results in the collection of less plant material and samples consequently take less time to process. It is expected that the RBA protocol tested in this study could be used to monitor other impacts across a wide range of lakes within the boreal shield.

## Tables and Figures

Table 3.1 Range of morphometric and chemical values observed in the 54 reference lakes sampled in Whiteshell and Nopiming Provincial Parks, MB, during the fall of 2007 or 2008. Shoreline development index was calculated by dividing the shoreline's perimeter by the perimeter of a circle of equal area for each lake (Wetzel 2001c).

| | **Surface area (ha)** | **Shoreline development index** | **Total phosphorus (µg / l)** | **Total nitrogen (µg / l)** | **Chlorophyll a (µg / l)** | **Secchi depth (m)** |
|---|---|---|---|---|---|---|
| Minimum | 22 | 1.3 | 7 | 350 | 1 | 0.4 |
| Maximum | 5578 | 7.1 | 64 | 1659 | 60 | 5.3 |
| Median | 142 | 2.6 | 18 | 618 | 5 | 1.8 |
| Mean | 377 | 2.9 | 20 | 694 | 9 | 2.0 |

Table 3.2 Lakes with cottages (test lakes) that were sampled in Whiteshell and Nopiming Provincial Parks, MB, during the fall of 2007 or 2008. Shoreline development index was calculated by dividing the shoreline's perimeter by the perimeter of a circle of equal area for each lake (Wetzel 2001c).

| Lake | % developed shoreline | Number of cottages | Sampling year | | Surface area (ha) | Shoreline development index |
|---|---|---|---|---|---|---|
| | | | 2007 | 2008 | | |
| Brereton | 71 | 348 | | x | 886 | 3.1 |
| Falcon | 42 | 813 | x | | 1572 | 3.1 |
| West Hawk | 41 | 525 | x | | 1462 | 3.0 |
| Caddy | 39 | 154 | x | x | 312 | 2.7 |
| Star | 36 | 129 | x | | 154 | 2.8 |
| Betula | 31 | 170 | | x | 382 | 5.1 |
| Red Rock | 30 | 123 | x | x | 152 | 2.4 |
| Florence | 29 | 30 | x | x | 70 | 1.6 |
| Big Whiteshell | 21 | 181 | x | | 1752 | 3.0 |
| Barren | 20 | 25 | x | | 73 | 2.7 |
| Bird | 19 | 123 | x | x | 724 | 3.2 |
| Davidson | 19 | 48 | x | | 279 | 5.4 |
| Hunt | 19 | 8 | | x | 18 | 2.4 |
| Jessica | 18 | 102 | x | | 880 | 2.4 |
| Flanders | 10 | 45 | | x | 177 | 3.7 |
| White | 10 | 83 | | x | 809 | 3.1 |
| Nora | 8 | 20 | x | x | 315 | 4.0 |

Table 3.3 List of taxa collected in Whiteshell and Nopiming Provincial Parks, MB, and their corresponding taxonomic level of identification (ID).

| **Phylum** | **Class** | **Order** | **ID level** | **Taxa** |
|---|---|---|---|---|
| Annelida | Clitellata | Arhynchobdellida | Order | Arhynchobdellida |
| | | Rhynchobdellida | Family | Glossiphoniidae |
| | | Branchiobdellida | Family | Branchiobdellidae |
| | | Haplotaxida | Subfamily | Naidinae |
| | | | | Tubificinae |
| | | Lumbriculida | Family | Lumbriculidae |
| Arthropoda | Arachnida | Acari | Order | Acari |
| | | Araneae | Genus | Pisauridae: *Dolomedes* |
| | Insecta | Coleoptera | Genus | Chrysomelidae: *Donacia* |
| | | | Subfamily | Curculionidae: |
| | | | Family | Dytiscidae |
| | | | | Elmidae |
| | | | | Georyssidae |
| | | | | Gyrinidae |
| | | | | Haliplidae |
| | | | | Hydraenidae |
| | | | | Hydrophilidae |
| | | | | Psephenidae |
| | | | | Scirtidae |
| | | | | Staphylinidae |
| | | Collembola | Family | Entomobryidae |
| | | | | Hypogastruridae |
| | | | | Isotomidae |
| | | | | Poduridae |
| | | | | Sminthuridae |
| | | Diptera | Family | Ceratopogonidae |
| | | | | Chaoboridae |
| | | | | Chironomidae |
| | | | | Dixidae |
| | | | | Dolichopodidae |
| | | | | Empididae |
| | | | | Muscidae |
| | | | | Psychodidae |
| | | | | Sciomyzidae |
| | | | | Stratiomyidae |
| | | | | Tabanidae |
| | | | | Tipulidae |
| | | Ephemeroptera | Family | Baetidae |
| | | | | Caenidae |
| | | | | Ephemerellidae |
| | | | | Ephemeridae |
| | | | | Heptageniidae |
| | | | | Leptophlebiidae |
| | | | | Siphloneuridae |
| | | Hemiptera | Family | Belostomatidae |
| | | | | Corixidae |
| | | | | Gerridae |
| | | | | Mesoveliidae |
| | | | | Nepidae |
| | | | | Notonectidae |
| | | | | Pleidae |
| | | | | Veliidae |

Table 3.3 (continued).

| Phylum | Class | Order | ID level | Taxa present |
|---|---|---|---|---|
| Arthropoda | Insecta | Lepidoptera | Family | Cosmopterigidae |
| | | | | Pyralidae |
| | | Megaloptera | Family | Corydalidae |
| | | | | Sialidae |
| | | Neuroptera | Family | Sisyridae |
| | | Odonata | Family | Aeshnidae |
| | | | | Corduliidae / Libellulidae |
| | | | | Gomphidae |
| | | | | Coenagrionidae |
| | | | | Lestidae |
| | | Trichoptera | Family | Brachycentridae |
| | | | | Dipseudopsidae |
| | | | | Helicopsychidae |
| | | | | Hydropsychidae |
| | | | | Hydroptilidae |
| | | | | Lepidostomatidae |
| | | | | Leptoceridae |
| | | | | Limnephilidae |
| | | | | Molannidae |
| | | | | Odontoceridae |
| | | | | Phryganeidae |
| | | | | Polycentropodidae |
| | | | | Psychomyiidae |
| | | | | Sericostomatidae |
| | Malacostraca | Amphipoda | Family | Crangonyctidae |
| | | | | Gammaridae |
| | | | | Hyalellidae |
| | | Decapoda | Family | Cambaridae |
| | Maxillopoda | Harpacticoida | Order | Harpacticoida |
| | Ostracoda | | Class | Ostracoda |
| Cnidaria | Hydrozoa | Hydroida | Family | Hydridae |
| Mollusca | Bivalvia | Veneroida | Family | Pisidiidae |
| | | Unionoida | Family | Unionidae |
| | Gastropoda | Basommatophora | Family | Ancyliidae |
| | | | | Lymnaeidae |
| | | | | Physidae |
| | | | | Planorbidae |
| | | Heterostropha | Family | Valvatidae |
| | | Neotaenioglossa | Family | Hydrobiidae |
| Nematoda | | | Phylum | Nematoda |
| Platyhelminthe | Turbellaria | | Class | Turbellaria |
| Porifera | | | Phylum | Porifera |

Table 3.4 Macroinvertebrate metrics evaluated for inclusion in rapid bioassessment protocols. Measures of abundance, relative proportion, richness and diversity were selected based previous recommendations for bioassessment and on the dominant taxa collected.

| Abundance | Relative proportions | Richness | Diversity |
|---|---|---|---|
| Scrapers | % scrapers | All taxa | Shannon index |
| Insecta | % Insecta | Scrapers | Simpson's D |
| ETO | % ETO | ETO | Hurlbert's PIE |
| ET | % ET | ET | % 1 dominant taxon |
| Ephemeroptera | % Ephemeroptera | Ephemeroptera | % 3 dominant taxa |
| Trichoptera | % Trichoptera | Trichoptera | % 5 dominant taxa |
| Odonata | % Odonata | Odonata | |
| Coleoptera | % Diptera | Diptera | |
| Diptera | % Chironomidae | Gastropoda | |
| Acari | % Acari | | |
| Amphipoda | % Amphipoda | | |
| Gastropoda | % Gastropoda | | |
| Hirudinea | % Oligochaeta | | |
| Oligochaeta | | | |

Table 3.5 Results of one-way ANOVA for log $_{10}$ processing times. This analysis was performed to determine if habitat type significantly affected the time needed to process samples collected from lakes in Whiteshell and Nopiming Provincial Parks, MB. The factor 'habitat' (cobble or mud sites) was used in the model and significant ($P < 0.05$) effects are followed by an asterisk (*).

| **Source** | **DF** | **SS** | **MS** | ***F*** | ***P*** |
|---|---|---|---|---|---|
| Habitat | 1 | 0.2261 | 0.2261 | 4.92 | 0.031* |
| Error | 56 | 2.5729 | 0.0459 | | |
| Total | 57 | 2.7990 | | | |

Table 3.6 Spearman correlation coefficients for measured open-water variables and measures of shoreline development from lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. Abbreviations used for development measures are % = per cent of shoreline developed with cottages, # per km = number of cottages per kilometre of shoreline, and # = number of cottages per lake. Only significant ($P < 0.05$) correlations are listed.

| Open-water variables | % | # per km | # |
|---|---|---|---|
| Total phosphorus | | | |
| Suspended phosphorus | | | |
| Total dissolved phosphorus | | | |
| Total nitrogen | | | |
| Suspended nitrogen | | | |
| Total dissolved nitrogen | | | |
| Nitrate | -0.23 | -0.23 | |
| Nitrite | | | |
| Ammonia | | | |
| Total carbon | | | |
| Suspended carbon | | | |
| Dissolved inorganic carbon | | | |
| Dissolved organic carbon | | | |
| Chlorophyll a | | | |
| Total suspended solids | | | |
| Conductivity | 0.43 | 0.45 | 0.43 |
| Secchi depth | | | |
| pH | 0.29 | 0.32 | 0.30 |
| Alkalinity | | | 0.23 |
| Calcium | 0.39 | 0.41 | 0.40 |
| Chloride | 0.65 | 0.64 | 0.63 |
| Iron | | | |
| Suspended iron | | | |
| Magnesium | 0.28 | 0.30 | 0.32 |
| Manganese | | | |
| Potassium | 0.41 | 0.43 | 0.39 |
| Soluble reactive silica | 0.31 | 0.31 | 0.30 |
| Sodium | 0.52 | 0.53 | 0.48 |
| Sulfate | 0.54 | 0.52 | 0.51 |

Table 3.7 Significant ($P < 0.05$) Spearman correlation coefficients between macroinvertebrate metrics calculated from cobble and mud sample data and measures of development or open-water variables from lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. Abbreviations used for shoreline development measures are % = per cent of shoreline developed with cottages, # per km = number of cottages per kilometre of shoreline, and # = number of cottages per lake. Abbreviations used for open-water variables are TP = total phosphrus, TN = total nitrogen, and Chl*a* = chlorophyll *a*.

| | **Cobble** | | | | | | | **Mud** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Shoreline** | | | **Open-water** | | | | **Shoreline** | | | **Open-water** | | | |
| **Metric** | **%** | **# per km** | **#** | **TP** | **TN** | **Chl*a*** | **Secchi depth** | **%** | **# per km** | **#** | **TP** | **TN** | **Chl*a*** | **Secchi depth** |
| Scraper abundance | 0.23 | | | | | | | 0.42 | 0.42 | 0.44 | | | | |
| ETO abundance | | | | 0.24 | 0.23 | 0.26 | | 0.41 | 0.41 | 0.42 | | | | |
| ET abundance | | | | 0.26 | 0.25 | 0.25 | | 0.40 | 0.41 | 0.42 | | | | |
| Ephemeroptera abundance | | | | 0.31 | 0.24 | 0.29 | | 0.34 | 0.33 | 0.35 | | | | |
| Trichoptera abundance | | | | | | | | 0.33 | 0.36 | 0.34 | | | | |
| Odonata abundance | | | | | | | | | | 0.23 | | | | |
| Coleoptera abundance | | | | | | | | 0.35 | 0.36 | 0.38 | | | | |
| Acari abundance | | | | | 0.23 | | | 0.27 | 0.31 | 0.35 | | | | |
| Amphipoda abundance | | | | | | | | 0.35 | 0.34 | 0.42 | | | | |
| Gastropoda abundance | 0.28 | 0.28 | 0.24 | | | | | 0.40 | 0.40 | 0.41 | | | | |
| Hirudinea abundance | | | | 0.29 | 0.29 | 0.24 | -0.26 | 0.28 | 0.29 | 0.31 | | | | |
| Oligochaeta abundance | | | | | | | | 0.26 | 0.29 | 0.27 | | | | |
| % Scrapers | | | | | | | | 0.34 | 0.32 | 0.31 | | | | |
| % Insecta | -0.26 | -0.26 | -0.23 | | | | | -0.27 | -0.25 | -0.30 | | | | |
| % Trichoptera | -0.36 | -0.33 | -0.31 | -0.24 | | -0.30 | | | | | | | | |
| % Diptera | -0.31 | -0.30 | -0.35 | | | | | -0.34 | -0.32 | -0.36 | | | | |
| % Chironomidae | -0.29 | -0.27 | -0.33 | | | | | -0.35 | -0.32 | -0.36 | | | | |
| % Gastropoda | 0.29 | 0.28 | 0.25 | | | | | 0.37 | 0.35 | 0.34 | | | | |
| Taxa richness | | | | | | | | 0.25 | 0.27 | 0.27 | | | | |
| Scraper richness | | | | | | | | | | 0.24 | | | | |
| ET richness | | | | | | | | | | | | -0.27 | | |
| Ephemeroptera richness | | | 0.24 | | | 0.26 | | | | | | -0.28 | | |
| Gastropoda richness | | | | 0.28 | 0.25 | | | 0.33 | 0.32 | 0.35 | | | | |
| Shannon index | | | | | | | | 0.29 | 0.32 | 0.27 | | | | |
| Simpson’s D | | | | | | | | -0.34 | -0.34 | -0.31 | | | | |
| Hurlbert’s PIE | | | | | | | | 0.34 | 0.34 | 0.31 | | | | |
| % 1 dominant taxon | | | | | | | | -0.30 | -0.31 | -0.29 | | | | |
| % 3 dominant taxa | | | | | | | | -0.31 | -0.31 | -0.28 | | | | |
| % 5 dominant taxa | | | | | | | | -0.29 | -0.30 | -0.27 | | | | |

