



The effects of sampling technique on the ecological characterization of shallow, benthic macroinvertebrate communities in two Newfoundland ponds

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Abstract

The influence of sampling technique on the characterization of benthic macroinvertebrate communities on boulder-cobble substrate in two shallow freshwater ponds was analysed. Sweep-net and rock-bag sampling techniques were used to collect macroinvertebrates from two ponds in Newfoundland, Canada. Abundance and diversity of macroinvertebrates were compared in relation to the nature of the substrate and the technique. The two sampling techniques provided different estimates of diversity and density of the benthos. Neither method truly represented the benthic community as neither collected all taxa and each method typically over- or under-estimated the abundance of taxa. The difficulty of interpreting such data is discussed, with special reference to the rapid assessment of water quality in biomonitoring studies.

Introduction

The structure and composition of benthic macroinvertebrate communities in freshwater ecosystems are very often used for the biological assessment of water quality and to evaluate the impact of chemical and other pollutants (Wiens & Rosenberg, 1984; Hellowell, 1986; Mance, 1987; Rosenberg & Resh, 1993). The data used for these characterizations are acquired by a variety of sampling techniques, which have been developed and modified over time (Flanagan & Rosenberg, 1982; Mackay et al., 1984; Rosenberg & Resh, 1993; Merritt et al., 1996). However, all methods have biases and problems associated with them. Some techniques, such as the Ekman grab sampler, provide quantitative information while others such as the sweep-net provide only semi-quantitative data (Mackay et al., 1984; Rosenberg & Resh, 1993). An additional problem is the effect of substrate types and habitat on the sampling method (Clements et al., 1989; McCreddie & Colbo, 1991; Francis & Kane, 1995). Grab samplers, for example, do not function

properly on coarse, rocky substrates, while surber samplers are only useful for sampling from shallow streams. Artificial substrates have also been used to sample macroinvertebrates, but their use is restricted to shallow water (Rosenberg & Resh, 1982). Direct comparisons of data from the different techniques are often difficult to make and interpret (Rosenberg & Resh, 1993; Merritt et al., 1996; Colbo et al., 1997).

Protocols for the rapid assessment of freshwater ecosystems using macroinvertebrates recommend using the same sampling technique in different localities (Resh & Jackson, 1993; Resh, 1995). The method chosen should collect representative samples of the benthic community (Resh, 1995). In reality, collecting representative samples that are proportional to the natural community structure is extremely difficult or impossible since the distributions of many macroinvertebrates vary spatially and temporally (Resh & Jackson, 1993; Merritt et al., 1996).

Relatively few studies have been conducted on shallow lentic habitats in comparison to lotic systems (Ward, 1992; Rosenberg & Resh, 1993). Resh (1995)

recommended the use of dip-nets and artificial substrate samplers (in addition to others) for the rapid assessment of freshwater ecosystems. While sweep-nets are among the most commonly used sampling devices in rapid assessment studies, artificial substrate samplers have been used widely in freshwater benthic macroinvertebrate studies (Rosenberg & Resh, 1982; Resh & Jackson, 1993). Their use allows standardised sampling by reducing variability between samples, which many other samplers, including sweep-nets, fail to accomplish (Rosenberg & Resh, 1982; Resh & Jackson, 1993). The present study compared benthic macroinvertebrate data collected over hard substrate in the shallow, littoral zones of two ponds using sweep-net and artificial substrate samplers. The purpose was two-fold: (a) to compare the shallow benthic macroinvertebrate community on cobble-boulder substrate in the two ponds as determined by two sampling techniques, and (b) to evaluate the information on the benthic macroinvertebrate communities provided by the two methods used.