Table 3.8 Coefficient of variation (CV) of metrics calculated using cobble and mud habitat data from reference lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. Sources of spatial (among- and within-lake) and temporal (annual) variability are listed for all metrics.

| | CV | | | | | |
|---|---|---|---|---|---|---|
| | Cobble | | | Mud | | |
| Metric | Among-lake | Within-lake | Temporal | Among-lake | Within-lake | Temporal |
| Scraper abundance | 88 | 54 | 68 | 174 | 98 | 96 |
| Insect abundance | 68 | 63 | 56 | 135 | 101 | 85 |
| ETO abundance | 80 | 86 | 61 | 127 | 83 | 62 |
| ET abundance | 81 | 87 | 56 | 132 | 85 | 63 |
| Ephemeroptera abundance | 95 | 97 | 59 | 105 | 80 | 50 |
| Trichoptera abundance | 62 | 63 | 47 | 186 | 113 | 75 |
| Odonata abundance | 126 | 126 | 109 | 169 | 134 | 34 |
| Coleoptera abundance | 163 | 118 | 76 | 164 | 82 | 75 |
| Diptera abundance | 90 | 48 | 76 | 151 | 116 | 111 |
| Acari abundance | 128 | 49 | 89 | 129 | 96 | 93 |
| Amphipoda abundance | 98 | 66 | 81 | 133 | 86 | 86 |
| Gastropoda abundance | 153 | 66 | 84 | 211 | 104 | 132 |
| Hirudinea abundance | 203 | 126 | 136 | 220 | 172 | n.a. |
| Oligochaeta abundance | 130 | 60 | 50 | 129 | 132 | 116 |
| % scrapers | 45 | 24 | 43 | 106 | 26 | 12 |
| % Insecta | 23 | 21 | 24 | 33 | 30 | 13 |
| % ETO | 44 | 47 | 47 | 63 | 47 | 42 |
| % ET | 45 | 48 | 50 | 67 | 50 | 41 |
| % Ephemeroptera | 56 | 58 | 59 | 86 | 70 | 51 |
| % Trichoptera | 59 | 57 | 57 | 72 | 61 | 52 |
| % Odonata | 93 | 93 | 73 | 121 | 103 | 88 |
| % Diptera | 58 | 64 | 36 | 58 | 58 | 37 |
| % Chironomidae | 60 | 63 | 35 | 58 | 58 | 33 |
| % Acari | 88 | 66 | 68 | 106 | 51 | 62 |
| % Amphipoda | 63 | 55 | 53 | 83 | 38 | 21 |
| % Gastropoda | 106 | 61 | 89 | 185 | 44 | 102 |
| % Oligochaeta | 77 | 30 | 64 | 90 | 84 | 62 |
| Taxa richness | 15 | 17 | 4 | 25 | 17 | 5 |
| Scraper richness | 32 | 23 | 20 | 42 | 21 | 16 |
| ETO richness | 23 | 19 | 20 | 31 | 20 | 6 |
| ET richness | 24 | 16 | 28 | 29 | 15 | 8 |
| Ephemeroptera richness | 22 | 11 | 18 | 39 | 35 | 17 |
| Trichoptera richness | 38 | 24 | 59 | 40 | 20 | 5 |
| Odonata richness | 58 | 48 | 9 | 64 | 68 | 19 |
| Diptera richness | 41 | 50 | 25 | 38 | 41 | 31 |
| Gastropoda richness | 47 | 29 | 38 | 46 | 31 | 38 |
| Shannon index | 12 | 8 | 9 | 22 | 12 | 3 |
| Simpson's D | 36 | 21 | 28 | 45 | 41 | 10 |
| Hurlbert's PIE | 7 | 6 | 6 | 19 | 9 | 2 |
| % 1 dominant taxon | 31 | 22 | 29 | 32 | 36 | 7 |
| % 3 dominant taxa | 17 | 8 | 13 | 15 | 19 | 8 |
| % 5 dominant taxa | 12 | 5 | 6 | 9 | 10 | 3 |
| **Mean CV:** | | | | | | |
| Abundance metrics | 112 | 79 | 75 | 155 | 106 | 83 |
| % metrics | 63 | 53 | 54 | 87 | 55 | 47 |
| Richness metrics | 33 | 26 | 25 | 39 | 30 | 16 |
| Diversity metrics | 19 | 12 | 15 | 24 | 21 | 6 |

Table 3.9 Percentage of lakes sampled in Whiteshell and Nopiming Provincial Parks, MB, assessed as impacted using macroinvertebrate metrics from cobble and mud habitat data.

| | % of lakes assessed as impacted | | | | | |
|---|---|---|---|---|---|---|
| | Cobble | | | Mud | | |
| Metric | Lake with >25% developed shoreline (N = 10) | Lakes with cottages (N = 21) | Reference lakes (N = 53) | Lake with >25% developed shoreline (N = 11) | Lakes with cottages (N = 21) | Reference lakes (N = 53) |
| Scraper abundance | 10 | 10 | 4 | 9 | 9 | 4 |
| Insect abundance | 10 | 5 | 4 | 0 | 0 | 4 |
| ETO abundance | 0 | 0 | 4 | 36 | 23 | 4 |
| ET abundance | 0 | 0 | 4 | 36 | 23 | 4 |
| Ephemeroptera abundance | 10 | 5 | 4 | 36 | 18 | 4 |
| Trichoptera abundance | 20 | 19 | 4 | 18 | 18 | 4 |
| Odonata abundance | 20 | 24 | 4 | 18 | 14 | 4 |
| Coleoptera abundance | 0 | 10 | 4 | 36 | 23 | 4 |
| Diptera abundance | 10 | 10 | 4 | 0 | 0 | 4 |
| Acari abundance | 0 | 0 | 4 | 9 | 14 | 4 |
| Amphipoda abundance | 30 | 24 | 4 | 9 | 14 | 4 |
| Gastropoda abundance | 30 | 24 | 4 | 18 | 14 | 4 |
| Hirudinea abundance | 10 | 5 | 4 | 36 | 23 | 4 |
| Oligochaeta abundance | 10 | 10 | 4 | 9 | 14 | 4 |
| % scrapers | 20 | 29 | 2 | 18 | 18 | 4 |
| % Insecta | 40 | 38 | 6 | 18 | 27 | 4 |
| % ETO | 30 | 29 | 6 | 0 | 0 | 4 |
| % ET | 20 | 29 | 6 | 0 | 0 | 6 |
| % Ephemeroptera | 10 | 10 | 6 | 9 | 5 | 6 |
| % Trichoptera | 20 | 33 | 4 | 18 | 9 | 4 |
| % Odonata | 10 | 10 | 6 | 9 | 9 | 4 |
| % Diptera | 50 | 33 | 6 | 0 | 5 | 6 |
| % Chironomidae | 50 | 33 | 4 | 0 | 9 | 6 |
| % Acari | 0 | 0 | 0 | 18 | 14 | 4 |
| % Amphipoda | 40 | 29 | 6 | 9 | 9 | 4 |
| % Gastropoda | 20 | 14 | 4 | 18 | 23 | 6 |
| % Oligochaeta | 10 | 10 | 4 | 9 | 5 | 6 |
| Taxa richness | 0 | 0 | 4 | 9 | 5 | 4 |
| Scraper richness | 0 | 5 | 2 | 0 | 5 | 6 |
| ETO richness | 0 | 0 | 0 | 0 | 0 | 4 |
| ET richness | 0 | 5 | 4 | 0 | 0 | 4 |
| Ephemeroptera richness | 0 | 0 | 0 | 0 | 0 | 0 |
| Trichoptera richness | 0 | 5 | 0 | 0 | 5 | 4 |
| Odonata richness | 10 | 14 | 0 | 0 | 0 | 0 |
| Diptera richness | 0 | 0 | 0 | 0 | 0 | 0 |
| Gastropoda richness | 0 | 0 | 0 | 0 | 0 | 2 |
| Shannon index | 10 | 10 | 4 | 18 | 14 | 6 |
| Simpson's D | 10 | 10 | 4 | 27 | 18 | 6 |
| Hurlbert's PIE | 10 | 10 | 4 | 27 | 18 | 6 |
| % 1 dominant taxon | 0 | 0 | 6 | 9 | 5 | 4 |
| % 3 dominant taxa | 10 | 10 | 6 | 18 | 9 | 4 |
| % 5 dominant taxa | 20 | 14 | 6 | 36 | 27 | 4 |

Table 3.10 Metrics selected for further investigation in cobble and mud habitat types and their response to increased shoreline development for lakes sampled in Whiteshell and Nopiming Provincial Parks, MB.

| **Cobble** | | | **Mud** | |
|---|---|---|---|---|
| **Metric** | **Response to increased shoreline development** | | **Metric** | **Response to increased shoreline development** |
| Hurlbert's PIE | + | | Hurlbert's PIE | + |
| % 5 dominant taxa | + | | % 5 dominant taxa | - |
| % scrapers | + | | % scrapers | + |
| % Insecta | - | | % Insecta | - |
| Gastropoda abundance | + | | % Gastropoda | + |
| % Amphipoda | + | | Amphipoda abundance | + |
| % Chironomidae | - | | % Acari | + |
| % Trichoptera | - | | ETO abundance | + |
| Odonata abundance | + | | Coleoptera abundance | + |
| Odonata richness | + | | Hirudinea abundance | + |

Table 3.11 Regression-based metrics for cobble habitat, and their corresponding correlation with per cent shoreline development and accuracy at detecting impacts in lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. Corresponding $r^2$ and *P* values are listed for each regression and significant correlations with development are followed by an asterisk (*). The predictor variable(s) used to create each regression-based metric are listed. Abbreviations used are: $NO_2$ = nitrite, $NH_4$ = ammonia, and TSI Chl*a* = Trophic State Index for chlorophyll *a* (Carlson 1977).

| Regression-based metric | | | | Spearman correlation with % developed shoreline | % of lakes assessed as impacted | | |
|---|---|---|---|---|---|---|---|
| **Response variable (metric)** | **Predictor variable(s)** | $r^2$ | *P* | | **Lakes with > 25% developed shoreline** | **Lakes with cottages** | **Reference lakes** |
| Hurlbert's PIE | $NO_2$ | 0.202 | 0.001 | 0.00 | 10 | 10 | 2 |
| % 5 dominant taxa | $NO_2$ | 0.186 | 0.001 | 0.10 | 20 | 14 | 2 |
| % scrapers | $NO_2$<br>Surface area | 0.259 | 0.001 | 0.17 | 20 | 29 | 2 |
| % Insecta | Latitude | 0.079 | 0.042 | -0.18 | 30 | 33 | 4 |
| Gastropoda abundance | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| % Amphipoda | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| % Chironomidae | Year | 0.247 | < 0.001 | -0.17 | 30 | 24 | 4 |
| % Trichoptera | TSI Chl*a*<br>Year<br>$NH_4$<br>TSI Chl*a* × year<br>TSI Chl*a* × $NH_4$ | 0.292 | 0.006 | -0.22 | 10 | 14 | 4 |
| Odonata abundance | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Odonata richness | Longitude | 0.197 | 0.001 | 0.09 | 20 | 19 | 4 |

Table 3.12 Regression-based metrics for mud habitat, and their corresponding correlation with per cent shoreline development and accuracy at detecting impacts in lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. Corresponding $r^2$ and *P* values are listed for each regression and significant correlations with development are followed by an asterisk (*). The predictor variable(s) used to create each regression-based metric are listed. Abbreviations used are: $NO_2$ = nitrite, $NH_4$ = ammonia, DIC = dissolved inorganic carbon, and DOC = dissolved organic carbon.

| Regression-based metric | | | | Spearman correlation with % developed shoreline | % of lakes assessed as impacted | | |
|---|---|---|---|---|---|---|---|
| **Response variable (metric)** | **Predictor variable(s)** | ***r*²** | ***P*** | | **Lakes with > 25% developed shoreline** | **Lakes with cottages** | **Reference lakes** |
| Hurlbert's PIE | $NH_4$<br>$NO_2$<br>$NH_4 \times NO_2$ | 0.281 | 0.001 | 0.34* | 0 | 0 | 2 |
| % 5 dominant taxa | $NH_4$ | 0.203 | 0.001 | -0.23 | 18 | 14 | 4 |
| $\text{Log}_{10}$ (% scrapers + 1) | Julian date<br>$NO_2$ | 0.397 | < 0.001 | 0.21 | 9 | 14 | 2 |
| % Insecta | $NH_4$<br>Year<br>DIC<br>Year × DIC | 0.462 | < 0.001 | -0.16 | 18 | 23 | 4 |
| $\text{Log}_{10}$ (% Gastropoda + 1) | Year<br>DIC<br>Year × DIC | 0.266 | 0.002 | 0.31* | 27 | 27 | 2 |
| Amphipoda abundance | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| $\text{Log}_{10}$ (% Acari +1) | Year | 0.233 | < 0.001 | -0.25* | 0 | 0 | 4 |
| $\text{Log}_{10}$ (ETO abundance + 1) | DOC | 0.126 | 0.010 | 0.34* | 36 | 23 | 2 |
| Coleoptera abundance | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Hirudinea abundance | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |

3.13 Multimetric indices created for cobble and mud habitat types and their component metrics for lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. The discrimination of lakes with cottages as impacted is given for each index, along with Spearman correlation with shoreline development. Significant ($P < 0.05$) correlations are followed by an asterisk (*).

| | **Cobble** | **Mud** |
|---|---|---|
| | Hurlbert's PIE | % 5 dominant taxa |
| | % 5 dominant taxa | % scrapers |
| | % scrapers | % Insecta |
| | % Insecta | ETO abundance |
| | % Trichoptera | Coleoptera abundance |
| | % Chironomidae | Hirudinea abundance |
| | % Amphipoda | |
| | Odonata abundance | |
| | Gastropoda abundance | |
| | Odonata richness | |
| **% of lakes assessed as impacted:** | | |
| lakes with > 25 % developed shoreline | 70 | 64 |
| lakes with cottages | 57 | 41 |
| reference lakes | 2 | 4 |
| **Spearman correlation with shoreline development:** | | |
| % developed shoreline | 0.62* | 0.64* |
| cottages per km or shoreline | 0.60* | 0.62* |
| number of cottages | 0.61* | 0.61* |

Table 3.14 Whiteshell and Nopiming Provincial Park, MB lakes designated as impacted using cobble (index score exceeding 0.2) or mud (index score exceeding 0.17) multimetric indices and their relative level of impact based on index score.

| Cobble | | | | Mud | | | |
|---|---|---|---|---|---|---|---|
| **Impacted lakes** | **% developed shoreline** | **Number of cottages** | **Index score** | **Impacted lakes** | **% developed shoreline** | **Number of cottages** | **Index score** |
| Brereton 2008 | 71 | 348 | 0.30 | Brereton 2008 | 71 | 348 | 0.33 |
| Falcon 2007 | 42 | 813 | 0.30 | Falcon 2007 | 42 | 813 | 0.33 |
| West Hawk 2007 | 41 | 525 | 0.30 | | | | |
| Caddy 2008 | 39 | 154 | 0.40 | Caddy 2008 | 39 | 154 | 0.83 |
| | | | | Caddy 2007 | 39 | 154 | 0.33 |
| Star 2007 | 36 | 129 | 0.40 | Star 2007 | 36 | 129 | 0.33 |
| Betula 2008 | 31 | 170 | 0.50 | Betula 2008 | 31 | 170 | 0.33 |
| | | | | Red Rock 2007 | 30 | 123 | 0.50 |
| Florence 2007 | 29 | 30 | 0.30 | | | | |
| Barren 2007 | 20 | 25 | 0.40 | Barren 2007 | 20 | 25 | 0.33 |
| Hunt 2008 | 19 | 8 | 0.60 | | | | |
| Jessica 2007 | 18 | 102 | 0.50 | Jessica 2007 | 18 | 102 | 0.33 |
| White 2008 | 10 | 83 | 0.30 | | | | |
| Nora 2007 | 8 | 20 | 0.30 | | | | |
| Elbow 2008 | 0.2 | 1 | 0.30 | | | | |
| | | | | Camp 2008 | 0 | 0 | 0.33 |
| | | | | Marion 2007 | 0 | 0 | 0.50 |

Table. 3.15 Test lakes assessed as impacted (marked with an x) using metrics, regression-based metrics and multimetric indices calculated using data from cobble sites in Whiteshell and Nopiming Provincial Parks, MB.

| | | **Assessment method** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Metrics** | | | | | | | | | | **Regression-based metrics** | | | | | | | **Index** |
| **Test lake** | % developed shoreline | Hurlbert's PIE | % 5 dominant taxa | % scrapers | % Insecta | Gastropoda abundance | % Amphipoda | % Chironomidae | % Trichoptera | Odonata abundance | Odonata richness | Hurlbert's PIE | % 5 dominant taxa | % scrapers | % Insecta | % Chironomidae | % Trichoptera | Odonata richness | Multimetric |
| Brereton | 71 | | | x | | x | | x | | | | x | | x | | x | | | x |
| Falcon | 42 | | x | x | | | | x | | | | | x | x | | | | | x |
| West Hawk | 41 | x | | | | | | x | | x | | | | | | | | | x |
| Caddy 2007 | 39 | | | | | | | | x | | | | | | | | | | |
| Caddy 2008 | 39 | | | | x | x | x | x | | | | | | | x | x | x | | x |
| Star | 36 | | | | x | x | x | | | | x | | | | | | | x | x |
| Betula | 31 | | | | x | | x | x | x | x | | | | | x | x | | x | x |
| Red Rock 2007 | 30 | | | | | | | | | | | | | | | | | x | |
| Florence 2007 | 29 | | x | | x | | x | | | | | | x | | x | | | | x |
| Florence 2008 | 29 | | | | | | | | | | | | | | | | | | |
| Big Whiteshell | 21 | x | | | | | | | | | | | | | | | | | |
| Barren | 20 | | | x | | | | | x | x | x | | | x | | | | x | x |
| Bird (2007) | 19 | | | | | | | | | | | | | | | | | | |
| Bird (2008) | 19 | | | | | | | x | | | | | | | | x | | | |
| Davidson | 19 | | | | | | | | | | | | | | | | | | |
| Hunt | 19 | | | x | x | x | | x | x | x | | | | x | x | x | x | | x |
| Jessica | 18 | | x | | x | x | x | | | x | | | x | | x | | | | x |
| Flanders | 10 | | | x | | | | | | | | x | | x | | | | | |
| White | 10 | | | | x | | x | | x | | | | | | x | | | | x |
| Nora 2007 | 8 | | | x | | | | | x | | x | | | x | | | | x | x |
| Nora 2008 | 8 | | | | x | | | | x | | | | | | x | | x | | |
| **% of lakes assessed as impacted:** | | | | | | | | | | | | | | | | | | | |
| > 25% developed shoreline | | 10 | 20 | 20 | 40 | 30 | 40 | 50 | 20 | 20 | 10 | 10 | 20 | 20 | 30 | 30 | 10 | 20 | 70 |
| Lakes with cottages | | 10 | 14 | 29 | 38 | 24 | 29 | 33 | 33 | 24 | 14 | 10 | 14 | 29 | 33 | 24 | 14 | 19 | 57 |
| Reference lakes | | 4 | 6 | 2 | 6 | 4 | 6 | 4 | 4 | 4 | 0 | 2 | 2 | 2 | 4 | 4 | 4 | 4 | 2 |

Table. 3.16 Test lakes assessed as impacted (marked with an x) using metrics, regression-based metrics and multimetric indices calculated using data from mud sites in Whiteshell and Nopiming Provincial Parks, MB.

| | | **Assessment method** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Metrics** | | | | | | | | | | **Regression-based metrics** | | | | | | | **Index** |
| **Test lake** | % developed shoreline | Hurlbert's PIE | % 5 dominant taxa | % scrapers | % Insecta | % Gastropoda | Amphipoda abundance | % Acari | ETO abundance | Coleoptera abundance | Hirudinea abundance | Hurlbert's PIE | % 5 dominant taxa | % scrapers | % Insecta | % Gastropoda | % Acari | ETO abundance | Multimetric |
| Brereton | 71 | | | | | | | | x | | x | | | x | | x | | x | x |
| Falcon | 42 | | | | x | | | x | | x | | | | | x | | | | x |
| West Hawk | 41 | x | x | | | | | x | | | | | | | | | | | |
| Caddy 2007 | 39 | | | | | | | | x | x | | | | | | | | x | x |
| Caddy 2008 | 39 | | x | x | | x | | | x | x | x | | x | | | x | | x | x |
| Star | 36 | x | x | x | | x | | | | | | | x | | | | | | x |
| Betula | 31 | | | | x | | x | | | | x | | | | x | x | | | x |
| Red Rock 2007 | 30 | x | x | | | | | | x | x | | | | | | | | x | x |
| Red Rock 2008 | 30 | | | | | | | | | | | | | | | | | | |
| Florence 2007 | 29 | | | | | | | | | | | | | | | | | | |
| Florence 2008 | 29 | | | | | | | | | | x | | | | | | | | |
| Big Whiteshell | 21 | | | | x | x | | x | | | | | | | x | x | | | |
| Barren | 20 | | | x | x | x | | | | | | | | | | x | | | x |
| Bird (2007) | 19 | | | | | | | | | | | | | | | | | | |
| Bird (2008) | 19 | | | x | | | | | | | | | | x | | x | | | |
| Davidson | 19 | | x | | | | | | | | | | x | | | | | | |
| Hunt | 19 | | | | x | x | | | | | | | | | | | | | |
| Jessica | 18 | | | | | | x | | x | x | | | | | | | | x | x |
| Flanders | 10 | | | | | | | | | | x | | | | x | | | | |
| White | 10 | | | | x | | x | | | | | | | | x | | | | |
| Nora 2007 | 8 | x | x | | | | | | | | | | | | | | | | |
| Nora 2008 | 8 | | | | | | | | | | | | | x | | | | | |
| **% of lakes assessed as impacted:** | | | | | | | | | | | | | | | | | | | |
| > 25% developed shoreline | | 27 | 36 | 18 | 18 | 18 | 9 | 18 | 36 | 36 | 36 | 0 | 18 | 9 | 18 | 27 | 0 | 36 | 64 |
| Lakes with cottages | | 18 | 27 | 18 | 27 | 23 | 14 | 14 | 23 | 23 | 23 | 0 | 14 | 14 | 23 | 27 | 0 | 23 | 41 |
| Reference lakes | | 6 | 4 | 4 | 4 | 6 | 4 | 4 | 4 | 4 | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 |

Table 3.17 Assessment precision using metrics calculated with cobble data. Precision is quantified as the percentage agreement of assessments between each possible pair of sites sampled in Barren, Caddy, Red Rock, Star, Bedford, Cabin and Ritchey Lakes, Whiteshell Provincial Park, MB. Abbreviations used are M = metrics and RM = regression-based metrics.

| | **Lake with cottages** | | | | | | | | | | **Reference lakes** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Barren** | | **Caddy** | | **Red Rock** | | **Star** | | **Mean** | | **Bedford** | | **Cabin** | | **Ritchey** | | **Mean** | |
| | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** |
| Hurlbert's PIE | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| % 5 dominant taxa | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 50 | 100 | 88 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | 83 |
| % scrapers | 50 | 50 | 33 | 33 | 100 | 33 | 50 | 50 | 58 | 42 | 100 | 100 | 100 | 100 | 33 | 50 | 78 | 83 |
| % Insecta | 100 | 100 | 100 | 100 | 100 | 100 | 50 | 100 | 88 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Gastropoda abundance | 100 | n.a. | 100 | n.a. | 33 | n.a. | 50 | n.a. | 71 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. |
| % Amphipoda | 33 | n.a. | 100 | n.a. | 100 | n.a. | 50 | n.a. | 71 | n.a. | 50 | n.a. | 100 | n.a. | 100 | n.a. | 83 | n.a. |
| % Chironomidae | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 50 | 100 | 50 | 100 | 67 | 100 |
| % Trichoptera | 33 | 100 | 33 | 100 | 100 | 100 | 50 | 100 | 54 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | 83 | 100 |
| Odonata abundance | 50 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 88 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. |
| Odonata richness | 50 | 50 | 100 | 100 | 100 | 33 | 50 | 50 | 75 | 58 | 100 | 100 | 50 | 33 | 100 | 100 | 83 | 78 |
| **Mean** | 72 | 86 | 87 | 90 | 93 | 81 | 70 | 79 | | | 90 | 93 | 90 | 90 | 88 | 93 | | |
| Multimetric index | 50 | | 100 | | 100 | | 50 | | | | 100 | | 100 | | 33 | | | |

Table 3.18 Assessment precision using metrics calculated with mud data. Precision is quantified as the per cent agreement of assessments between each possible pair of sites sampled in Barren, Caddy, Red Rock, Star, Bedford, Cabin and Ritchey Lakes, Whiteshell Provincial Park, MB. Abbreviations used are M = metrics and RM = regression-based metrics.

| | **Lake with cottages** | | | | | | | | | | **Reference lakes** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Barren** | | **Caddy** | | **Red Rock** | | **Star** | | **Mean** | | **Bedford** | | **Cabin** | | **Ritchey** | | **Mean** | |
| | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** | **M** | **RM** |
| Hurlbert's PIE | 100 | 100 | 100 | 100 | 50 | 100 | 33 | 100 | 71 | 100 | 100 | 100 | 33 | 100 | 50 | 100 | 61 | 100 |
| % 5 dominant taxa | 100 | 100 | 100 | 100 | 50 | 100 | 50 | 50 | 75 | 88 | 100 | 100 | 50 | 50 | 100 | 100 | 83 | 83 |
| % scrapers | 33 | 100 | 50 | 100 | 100 | 100 | 33 | 100 | 54 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| % Insecta | 50 | 100 | 50 | 50 | 100 | 100 | 50 | 100 | 63 | 88 | 50 | 100 | 100 | 100 | 100 | 100 | 83 | 100 |
| % Gastropoda | 33 | 33 | 50 | 50 | 100 | 100 | 50 | 100 | 58 | 71 | 50 | 100 | 100 | 100 | 100 | 100 | 83 | 100 |
| Amphipoda abundance | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. |
| % Acari | 100 | 100 | 100 | 100 | 50 | 100 | 33 | 100 | 71 | 100 | 100 | 100 | 50 | 50 | 100 | 100 | 83 | 83 |
| ETO abundance | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 100 | 100 | 100 | 100 | 50 | 100 | 83 | 100 |
| Coleoptera abundance | 50 | n.a. | 50 | n.a. | 50 | n.a. | 50 | n.a. | 50 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. |
| Hirudinea abundance | 100 | n.a. | 100 | n.a. | 50 | n.a. | 100 | n.a. | 88 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. | 100 | n.a. |
| **Mean** | 72 | 83 | 75 | 79 | 70 | 93 | 55 | 86 | | | 90 | 100 | 83 | 86 | 90 | 100 | | |
| Multimetric index | 33 | | 50 | | 33 | | 50 | | | | 100 | | 100 | | 100 | | | |