Study area

This study was carried out on two ponds near Hawke's Bay, Northern Peninsula, Newfoundland. The ponds are about 1 km apart and located approximately 4 km from the Northern Peninsula Highway (Route # 430) in relatively unperturbed forested areas. Pond 1 (50° 28' 15.8" N, 57° 09' 12.9" W) has an approximate area of 1 ha and is <1 m deep with a cobble boulder bottom overlain in part by a thick layer of fine organic ooze. The pond shoreline vegetation grades from a moss-sedge (*Carex* sp.) cover to a bog myrtle (*Myrica gale*)-leatherleaf (*Chamaedaphne calyculata*) shrub complex, which grades into spruce-fir forest. Pond 2 (50° 27' 49.9" N, 57° 09' 02.7" W) is also about 1 ha in area but its maximum depth is 2 m. The littoral substrate in the sample area is primarily coarse gravel and cobble with a few boulders. Organic debris overlies extensive areas of this substrate on each side of the sampling site. The pond shoreline vegetation is dominated by bog myrtle overhung by spruce-fir forest. The two ponds are relatively isolated and free from human disturbance.

Methods and materials

Sweep samples were taken at approximate depths of 0.3 m over gravel-cobble substrate with a standard aquatic D-net with an opening of 30 cm and a mesh size of 0.8 mm. A metre-long marker was placed on the bottom of the pool and six back and forth sweeps were made along the length of the marker. The material collected in the sweep net was placed in a ziplock bag and preserved in 95% ethanol.

The artificial samplers were rock-bags made from a 38-cm length of 6-cm diameter tube of expansible plastic webbing which stretched to 10 cm in diameter, with a maximum mesh opening (pore size) of approximately 2.5 cm. Coarse gravel, ranging in size from 3 to 7 cm in length was collected from the bottom of each pond. A 1-l plastic container (capable of holding between 90 and 110 stones) was filled with these stones and each stone was cleaned with a brush to remove pre-existing macroinvertebrates. One end of the tubing was tied with plastic fasteners and the stones were poured into the tubing and other end fastened leaving approximately a 30-cm length of the tubing filled with stones. This bag was placed on the rocky substrate.

On a sampling date, a sweep-net was placed at the edge of the rock-bag, which was lifted into the net and then lifted out of the water. The rock bag and the contents of the net were placed in a 4-l plastic bucket with water. The ends of the rock-bag were then opened to release the stones into the container with water. The stones were scrubbed with a brush to remove invertebrates and debris and were then discarded. The debris and invertebrates in the container were passed through a fine cloth sieve (mesh size <0.25 mm) before being placed in a labeled ziplock bag and preserved with 95% ethanol.

Fifteen rock-bags were placed in each pond on 17 June, 1996. Three of these rock-bags were removed from each pond on each of the following dates: 3, 12, 15 and 23 July and 5 August, 1996. Three sweep samples were also taken on these sampling dates. In Pond 1, the sweeps were taken along side the rock-bag samples on coarse gravel-cobble-boulder substrate with the gravel of the size used in the rock-bags. In Pond 2 we used two adjacent narrow patches of boulder, cobble and gravel substrate about 20 m apart. The sweep samples in Pond 2 had to be spatially separated from the rock-bags due to the abundance of twigs and other debris in the immediate vicinity of

the rock-bags. In both ponds rock-bags were placed at approximately 0.3 m depth.

In the laboratory the total volume of each sample was measured in a 1000 ml graduated cylinder. All macroinvertebrates were picked out of the samples and counted under a stereoscope. Identifications were made to lowest possible taxonomic level using the following references: Thorp & Covich, 1991; Wiggins, 1996; Merritt & Cummins, 1996. The Chironomidae, Oligochaeta, Hydrachnidia and early-instar insects were only identified to higher taxonomic levels.