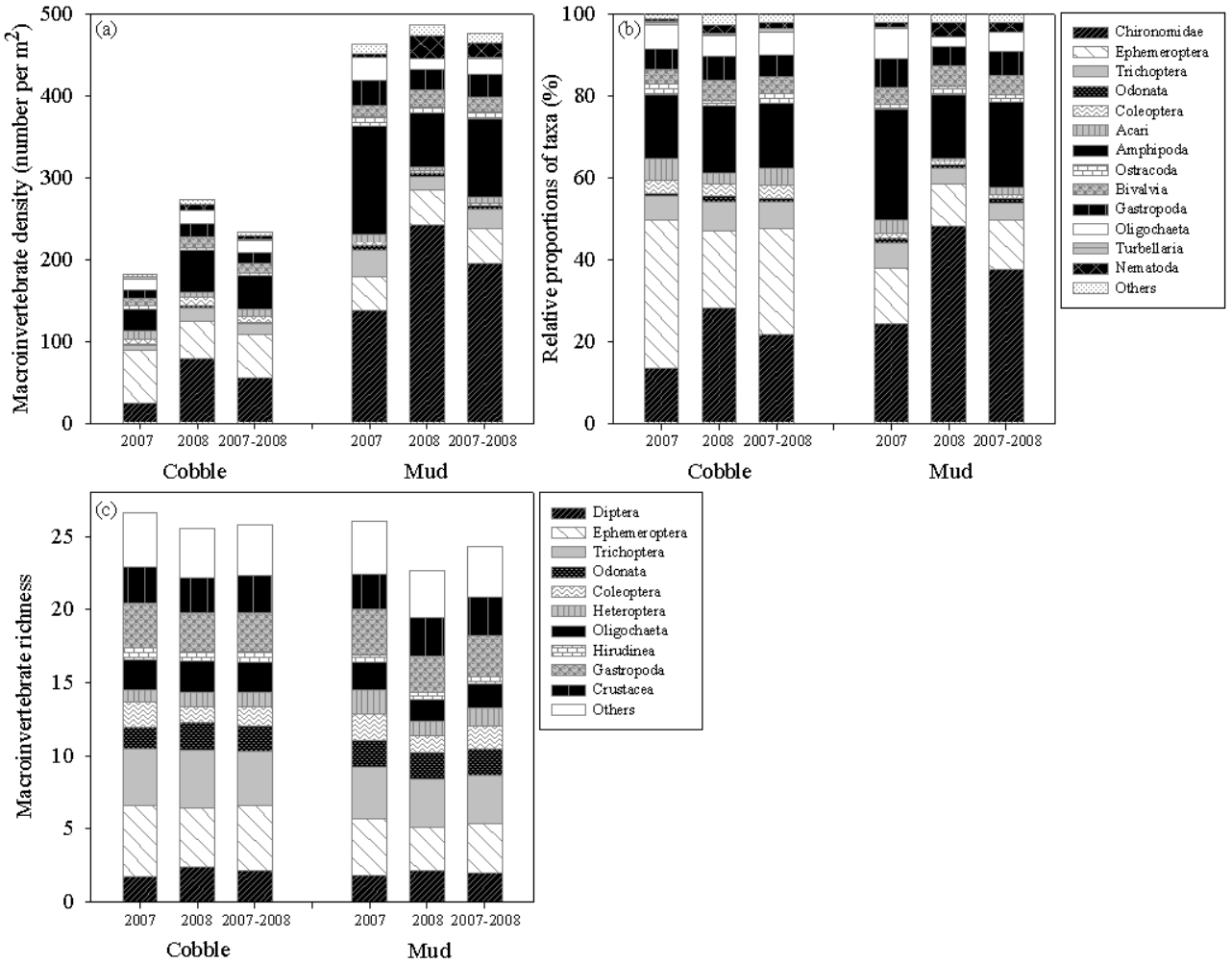


Figure 3.1 Mean density (number per metre squared) (a), relative proportions of taxa (b) and richness (c) of macroinvertebrates in undeveloped boreal shield lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. Benthic data are separated by habitat type (cobble, mud) and sampling year (2007, 2008 and 2007-2008 combined).

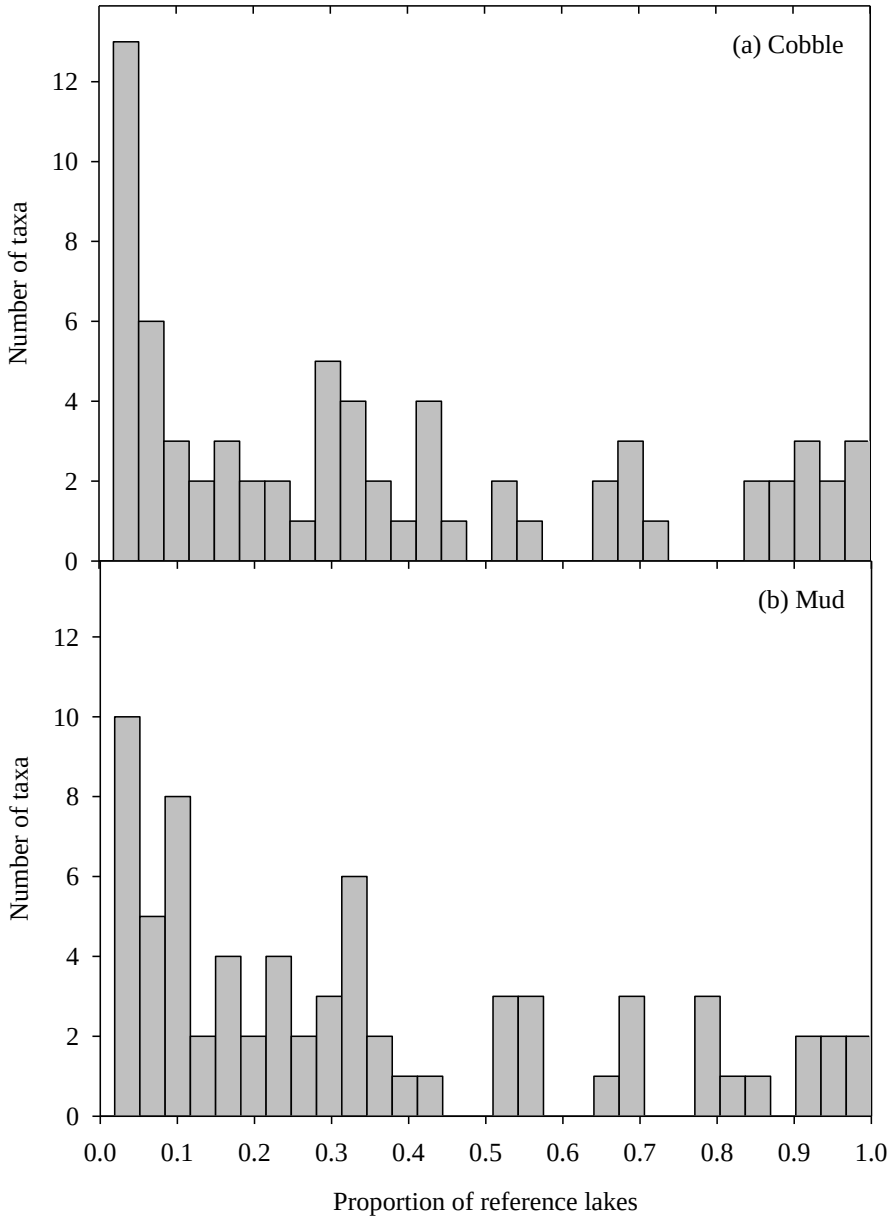


Figure 3.2 Histogram of the number of taxa observed across different proportions of reference lakes in cobble (a) and mud (b) habitats sampled in Whiteshell and Nopiming Provincial Parks, MB.

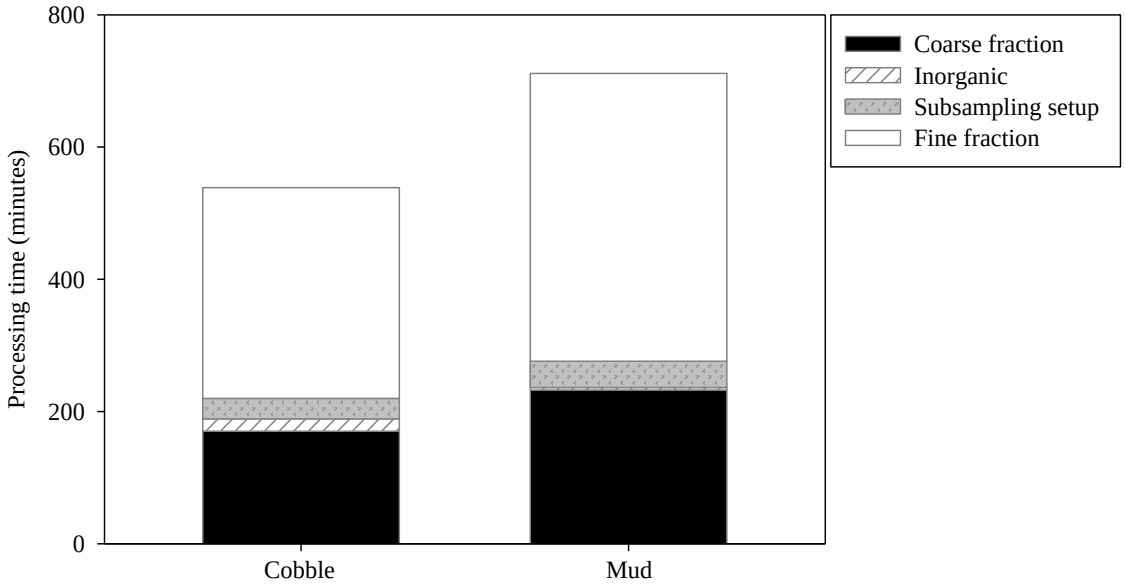


Figure 3.3 Mean time required for one person to process samples collected from 2008 reference lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. 'Coarse fraction' is the time taken to wash, plate and sort coarse material; 'inorganic fraction' is the time take to elutriate inorganic material from organic material, and sort through 20% of the inorganic material without collecting any invertebrates (with the exception of Bivalvia, Gastropoda, and encased Trichoptera); 'subsampling setup' is the time taken to determine the amount of fine material that should be sorted; and 'fine fraction' is the time take to plate and sort a subsample of fine material, perform a large-rare search and identify invertebrates.

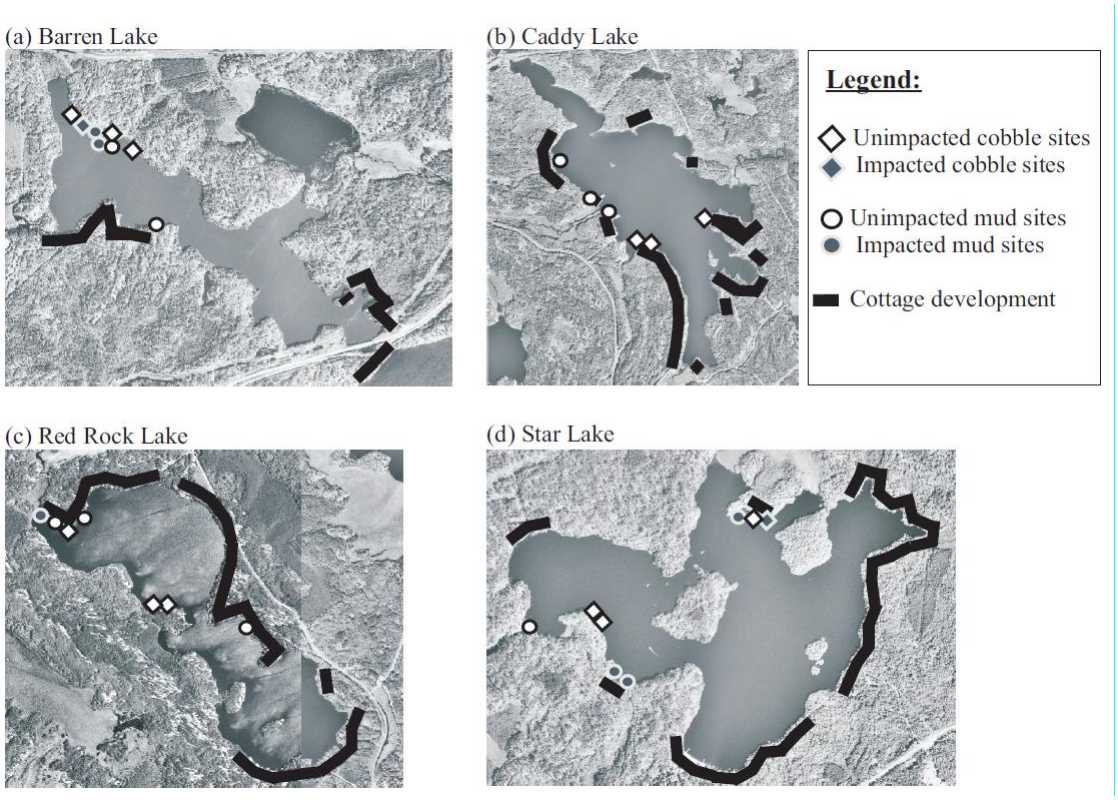


Figure 3.4 Cobble and mud sites sampled in Barren, Caddy, Red Rock and Star Lakes, Whiteshell Provincial Park, MB in 2007. Sites were assessed as unimpacted or impacted using multimetric indices.

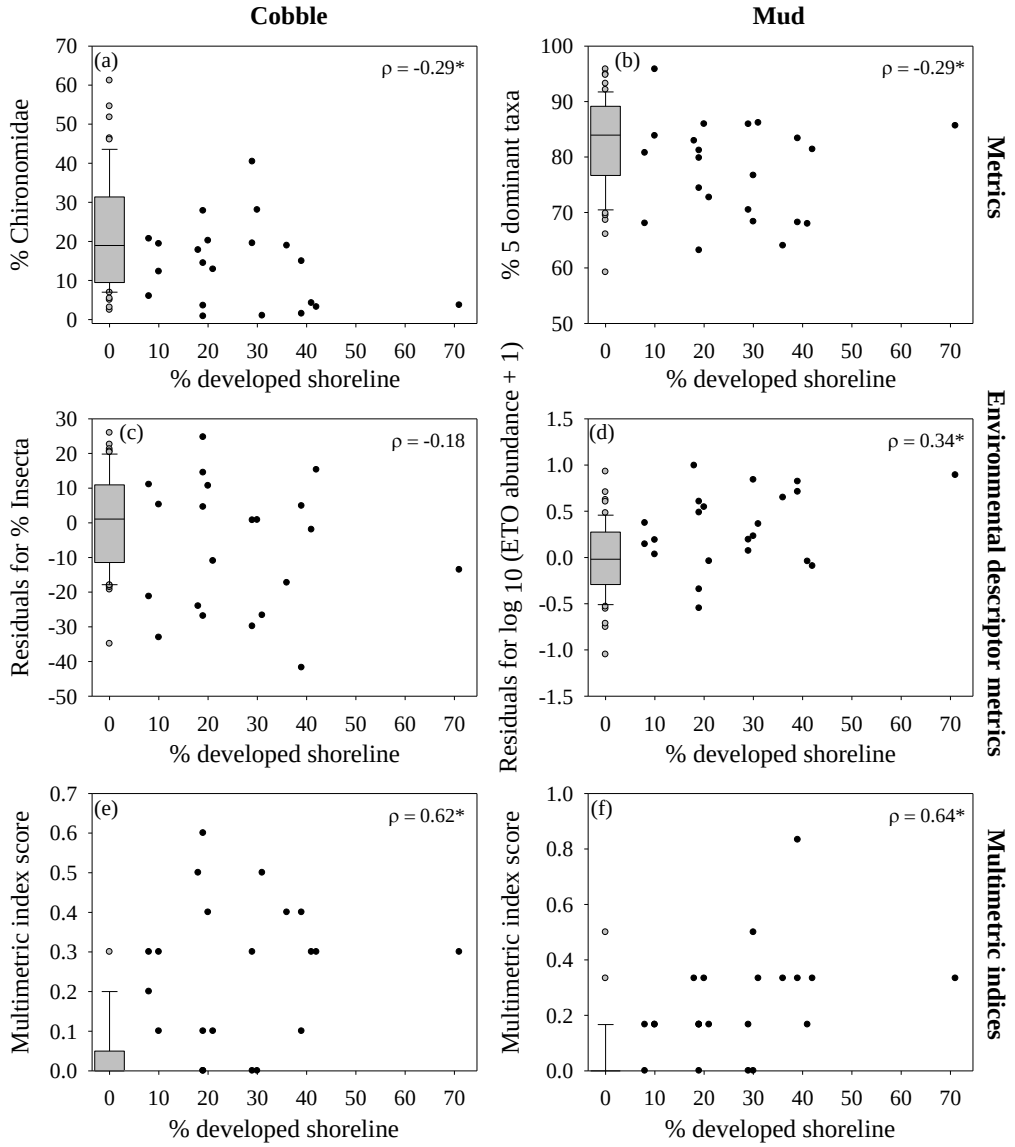


Figure 3.5 Most effective metrics (a and b), regression-based metrics (c and d) and multimetric indices (e and f) for distinguishing between lakes with (black circles) and without (grey boxplots) cottages *versus* per cent developed shoreline in lakes sampled in Whiteshell and Nopiming Provincial Parks, MB. Community measures calculated using cobble (a, c and e) and mud (b, d and f) data are presented for each bioassessment method. Spearman correlation coefficients (ρ) between each measure and per cent developed shoreline are marked on each plot and followed by an asterisk (*) when correlations were significant ($P < 0.05$).

# CHAPTER 4. Summary

## 4.1 Introduction

Freshwater impacts are a significant concern for lotic and lentic systems; however, biomonitoring programs that survey large geographic areas have largely been restricted to streams and rivers. Rapid bioassessment (RBA) protocols can be used to assess water quality across a number of sites at reduced time and cost. Using rapid methods, fewer data are collected per site and the accuracy and precision of impact detection will be reduced, but these methods can be an effective screening tool to identify sites warranting further investigation (David *et al.* 1998; Resh *et al.* 1995). RBA protocols are a valuable tool for water resource managers who need to monitor large areas with limited funding. Unfortunately, RBA methods have had limited application to lakes because there has been less protocol development and testing.

The RBA of lakes has largely been restricted to Canada (Figure 1.2), where protocols have been published by the Ontario Ministry of Environment (OMOE) (David *et al.* 1998) and the Ontario Benthos Biomonitoring Network (OBBN) (Jones *et al.* 2004). These protocols recommend collecting macroinvertebrate kick-samples from the littoral zone and assessing sites using the Reference Condition Approach (Bailey *et al.* 2004). The application of these methods has been relatively limited and appears to have been restricted to the protocols recommended by the OMOE (e.g., David *et al.* 1998); OMOE protocols have been used to monitor lakes impacted by acidification (e.g., Lento *et al.* 2011; Lento *et al.* 2008; Reid *et al.* 1995; Reid *et al.* 1999; Somers *et al.* 1998), urbanization (e.g., Hynes 1998), and metals (e.g., Wesolek *et al.* 2010), as well as for monitoring temporal changes associated with climate change (e.g., Bowman

*et al.* 2008). While there has been some success using these lake RBA methods for monitoring, it is unclear why certain methods were chosen and whether or not the sensitivity of protocols could be improved using alternative methods.

A number of sampling and processing methods recommended by the OMOE could reduce the sensitivity of their RBA method. The OMOE protocol suggests that five sites are to be sampled per lake. Sites are chosen randomly from those available based on the relative proportion of the different habitat types present (e.g., in a lake with 60% of its shoreline occupied by mud, 20% occupied by cobble and 20% occupied by sand, three sites would be sampled in mud, one site would be sampled in cobble and one site would be sampled in sand). Operationally this increases field time because it requires a complete survey of shorelines prior to sampling, which could involve substantial effort if access by motorized vessel is not possible. It is unlikely that all lakes would all have the same proportional habitat composition and it is also possible that the selection of lakes for a particular type of activity, for example recreational development, might be biased towards lakes with a particular character of shorelines. In either case, the method results in comparisons across lakes of samples created by pooling different proportions of habitat types from one lake to the next. Habitat preferences of benthos will influence the composition of the communities sampled and thus the variability in benthic samples that are collected. David *et al*. (1998) also recommend standardizing the size of samples by sampling time, a method that can further increase the variability of benthic samples collected because different micro-habitats and different collectors will require more or less time for effective kick-sampling. Further reductions in assessment sensitivity may be caused by the live-

sorting of samples which can create a bias towards the collection of large, conspicuous invertebrates (Nichols and Norris 2006), the processing of only 100 individuals (i.e., sample data based on collections of fewer than 200 individuals have been considered too variable for assessment) (Doberstein *et al.* 2000), and the identification of many taxa to order or higher (i.e., has not been supported as widely as family-, genus- or species-level identifications).

In the OBBN manual, a range of methods is provided, allowing a customized protocol to be chosen that can meet each monitoring program's financial constraints (Jones *et al.* 2004). The range of methods recommended could create difficulty in the comparison of samples collected by different groups; however, it is unclear how often any of these methods have been used based on the lack of published literature using this protocol.

Development in a watershed can impact lakes and degrade the quality of water. This can have important implications as human development spreads across formerly remote and unimpacted regions, reducing the amount of pristine freshwater resources. Lake assessment protocols that are sensitive and practical should be available so stressors can be identified early before impacts become widespread.

### 4.2 Rationale and objectives

To address the lack of information supporting the design choices of existing RBA protocols for lakes, a new study was undertaken with the main objective of designing a RBA protocol for boreal shield lakes. The Canadian boreal shield is an extensive ecoregion with vast freshwater resources that are being degraded by human

development. Methods for monitoring boreal shield lakes were developed by first reviewing literature to determine which techniques had the most potential to increase the sensitivity of bioassessment and then by testing methods in a survey within Manitoba's boreal shield.

The literature review (Chapter 1) allowed design choices to be evaluated and some protocol decisions to be made. Based on this review, it was determined that macroinvertebrate samples should be collected in fall when macroinvertebrate richness is high and there are fewer logistical considerations for sampling a large number of lakes (i.e., safety concerns in winter, short sampling period in spring, low richness in summer). It was also decided that samples should be collected from the littoral zone using a hand-held net so that the indicator value of macroinvertebrates could be maximized (i.e., the richness of macroinvertebrates is much higher in the littoral *versus* the profundal zone) and so that the sampler used would be versatile across different habitat types. The decisions to wash samples in a 500 µm sieve, to randomly subsample benthic samples until at least 200 macroinvertebrates were processed, and to identify taxa to family instead of genus or species were made to reduce the time required to process samples while minimizing reductions in assessment accuracy.

The literature review (Chapter 1) did not provide enough information for all protocol decisions to be made. The restriction of sampling to one habitat type appeared to be an effective way of reducing the natural variability associated with macroinvertebrate samples; however, it was unclear which habitat type would allow the collection of samples that were more sensitive for the detection of impacts. Standardizing kick-samples to a specific area appeared to be an effective way of

ensuring the majority of macroinvertebrate taxa present could be collected; however, the literature did not allow a sufficient sample area to be determined. Lastly, it was unclear how sites should be assessed based on the literature review. Multimetric metric indices appeared to be a practical choice for the assessment of lakes (i.e., they appeared less complicated and time-intensive for the initial design of protocols in comparison with multivariate methods); however, the specific metrics that should be included in such an index was unclear.

The survey of Malloy Lake (Chapter 2) and the survey of a large group of boreal shield lakes (Chapter 3) were designed to answer the questions that remained concerning kick-sampling and analysis methods.

Questions that were addressed in Chapter 2, the Malloy Lake study, were:

1. How are macroinvertebrate data influenced by the habitat type sampled?
2. Is sample data affected by the collection of benthic samples across the relatively narrow depth range of 0 to 1 m accessed by kick sampling in the littoral zone?
3. What sample area is required to collect all commonly and intermediately occurring macroinvertebrate taxa?

These questions were answered by collecting samples from many small plots placed in different habitats and depths and combining data to allow comparisons to be made for different sample areas. The abundance, relative proportions and richness of macroinvertebrate taxa were compared across three habitats and depths, and significant effects of habitat type and depth on macroinvertebrate taxa were assessed using a two-way ANOVA. The relationship between sample area and the number of taxa collected

was then investigated by creating richness-area curves for commonly, intermediately and rarely occurring macroinvertebrate taxa.

The larger survey of lakes (Chapter 3) incorporated the techniques identified in the literature survey (Chapter 1) and the Malloy Lake study (Chapter 2) and allowed the following questions to be investigated:

1. Can RBA methods be used to detect impacts in boreal shield lakes?
2. Is impact detection facilitated by collecting samples in different habitat types?
3. Does the analysis method used to summarize macroinvertebrate data affect the accuracy and precision of RBA?
4. Does temporal and spatial variation influence the RBA of lakes?
5. How are macroinvertebrate communities affected by cottage impacts?

Questions were addressed by collecting samples from two habitats in 69 lakes, 17 of which had variable levels of cottage development, and then comparing the macroinvertebrate data collected in 2007 and 2008. The natural character of benthic macroinvertebrate communities was defined by the compositions observed across reference lakes, allowing sites to be assessed as impacted when they fell outside of this normal distribution. Within some lakes, additional samples were collected to investigate the spatial variability of assessments, while in other lakes samples were collected in both 2007 and 2008 to investigate temporal changes. Sample processing time and accuracy of assessments were compared between data collected from cobble and mud sediments to determine if one habitat type would be more cost-effective and sensitive than the other for the RBA of lakes. The accuracy and precision of three

analytical methods (metrics, regression-based metrics, multimetric indices) were also compared to determine how the assessment of lakes would be influenced by analysis method. The correlation between macroinvertebrate metrics and development intensity was compared among taxa to determine how benthic community composition is affected by cottages.

## 4.3 Summary of results

### *4.3.1 Malloy Lake study (Chapter 2)*

In my study of Malloy Lake, I found that habitat type, depth and sample area influenced the macroinvertebrate data collected. As expected, macroinvertebrate taxa were present in different abundance, proportion and richness when samples were collected from different habitat types. Somewhat unexpectedly, the collection of samples across the narrow depth range of 0 to 1 m also affected the composition of macroinvertebrate communities. These findings have important implications for the design of a RBA protocol, where sources of variability that are not associated with impacts should be minimized to increase protocol sensitivity. These findings support the stratification of sampling to a specific habitat type, and emphasize the importance of ensuring that field crews expend equal sampling effort across the narrow depth range accessible by kick sampling. Richness-area curves showed that large kick-sample areas (e.g., > 20 $m^2$) were required to collect all of the taxa present. The exclusion of rare taxa was found to reduce the required sample area greatly to approximately 8 $m^2$. This was an important finding as this sample area is much higher than those used in most

RBAs (Figure 1.6) as well as the sample area recommended for the collection of benthos from the littoral zone of German lakes (e.g., Schreiber and Brauns 2010).