Quantification

Density

To calculate densities, data from both sample types were standardized to numbers/m². The area sampled by the sweep-net was estimated to be one 0.33 m² (D-net opening was 30 cm and was swept over a metre of substrate). An estimate of the total surface area of the stones in the rock-bags was obtained by the following method. Three rock-bags were randomly selected and the number of stones in each determined. Five stones were chosen randomly from each of these rock-bags. The dimensions of each stone were determined by measuring the greatest width and greatest length and from this the total area estimated (McCreadie & Colbo, 1991). An estimate of the total surface area of the stones in a rock-bag was made by multiplying the mean area per stone from the five measured stones by the number of stones in the rock-bag. The mean total area of stone surface from three estimates of rock-bag samplers was 0.25 m² and this was used as an estimate of the surface area sampled by each rock-bag. The density of the abundant taxa (those that represented more than 5% of the total macroinvertebrates collected) and the total macroinvertebrates collected were calculated for both sampling techniques in both ponds.

Several measures of freshwater benthic macroinvertebrate communities have been recommended for the rapid assessment of the communities (Resh & Jackson, 1993; Resh 1995). The following measures were used to assess benthic biodiversity sampled by the two techniques.

Taxonomic richness and community diversity

- Taxonomic richness: the number of taxa collected by each sampling technique. The furthest level

of classification was determined for each taxon (usually genera and species, but families and subclasses in some cases).

- Shannon–Weiner’s Index of Diversity (Shannon, 1948): a measure of the relative proportions of the different taxa collected which provides information on the evenness of distribution of the different taxa.
- Number of insect families: the total number of insect families represented in the samples.
- Number EPT taxa: number of taxa within the Ephemeroptera, Plecoptera and Trichoptera represented in the samples.

Enumerations of relative abundance

- Percentage EPT: the proportion of Ephemeroptera, Plecoptera and Trichoptera represented in the benthic fauna collected by each sample.
- EPT/Chironomidae-ratio: number of EPT divided by the total number of chironomids in each sample.
- % Chironomidae: proportion of chironomids in each sample.
- % non-chironomid Diptera: proportion of Diptera, excluding the Chironomidae represented in the samples.
- % non-insect taxa: proportion of taxa in the samples other than insects.

Pollution tolerance

The Family Biotic Index (FBI) (Hilsenhof, 1988): this is based on the tolerance levels of families of arthropods. In the present study calculations were based on insect family tolerance levels given by Hilsenhof (1988).

Statistical analyses

Temporal variation occurs in all benthic communities, but since in the present study all sites were sampled on the same days they were all exposed to the same temporal changes. The prime questions in this paper were the influence of technique and pond on the estimated community structure and the interpretation of the environmental conditions based on that structure. Therefore data collected over the entire period were pooled prior to carrying out statistical analyses. The means of all the measures were compared between the two sampling techniques within each pond using randomization techniques (Monte Carlo simulations) with a

Table 1. Summary of substrate types and detrital material collected by the two sampling techniques: mean volume of detrital material is given in parentheses

Sample type	Nature of substrate	Type and volume of detrital material present in samples	
		Pond 1	Pond 2
Sweeps	Pebbles on pond bottom	Primarily fine organic matter; very little aquatic vegetation remains (coarse organic matter) (~400 ml)	A variety of leaf fragments, conifer needles, and other terrestrial/aquatic vegetation remains; little fine organic matter; some fine sand (~500 ml)
Rock-bags	Pebbles within extensible plastic net	Primarily fine organic matter; very little vegetation remains (<10 ml)	some vegetation remains; some fine organic ooze and fine sediments (<10 ml)

minimum of 1000 randomizations (Manly, 1991). The data for the two ponds were analyzed separately as a large difference in the benthic communities was observed between the ponds.

Results

Rock-bag samples were easier to sort as they had less detrital material compared to sweep samples (Table 1). A total of 67 taxa (Table 2) were identified from all the samples with a total of 59 taxa from Pond 1 and 51 taxa from Pond 2, with 43 taxa found in both ponds. The sweep-net samples in both ponds collected the highest number of taxa but did not collect all the taxa recovered by the rock-bag samplers in both ponds.