### *4.3.2 Testing RBA methods in a large group of lakes (Chapter 3)*

In my larger survey of boreal shield lakes, RBA methods were used to detect impacts from cottages, and the accuracy of impact detection was found to be influenced by the habitat type sampled and the assessment method used. Sampling cobble sediments allowed the collection of macroinvertebrate samples that took significantly less time to process than those collected from mud sediments, and provided data that allowed impacts to be detected more accurately. The benefits of reducing the time needed to complete assessments, as well as increasing the confidence in assessment results, strongly supports the sampling of cobble sediments for the RBA of lakes. Multimetric indices were more accurate for the detection of impacts than metrics or regression-based metrics. This finding supports the analysis of the macroinvertebrate community, in contrast with a single taxon, for the accurate assessment of impacts. The multimetric index designed for cobble-sediment sampling was accurate 70% of the time for the assessment of lakes with over 25% of their shorelines developed with cottages, and 57% of the time for the assessment of lakes with more than one cottage.

## 4.4 Significance and implications

Based on the assessment of most heavily-developed lakes as impacted, it appears that RBA methods can be used to monitor lakes in the boreal shield. This has important implications for water resource managers who are responsible for the quality

of lakes across large geographic regions such as parks. Because traditional assessment methods are too expensive and time consuming to be applied over large areas, the severity of impacts to most lakes has remained unknown. If lakes can be monitored in an affordable way, problems can be identified before impacts become severe and remediation efforts can be assessed.

These findings are particularly promising given the complexity of impacts associated with cottage development. Because multiple stressors of unknown and varying intensity can affect lakes with cottages, predicting how lake ecosystems will be affected is difficult. This has been reflected in the lack of consistent results reported in previous investigations of the impacts of residential development on benthic macroinvertebrates (e.g., Brauns *et al.* 2007b; De Sousa *et al.* 2008; Francis *et al.* 2007; Rosenberger *et al.* 2008) as well as in the variability of response observed in the 17 lakes with cottages that were sampled in this study. It is expected that the RBA protocols used here would have equivalent, if not greater success in the assessment of lakes impacted by one stressor (e.g., nutrient enrichment, sedimentation, salinity).

This study has furthered our understanding of how sampling choices affect the macroinvertebrate data we collect and consequently our ability to detect impacts. Other RBA protocols for lakes (e.g., David *et al.* 1998; Jones *et al.* 2004) have not emphasized the stratification of sampling by habitat type, the use of equal sampling effort across different water depths in the littoral zone or the standardization of sample plots to an area that allows most taxa present to be collected. My research has shown that ignoring these factors could lead to the collection of highly variable communities, making the detection of community changes associated with impacts more difficult to

detect. I believe that by addressing these issues in the design of my RBA protocol, the cost-efficiency of impact detection has been improved.

To my knowledge, this is the largest study on the RBA of lakes. Previous work has been published describing how littoral zone sampling methods can influence impact detection using macroinvertebrate communities (e.g., Schreiber and Brauns 2010; Tolonen and Hämäläinen 2010; White and Irvine 2003), and the development of assessment methods such as multimetric indices in lakes (e.g., Blocksom *et al.* 2002; Lewis *et al.* 2001) and ponds (e.g., Menetrey *et al.* 2011; Solimini *et al.* 2008; Trigal *et al.* 2009); however, my study examines both of these issues in the context of lake RBA. My study also involved the investigation of different processing methods, which can have a large influence on the time required to complete assessments, an important consideration for rapid protocols. My research involved testing protocols across the largest group of lakes yet surveyed and spanned a wider range of morphometric and chemical characteristics than is generally observed in earlier RBA lake surveys (e.g., Hynes 1998; Somers *et al.* 1998; Wesolek *et al.* 2010). Thus, this survey has contributed greatly to the development of lake RBA methods by studying a wide breadth of considerations across a large group of lakes.

### 4.5 Limitations of research

The application of RBA methods does show potential; however, the limitations of this research are observable in the high spatial variability of benthic samples between and within lakes. The community composition of samples collected from lakes with cottages did not show a consistent response to impact in relation to the

communities observed in reference lakes. This may be influenced by the complexity of cottage impacts and different stressors being more prominent in some lakes; however, in lakes where multiple samples were collected, variable results were obtained from sampling sites located in close proximity to one another. Kick-samples collected in cobble sediments from nearby sections of shoreline provided different assessment results in Barren and Star Lakes using a multimetric index (Figure 3.4). Based on these results in appears that there is a significant amount of variability associated with habitat characteristics other than dominant sediment size (e.g., wave exposure, types and biomass of macrophytes, periphyton, sediment embeddedness). Unfortunately, by making the habitat requirements for sampling more specific, it becomes less likely that adequate sites will be present in lakes.

The temporal variability of benthic samples presents another challenge for the application of RBA protocols into a long-term biomonitoring program. Lakes that were sampled in the fall of 2007 and 2008 did not always display consistent results from year to year. In Caddy Lake, all cobble and mud sites sampled in 2007 were assessed as unimpacted, while all of those sampled in 2008 were assessed as impacted. These annual differences can be attributed to different weather patterns, water level fluctuations, or shifts among other members of the aquatic food web (e.g., Bradley and Ormerod 2001; McElravy *et al.* 1989). However, this may present a problem if budgets do not allow many reference lakes to be sampled from year to year. Investigating whether or not older reference lake data can be retained for comparison with test lakes would be a valuable contribution to the protocols used to rapidly assess lakes.

In retrospect, there are other methods that could further reduce the variability of benthic kick-samples and potentially improve the RBA of lakes. These include:

1. sampling a smaller depth range (e.g., sampling to a water depth of 0.5 m *versus* 1 m) to limit the inclusion of sand and mud habitat that typically occurred at the deep end of cobble sites;
2. collecting multiple samples from each lake that are smaller in size and using the mean numbers of invertebrates collected for analysis;
3. characterizing sampling sites with greater detail (e.g., types of macrophytes present, amount of area covered with macrophytes or an estimation of their biomass, wave exposure) and using these data in a multivariate analysis of impacts;
4. collecting samples from an 8 $m^2$ area instead of 10 $m^2$ to reduce the collection of rare taxa as well as the size of samples.

Future studies on the RBA of boreal shield lakes should investigate how these methods affect the cost-efficiency of impact assessment to see if protocols could be improved with their use.

Future studies would also benefit from the investigation of other types of impacts. With so few studies using RBA methods in lakes, it is difficult to know if more consistent results would be obtained when addressing less complex stressors. Using these RBA methods to assess lakes impacted by wild rice harvest, forestry or mining within Whiteshell and Nopiming parks could contribute to our understanding of whether the lack of consistent results had its origin in the complexity of cottage impacts or the shortcuts used in RBA.

### 4.6 RBA protocol for lakes

Based on the findings of my research, I recommend the protocol described in Appendix 2 for the RBA of lakes. The protocol presented in Appendix 2 was designed based on a number of investigations in effort to improve the sensitivity and increase our understanding of lake RBA. To ensure that impact detection remains accurate using this protocol, I recommend at least ten reference lakes are sampled annually to account for temporal changes that may occur within the study area from year to year (e.g., Dillon *et al.* 1997; Lento *et al.* 2008).

It is also advised that a water sample is collected from each lake to aid in the interpretation of impacts. Within this study, sampling just the water chemistry or the benthos of lakes would not have allowed impacts to be detected with as much certainty as they were when these methods were combined. In cases where unexpectedly large impacts were detected in the benthic community (e.g., Hunt Lake), the water chemistry of lakes improved my understanding of why such changes were observed. The increased time and cost of including water sampling in a RBA protocol is negligible in comparison to the value it could add to an assessment.

For a better understanding of how cottages or other stressors affect lakes, it is recommended that whenever possible, RBA monitoring begins prior to development. The wide range of communities observed among reference lakes in this study highlights the possibility of lakes being incorrectly assessed as impacted if they possess a naturally distinct community. Knowing the community composition of benthic macroinvertebrates prior to development could ameliorate our understanding of the different ways that lakes can be affected by impact.

## 4.7 Conclusion

The methods developed and used in this RBA study show potential for biomonitoring lakes across large geographic areas. These methods were effective as a screening tool to identify lakes impacted by cottage development in the boreal shield and it is expected that the effectiveness of these methods may be improved when less complicated stressors are investigated. Further research on method development is recommended; however, based on the success rate observed here (70% of lakes with over 25% of their shorelines occupied with cottages assessed as impacted), there is good potential for water resource managers to use these protocols in long term biomonitoring programs.

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**APPENDIX 2:**

# A rapid bioassessment protocol for boreal shield lakes

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**August 2012**

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## INTRODUCTION

The rapid bioassessment (RBA) protocols outlined here were developed in a study of Manitoba's boreal shield lakes and can be used to rapidly assess impacts within lentic environments (Hynes 2012). Focus has been placed on the collection of kick-samples from one habitat type (cobble dominated sediments), using an equal amount of sampling effort across different water depths within the littoral zone, standardizing sample size by area and reducing processing time by subsampling and identifying macroinvertebrates to family instead of genus or species. These RBA protocols can be used to detect impacts to lakes by comparing the community composition of macroinvertebrates observed at a test site to the distributions observed across a group of reference (unimpacted or minimally impacted) lakes.

### *Rapid bioassessment (RBA)*

RBA protocols allow impacts to be monitored at reduced time and costs across large geographic regions. Time can be saved in the field and in the lab by collecting fewer samples, subsampling and identifying macroinvertebrates to a lower level of taxonomic resolution. RBA methods are sensitive enough to detect impacts; however, they are intended to be used as a screening tool to identify sites that require further investigation (David *et al.* 1998; Resh *et al.* 1995).

The use of RBA techniques has increased in popularity over the past few decades; however, the majority of RBA protocols have been designed for and have been used in streams or rivers. RBA research has been especially prominent in the United States, Australia and Europe where regional stream biomonitoring programs

have been developed and are run by government agencies (e.g., Barbour *et al.* 1999; Turak *et al.* 2004; Wright 2000). These RBA programs have allowed more streams to be monitored across wide geographic regions. Unfortunately, the development of equivalent protocols for lakes has been less prominent.

The protocols described here were designed based on existing RBA methods. Their development involved a literature review of methods that have been successfully used to rapidly assess freshwater sites, a sampling survey to investigate the influence of habitat type, water depth and sample area on the composition of benthic macroinvertebrate samples, and testing and refining protocols across a large group of lakes (Hynes 2012). These methods differ from other RBA protocols designed for lakes (e.g., David *et al.* 1998; Jones *et al.* 2004) by stratifying sampling by habitat type, standardizing sample size by area and emphasizing the use of equal sampling effort across different depths in the shallow littoral zone. The methods were selected to minimize sample variability and theoretically increase the sensitivity of assessments as a result.

*Benthic macroinvertebrate biomonitoring*

Benthic macroinvertebrate communities were selected for this, and the majority of other freshwater biomonitoring programs (Abel 1989; Rosenberg and Resh 1993), because they are a diverse assemblage with attributes ideally suited for the detection of impacts. These attributes include:

- adaptation to most freshwater habitats (Lenat *et al.* 1980);

- high diversity – a variety of species that respond to impacts in various ways and in different degrees (Abel 1989; Hellawell 1986);
- large literature base (e.g., taxonomic keys, tolerance levels, habitat requirements) (Abel 1989; Hellawell 1986);
- easily sampled - abundant and have small body size (Abel 1989; Lenat *et al.* 1980);
- sedentary – allowing the spatial extent of impacts to be defined (Abel 1989; Rosenberg and Resh 1993);
- lifespan – long enough for individuals to be affected by exposure to contaminants or pollution (Abel 1989; Reice and Wohlenberg 1993), and short enough for changes in community composition to be observed (Rosenberg and Resh 1993);
- proximity to sediments – increased exposure to certain contaminant through extended contact (Reice and Wohlenberg 1993).

More specifically, benthic macroinvertebrates inhabiting the littoral zone are the assemblage of interest for this RBA protocol. The richness of benthic macroinvertebrate communities is much higher in the littoral zone, in contrast with the profundal zone, and by collecting samples with more taxa, we increase the utility of benthic macroinvertebrates as indicators of impairment (Donohue *et al.* 2009). Littoral zone macroinvertebrates have been used for the bioassessment (e.g., Donohue *et al.* 2009; Tolonen and Hämäläinen 2010) and RBA of lakes (e.g., Hynes 2012; Somers *et al.* 1998; Wesolek *et al.* 2010).

*Reference lake selection*

A group of reference (unimpacted) lakes should be sampled within the study region to allow for the assessment of test sites. Unimpacted, or more often minimally impacted, lakes are sampled so that the natural range of benthic communities can be characterized. Macroinvertebrate metrics will be calculated using data from reference lakes, and the range of their scores are then used to define the reference condition (i.e., the range of metric scores than occur naturally at unimpacted sites). A minimum of 20 reference sites should be sampled to adequately describe the natural range of community composition observed for macroinvertebrates; however, 30 to 50 sites is preferable and it is recommended that as many unimpacted lakes are sampled per season as possible to improve assessment accuracy (Bowman and Somers 2005; Reynoldson and Wright 2000).

For long-term RBA programs, it is recommended that a set of reference lakes are sampled each year to assess temporal changes that may occur from year to year. Weather, water-level fluctuations or shifts in other biological communities can all influence benthos from year to year (e.g., Bradley and Ormerod 2001; McElravy *et al.* 1989).

*When to sample*

To minimize the effects of temporal variability, it is recommended that all samples are collected within as short of a time frame as possible, in one season. Fall is recommended for sampling because macroinvertebrate richness is high at this time (i.e., in comparison with summer) and there are fewer logistical issues in comparison with

sampling in spring (e.g., short time frame for sampling after ice-off and the emergence of aquatic insects) and winter (e.g., safety concerns with winter sampling and the need for more equipment) (Jones *et al.* 2004). Temporal variability is influenced by the emergence of aquatic insects, food supply, the feeding habits and abundance of predators, and weather patterns; however, previous work has shown that consistent samples can be collected over a period of three to four weeks (e.g., Hose *et al.* 2004; Reid *et al.* 1995).

*Important considerations to note before using this protocol*

The methods discussed here have for the most part been verified through field testing (Hynes 2012); however, this manual includes a few modifications that are expected to improve the protocol but have yet to be tested in lakes. Modifications that have been made from the original methods are:

- the depth range sampled in the littoral zone has been changed from 0 to 1 m to 0 to 50 cm. This change was made to reduce the spatial variability that is expected to be caused by the transition of cobble sediments to sand or mud at depths beyond 50 cm.
- the total area sampled has been reduced from 10 $m^2$ to 8 $m^2$. This is an attempt to reduce the time needed to process the coarse material collected in samples, while still allowing all commonly and moderately occurring taxa to be collected.
- one-sided boundaries for metrics were originally used – this has been modified to the used of two-sided boundaries (i.e., metric scores that fall both below and

above the 5th and 95th reference-lake percentiles, respectively, are now considered impacted). This modification was made to increase the versatility of methods across a variety of impacts.

## FIELD METHODS

The methods outlined here should be used within lakes that are suspected to be impacted by human development or that are of interest for long-term monitoring (test lakes), as well as within a group of reference lakes. Effort should be taken to be as consistent as possible across sampling sites and whenever possible one person should be responsible for all kick-sampling to minimize the variability that could be associated with different collectors.

*List of recommended field equipment*

- Water sampling
  - Acid-washed bottles made of high density plastic (e.g., polyethylene) for the collection of a water sample
- Measuring and marking sampling sites
  - Tape measure
  - Flagging tape
  - GPS unit (to record site location)
  - Gravelometer
  - Camera (optional)
- Macroinvertebrate sampling
  - D-net with a 500 µm mesh
  - Waders and wader boots
- Storing and preserving macroinvertebrate samples
  - Squirt bottle
  - Formaldehyde
  - Formaldehyde spill kit
  - Borax
  - Latex gloves
  - Safety glasses
  - Sample jars or bags

- Electric tape (e.g., black for sealing sample jars and coloured for labelling jars)
- Labels
- Field book
- Pencils
- Markers

*Collection of water sample*

A water sample should be collected from the epilimnion of each lake for the assessment of nutrient and ion concentrations. This can be very useful for the interpretation of impacts and will not significantly increase the time and costs required for assessment. The water sample should be collected from above the deepest area of the lake (if known) or near the lake center. Rinse the sample bottle twice in lake water before filling it approximately 30 cm below the lake-water surface. Keep the sample bottle cool and bring it back to the lab for analysis as soon as possible. The concentrations of nutrients and major ions can be assessed using methods described by Stainton *et al.* (1977).

*Site selection*

To minimize sources of natural variability on the benthic samples collected, effort should be taken to locate sites that are relatively similar across lakes. This requires sampling habitats with a consistent grain-size range because macroinvertebrate taxa have been observed to prefer certain sediments over others (e.g., Doeg *et al.* 1989; Minshall 1984; Wieser 1959; Williams and Mundie 1978). It is also recommended that sections of shoreline adjacent to stream inflows be avoided because these areas may be

affected by the influxes of sediment and organic matter to a greater degree than other regions (e.g., Hilton *et al.* 1986; Rau 1976)

A sampling site should be selected from a rocky section of the lakeshore where cobble and larger gravel (20-256 mm) sediments are dominant. Sediment size should be verified using a gravelometer (i.e., a handheld size analyzer for rocks) to ensure that the correct habitat type is sampled. Sites can be characterized by the dominant grain size present from the land-water interface to a water depth of approximately 50 cm. Beyond this depth, grain size along shorelines tend to decrease to sand or silt. This sediment type is recommended because it is common in the boreal shield, it supports a diverse macroinvertebrate community and it has been found to facilitate the detection of impacts in comparison with other sediment types (e.g., Donohue *et al.* 2009; Hynes 2012; Tolonen and Hämäläinen 2010; Tolonen *et al.* 2001).

The site that is sampled should be standardized to an area of 8 $m^2$ to allow all commonly and intermediately occurring taxa to be collected (Hynes 2012). Sample plots should be defined by measuring the perpendicular distance, or length of the plot ($l$), from the shoreline to a depth of 50 cm using a tape measure held along the lake bottom. This distance can then be used to determine the plot's width ($w$): $w = 8\ m^2 / l$. Plot edges should be marked using flags and the entire 8 $m^2$ area within them should be kick-sampled (Figure 1).

*Site description*

The percentage of bedrock, boulder, cobble, pebble, sand and silt, and the percentage of macrophyte coverage should be recorded for each site, before it is

disturbed by kick-sampling. Strict definitions of substrate sizes must be available for field crews along with a gravelometer for verification when sites are described. The size distribution of rocks and the amount of macrophyte growth can be estimated by eye and recorded along with any other notable site characteristics. It is also recommended that a simple sketch of the plot's dimensions and any of its notable features are recorded in a field book. A photo of each site and the adjacent riparian zone can also be taken; however, this is not required.

*Macroinvertebrate sampling*

Macroinvertebrates should be sampled using a D-net with a 500 μm mesh size and a traveling kick and sweep method (Figure 2) (e.g., David *et al.* 1998; Jones *et al.* 2004). When macrophytes are present in a sampling plot, the D-net should be swept through them before the area is kick-sampled, to maximize the collection of fast swimming invertebrates. Kick-sampling should continue until it is believed that all areas of the plot have been thoroughly covered (i.e., more time should be spent sampling difficult microhabitats such as crevices, reeds, logs, etc.). Precautions should also be taken to ensure that the collection of macroinvertebrates is unbiased; this requires that an equal amount of effort is used to kick-sample the shallow and deep regions of each plot. This is important because the distribution and density of certain invertebrate taxa vary by depth, even within the shallow depth range of 0 to 50 cm (Hynes 2012). Some of these differences that occur with depth could result in the misidentification of lakes as impacted or unimpacted, diligence sampling sites in a standardized way needs to be maintained. Constant movement of the net is required

when kick-sampling lentic habitats to ensure that all of the macroinvertebrates that are collected cannot swim out of the net.

*Washing samples in the field*

To reduce the amount of silt collected, samples can be washed in the field within the D-net. This is done by dunking the net (containing sample material) repeatedly, using caution to not lose any organisms or add new ones to the sample. Samples should be washed until the water draining from the net is clear.

*Preserving samples in formalin*

Samples should be preserved in a 10% formalin solution after they have been transferred to jars (or bags) for storage. Transferring sample material to jars is facilitated by concentrating the sample into the base of the net, and then using your hands to move the material to the sample jar. A squirt bottle filled with lake water can be used to dislodge any sample material that remains in the net into the sample jar. In a field situation, in the absence of any measuring equipment, an approximate 10% formalin solution is made by first assessing the total volume of the sample, and then adding enough formalin to cover approximately 10% of this total sample volume. Water is then added to fill the sample jar and dilute the formalin to a 10% formalin solution. It is recommended that latex gloves and safety glasses are worn whenever handling formalin. A few spoonfuls of borax should be added to each sample jar to buffer the solution. Sample jar lids should be tightened as much as possible and sealed

with a few layers of electric tape to prevent any formalin leaks. Proper care should be taken when working with formalin, including:

- keeping formalin in its original container and ensure the proper safety labels are present;
- containers should always be sealed tightly to prevent formaldehyde gas inhalation;
- formalin should be securely stored for transport in vehicles to prevent spills;
- formalin is highly flammable and should be kept away from heat or possible ignition sources.

## LABORATORY METHODS

*List of recommended lab equipment*

- Transferring samples from formalin to ethanol
    - 95% ethanol
    - formaldehyde neutralizer (e.g., Polyform-F)
    - 500 µm sieve
    - Latex gloves
    - Safety glasses
- Washing samples
    - Sieves of various sizes (e.g., 500 µm to 2cm)
- Elutriation of samples
    - 500 µm sieve
    - White pan
    - Spoon
    - Gridded sorting dishes
- Subsampling
    - Gridded subsampling frame (Figure 3) (e.g., Moulton *et al.* 2000)
    - Stereomicroscope
    - Gridded sorting dishes
- Identification of macroinvertebrates
    - Various taxonomic keys, for example:
        - Insects (Merritt *et al.* 2008)
        - Ephemeroptera (Edmunds *et al.* 1976)

- Trichoptera (Wiggins 1996)
- Oligochaeta (Kathman and Brinkhurst 1998)
- Mollusca (Clarke 1981)
- Non-insect invertebrates (Thorp and Covich 2001)

*Washing samples in the lab*

Samples should be re-washed in the laboratory to ensure all (or most) of the fine silt and formalin have been removed (i.e., water running out of sieves should be clear). Formalin should first be drained from samples in a well-ventilated room under a fume hood and neutralized before it is discarded. Thoroughly washing the sample will remove most of the formalin, which is toxic when inhaled, as well as reduce the time required to process samples by discarding fine material that is not of interest.

*Transferring samples to ethanol*

After washing, sample material should be stored in jars filled with 95% ethanol, which should be replaced with 70% ethanol approximately one week later. This is done because formalin is a good invertebrate fixative but will damage specimens after extended storage (i.e., will make them brittle) (Giere 2009). The second concentration of ethanol (70%) is recommended because the organic sample material will contain enough water to dilute the first addition of ethanol.

*Elutriation of samples*

When samples contain a large amount of inorganic material (e.g., sand, gravel), elutriation is recommended to reduce processing time. Sample material should be placed in a white pan with water, and gently agitated or circulated until a vortex is

created. This allows lighter organic material to be raised into the water column, while the heavier inorganic material remains on the bottom of the pan. This organic material should then be poured into a 500 µm sieve, leaving the heavier material behind. Repeat this process until only heavier-inorganic material remains in the pan. Approximately twenty per cent of the inorganic material should then be randomly selected and sorted within gridded sorting trays using a stereomicroscope. If any organisms are found, with the exception of molluscs and encased caddisfly larvae, the sample should be re-elutriated. If no organisms are found, a quick scan of the remaining inorganic material can be performed without magnification for the collection of any remaining molluscs or caddisflies. Invertebrates collected during this process should be added to the organic fraction of the sample before subsampling.

*Random subsampling*

To further reduce sample processing time, all samples should be randomly subsampled until at least 300 benthic invertebrates have been collected using methods such as those recommended by the USGS (Moulton *et al.* 2000). The USGS recommends randomly sorting a known proportion of sample material that has been divided by area in a gridded frame (Figure 3). To ensure that estimates of invertebrate density can be made for the entire sample, the proportion of sample processed must always be recorded. The number of invertebrates collected during subsampling should be divided by the proportion of sample that was processed for all taxa prior to data analysis; this will provide an estimate of total sample abundance.

After sorting subsamples, a search for large-rare specimens should be performed on the unprocessed portion of the sample by scanning the remaining material by eye (Moulton *et al.* 2000). Any large macroinvertebrates that were rare or absent in the sorted subsample should be collected. This search provides a more accurate measure of richness, as some taxa are missed when only a portion of the sample is sorted. The numbers of all large-rare specimens that are collected should be added to the estimates of abundance made from processed subsamples.

*Identification of macroinvertebrates*

Macroinvertebrates should primarily be identified to family level; however, when identification is more difficult, taxa may be identified at a lower resolution. Family is considered adequate for this rapid protocol because it is sufficient for the rapid assessment of streams (e.g., Chessman *et al.* 2007; Hilsenhoff 1988; Metzeling *et al.* 2006) and it can greatly reduce the time and costs associated with macroinvertebrate assessments (Jones 2008). Taxonomic keys that are recommended for the identification of macroinvertebrates are Merritt *et al.* (2008) for insects in general, Edmunds *et al.* (1976) for Ephemeroptera, Wiggins (1996) for Trichoptera, Kathman and Brinkhurst (1998) for Oligochaeta, Clarke (1981) for Mollusca, and Thorp and Covich (2001) for other non-insect invertebrates.

*Quality assurance / quality control (QA/QC)*

To ensure samples are sorted and identified properly, some processed portions of samples should be reprocessed with identifications verified by someone other than

the original sorter. The first ten samples processed by new sorters should be verified, followed by a randomly-chosen 10% of subsequent samples. This can be done by recording each sample that was processed by an individual sorter in the order that they were processed and then dividing these into blocks of ten. One sample should be randomly selected from each block of ten and resorted to see how many organisms were missed. Sorting efficiencies falling beneath 95% are considered a fail, and require the original sorter to reprocess the remaining nine samples within that block of ten. The identification of representatives of each taxon should be verified by an expert.

## ANALYSIS METHODS

### *Data summarization by site*

To summarize sample data, macroinvertebrate abundance estimates should first be used to calculate a variety of community metrics for each of the sites sampled. I recommend calculating metrics that measure different aspects of community composition (i.e., relative proportions, richness, diversity) that are not overly redundant with one another (i.e., calculating both ETO (Ephemeroptera, Trichoptera, Odonata) abundance and Ephemeroptera abundance is not recommended). I would also recommend that the chosen metrics are based on commonly occurring taxa in the study area. Within Manitoba's boreal shield, Hurlbert's PIE, % 5 dominant taxa, % scrapers, % Insecta, % Trichoptera, % Chironomidae, % Amphipoda, Odonata abundance, Gastropoda abundance and Odonata richness were found to be the most effective suite of metrics for the detection of lake impacts (Hynes 2012).