The density of several common taxa was significantly different between rock-bags and sweeps (Table 3). In Pond 1 five common taxa showed significant density differences, whereas seven taxa had significantly different densities ($P = \leq 0.05$) in Pond 2. Total macroinvertebrate and Chironomidae densities were significantly higher in rock-bags compared to sweeps in Pond 2 only. Numbers of Hydrachnidia and Ephemeroptera (young instars) were higher in rock-bags in both ponds while *Caenis simulans* showed the opposite pattern in both ponds. *Hyaella azteca* densities were significantly higher in the sweeps only in Pond 2. Numbers of the leech *Helobdella stagnalis*, were significantly different between the two techniques and between the two ponds.

Table 4 indicates that several community indices calculated from the data from the two techniques in each of the two ponds were significantly different (P

$= \leq 0.05$). In Pond 1, three out of the eight community structure indices were significantly different between the data from the two techniques and in Pond 2 six indices were significantly different. Furthermore the FBI also provided different assessments of water quality calculated from the two techniques and were not consistent between ponds (Table 5). FBI values from sweeps in Pond 1 suggested some organic pollution in the system, whereas those from rock bags suggested excellent water quality. In Pond 2, FBI values from sweeps suggested excellent water quality, while those from rock-bags suggested substantial organic pollution.

Discussion

The effect of sampling technique on the characterization of the macroinvertebrate community is clearly illustrated by both the qualitative and quantitative data (Tables 2–5). Neither sampling technique captured representatives of all the benthic macroinvertebrate taxa found on coarse rocky substrates within the littoral zone of the two ponds. Furthermore, density estimates for the abundant taxa collected by the two sampling techniques are not equal, illustrating the different selectivity of each technique even for the taxa collected by both techniques (Table 3).

Selection of sampling technique is among the most important decisions to be made prior to the sampling of aquatic habitats (Rosenberg & Resh, 1993; Merritt et al., 1996). The goal for comparing communities is to have sampling techniques that best capture samples that are representative of the community. A number

Table 2. Presence of taxa obtained by both sampling techniques in the two ponds; (+) indicates presence and (-) indicates absence

Taxon	Pond 1		Pond 2	
	Sweep	Rock bag	Sweep	Rock bag
Cnidaria				
Hydroidea	+	+	+	+
Annelida				
Oligochaeta	+	+	+	+
Hirudinea (unidentified)	+			+
<i>Helobdella stagnalis</i> (Linnaeus)	+	+	+	+
<i>Glossiphonia complanata</i> (Linnaeus)	+	+		
<i>Nepheleopsis obscura</i> (Verrill)	+		+	+
<i>Haemopsis</i> sp.		+	+	+
<i>Erpobdella</i> sp.				+
Mollusca				
Gastropoda		+	+	
<i>Valvata sincera</i> (Say)	+			
<i>Gyraulus</i> sp.	+	+	+	+
<i>Stagnicola</i> sp.	+	+		
<i>Physa heterostropha</i> (Say)	+	+		
<i>Ferrissia</i> sp.			+	
Bivalvia				
<i>Anodonta cataracta</i> (Lamarck)	+			
Sphaeriidae	+	+	+	
Arthropoda				
Acari				
Hydrachnidia	+	+	+	+
Crustacea				
Ostracoda		+	+	+
Copepoda	+	+	+	+
Daphniidae	+	+	+	+
<i>Hyalella azteca</i> (Saussure)	+	+	+	+
<i>Gammarus lacustris</i> (Sars)	+	+	+	+
Insecta				
Ephemeroptera (early instars)	+	+	+	+
<i>Caenis simulans</i> (McDunnough)	+	+	+	+
<i>Siphonurus</i> sp.	+	+	+	+
<i>Baetis pygmaeus</i> (Hagen)	+	+		
<i>Callibaetis skokianus</i> (Needham)	+			
<i>Aeshna</i> spp.		+	+	+
<i>Aeshna eremita</i> (Scudder)		+		+
<i>Aeshna interrupta</i> (Walker)		+		
<i>Aeshna umbrosa</i> (Walker)		+		

Table 2. contd.