To allow test sites to be assessed, the metric scores from reference lakes should be used to calculate confidence boundaries. This can be done by bootstrapping the scores for each metric using resampling software (e.g., StatTools 5.5, Palisade Corporation) and then calculating the $5^{th}$ and $95^{th}$ percentiles from the bootstrapped distribution. The $5^{th}$ and $95^{th}$ percentiles can then be used as boundaries; metric scores falling below the $5^{th}$ percentile score or above the $95^{th}$ percentile score would be considered impacted or not within the reference distribution (Figure 4).

*Calculating multimetric index scores*

The reference lake confidence boundaries that were calculated for each metric should next be used to condense metric data into a unitless score as a multimetric index. This can be done using a simple scoring method where a 1 is assigned to every metric score that falls below or above the $5^{th}$ and $95^{th}$ percentiles, respectively. Metric scores that fall within the $5^{th}$ and $95^{th}$ percentiles are assigned a score of 0. The multimetric index score for each site is then calculated by summing the scores (of 1 or 0) from each metric and dividing this sum by the total number of metrics (Hering *et al.* 2006).

*Comparing test sites to the reference distribution*

A test site can now be assessed as impacted or in reference condition based on its multimetric index score. Using the $95^{th}$ percentile of all reference site multimetric index scores as an assessment boundary, sites with index scores falling above the $95^{th}$

percentile score would be considered impacted. Sites with higher multimetric index scores are expected to be more severely impacted by anthropogenic stressors.

**FIGURES**

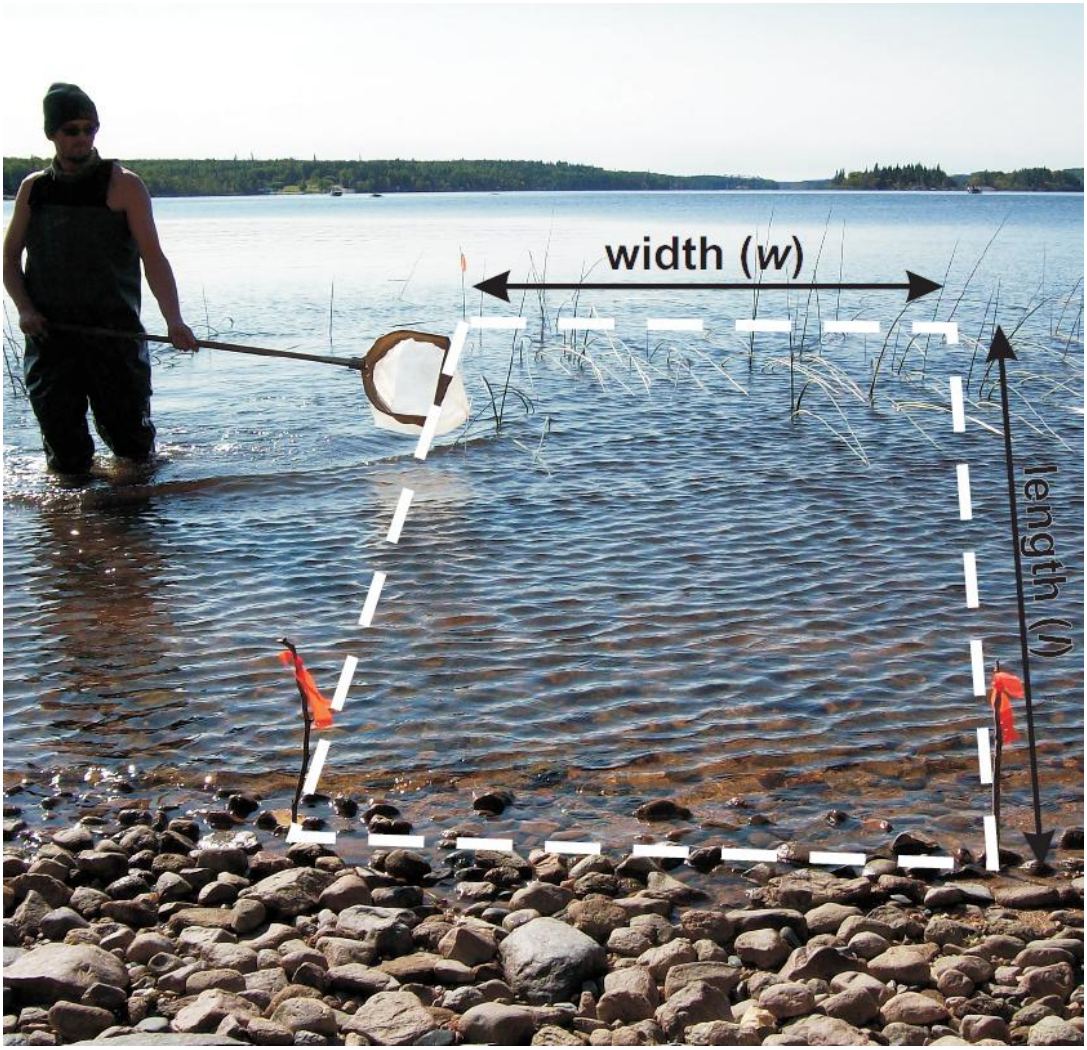


Figure 1  Layout of 8 m² kick-sampling plot. The length of the plot extends to a water depth of 50 cm and the width of the plot is determined by dividing 8 m² by the plot's length. Photo taken by J. Drysdale.

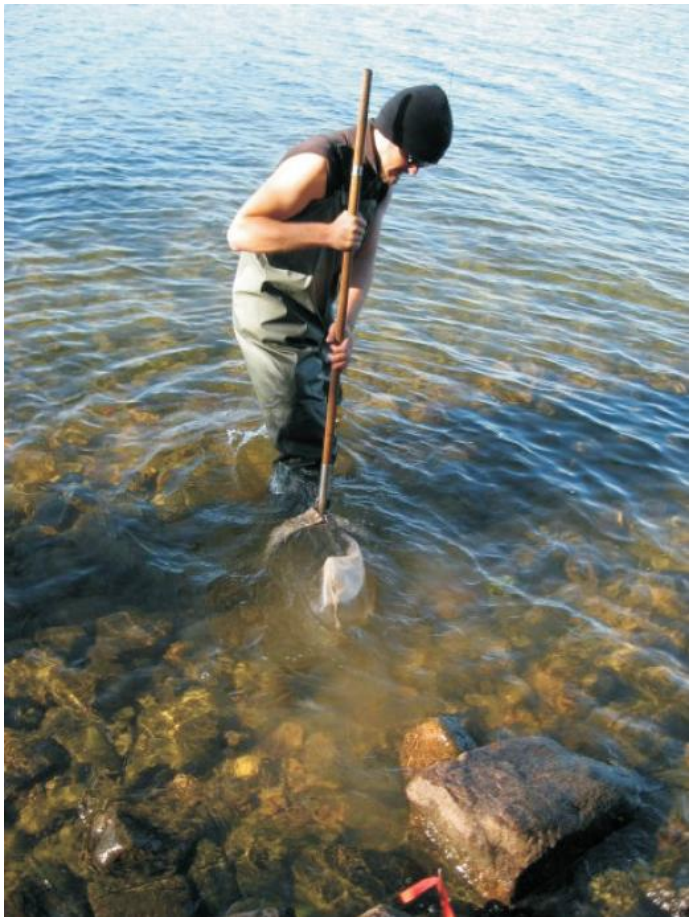

Figure 2  Kick-sampling along a cobble shoreline. Photo taken by J. Drysdale.

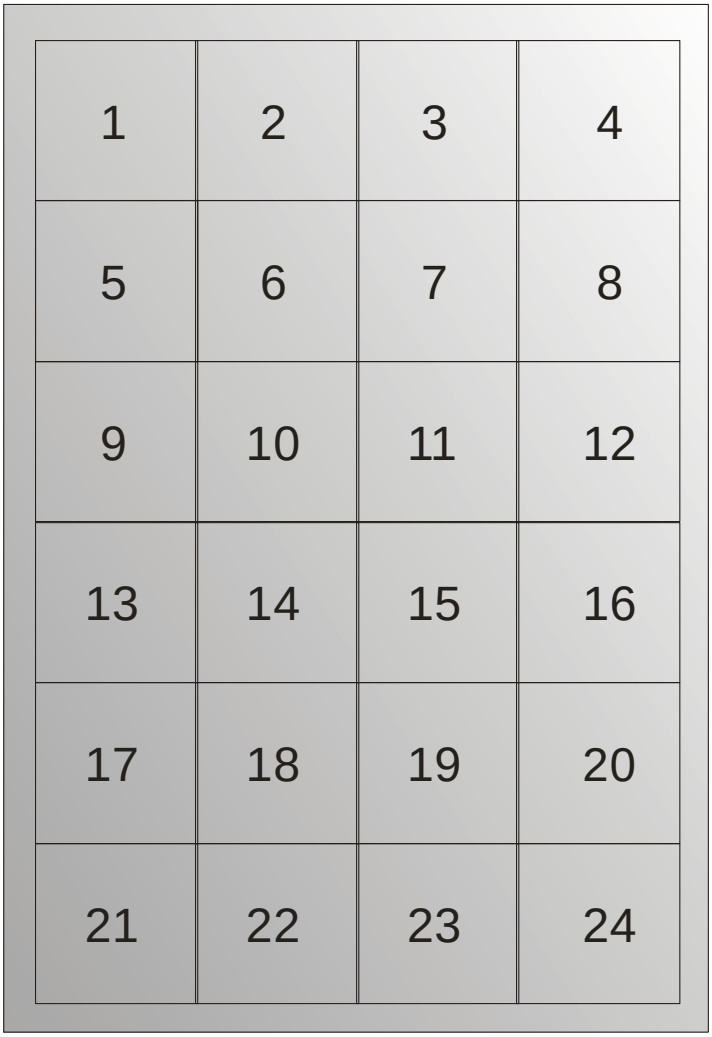


Figure 3 Example of gridded subsampling frame that can be used to divide sample material by area (e.g., Moulton *et al.* 2000). The outer walls of the frame can be made out of plastic and should be tall enough to prevent any sample material from being lost. The base of the frame should be covered in fine mesh (e.g., ≤ 200 µm) to prevent sample material from becoming caught within it and to allow water to escape. The grid can be made using thin metals rods. A waterproof epoxy should be used to ensure the mesh base is held firmly in place and will not allow any sample material to be lost. Grids should be sorted in the order that they are randomly selected.

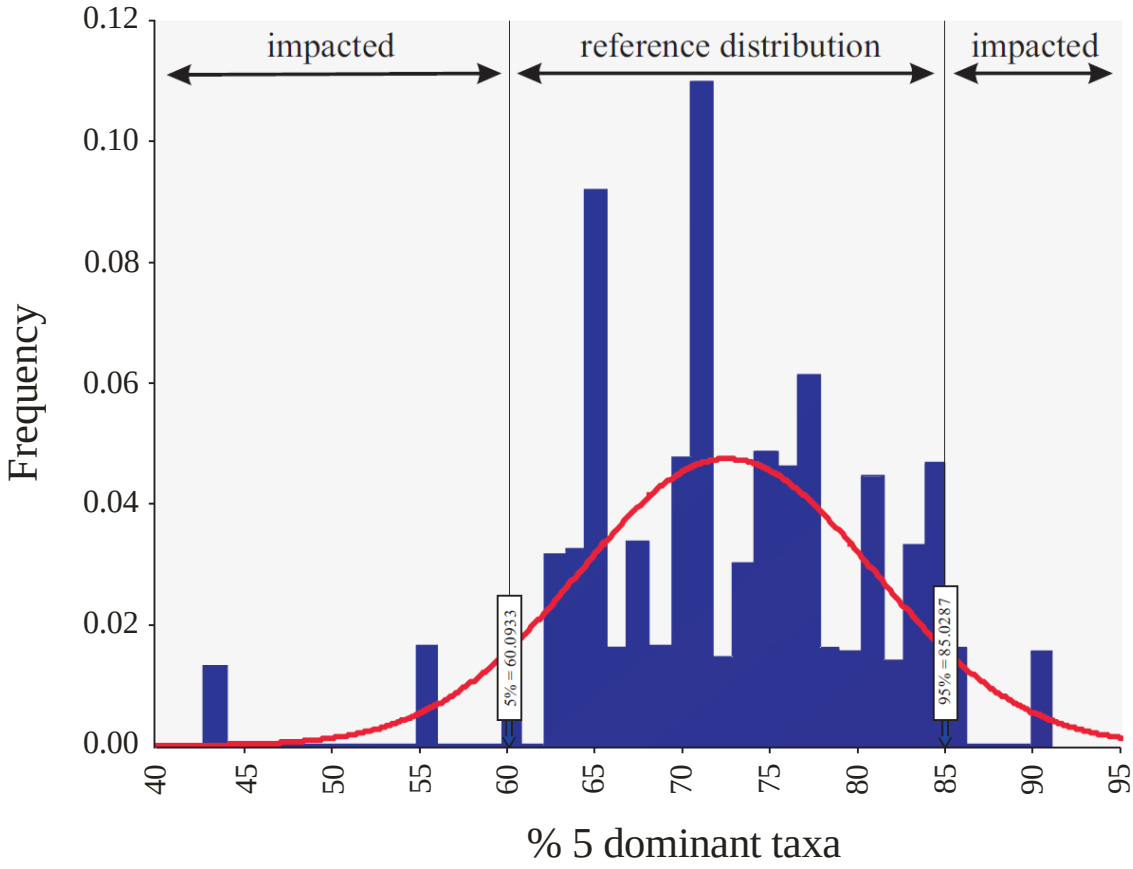


Figure 4 Metric score distribution across reference sites and corresponding boundaries used for assessment. The 5$^{th}$ and 95$^{th}$ percentiles of the reference distribution are the boundaries for the range of metric scores that are considered normal or unimpacted. Metric scores falling below or above the 5$^{th}$ and 95$^{th}$ percentiles, respectively, are considered impacted because they fall outside of the "normal" reference distribution.

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