Taxon	Pond 1		Pond 2	
	Sweep	Rock bag	Sweep	Rock bag
Insecta				
Cordulidae (unidentified)	+	+	+	
<i>Somatochlora albicincta</i> (Burmeister)	+	+	+	
<i>Cordulia shurtleffi</i> (Scudder)	+	+	+	
<i>Leucorrhinia proxima</i> (Calvert)			+	
Coenagrionidae (unidentified)	+		+	+
<i>Ischnura</i> sp.		+		
<i>Enallagma</i> sp.		+	+	+
<i>Leuctra</i> sp.		+		
Corixidae	+	+	+	
Trichoptera (young larvae)		+	+	+
<i>Oxyethira</i> sp.	+		+	+
<i>Hydropsyche</i> sp.	+		+	
<i>Cheumatopsyche pettiti</i> (Banks)	+	+		
<i>Chimarra</i> sp.			+	
<i>Polycentropus</i> sp.	+		+	
Limnephilidae (unidentified)	+			
<i>Limnephilus</i> sp.			+	+
<i>Glyphopsyche</i> sp.	+		+	
<i>Molanna</i> sp.			+	
Phryganeidae (unidentified)	+	+	+	+
<i>Banksiola</i> sp.			+	+
<i>Mytastoides sepulchralis</i> (Walker)	+		+	
<i>Oecetis</i> sp.	+	+	+	+
<i>Trienodes</i> sp.				+
<i>Gyrinus</i> sp. (larvae)		+	+	+
<i>Haliphys</i> sp.(adults)	+		+	
Dytiscidae (larvae) (unidentified)	+	+	+	
<i>Agabus</i> sp. (adults)	+			
<i>Hydroporus</i> sp.(adults)	+			
<i>Hygrotus</i> sp.(adults)	+			
<i>Bezzia</i> sp.	+	+	+	+
Chironomidae (larvae)	+	+	+	+
<i>Prosimulium fuscum /mixtum</i>			+	
<i>Simulium venustum / verecundum</i>		+	+	
<i>Simulium vittatum</i> (Zetterstedt)	+			
<i>Chelifera</i> sp.	+			
<i>Chrysops</i> sp.	+		+	
Total number of taxa	46	41	47	31

of factors such as substrate type, presence of emergent vegetation and depth influence the usefulness of different techniques, but as illustrated here useful samplers do not equally sample the natural community from the same substrate at any given site. The substrate has a profound influence on both the species present and the density of macroinvertebrates occupying a site; in addition each taxon has its own behavioural response to physical and biological processes occurring on a particular substrate type. Thus different techniques will

differentially collect taxa and individuals because of these different preferences. This results in biased data and a skewed characterization of the community.

The aquatic D-frame sweep-net is a commonly used semi-quantitative sampling device (Mackay et al., 1984). However, depending on the coarseness of the substrate, the macrobenthos from the interstitial spaces may be poorly represented in samples because they only enter the net if currents created by repeated sweeps draw them out. A Newfoundland stream study,

Table 3. A comparison of the density of the abundant (<5% of total) macroinvertebrate taxa collected by the two sampling techniques within each pond. Numbers in parentheses show one standard error calculated by bootstrap estimates using 1000 randomizations. *P* values are based on comparisons of mean densities based on 1000 randomizations

	Mean density (numbers/m ²)					
	Pond 1			Pond 2		
	Sweeps (N=15)	Rock bags (N=15)	<i>P</i> value	Sweeps (N=15)	Rock bags (N=15)	<i>P</i> value
Total	721.0 (±95.8)	826.3 (±104.6)	0.222	686.8 (±87.5)	1288.8 (±153.7)	0.001
Oligochaeta	37.8 (±5.3)	74.1 (±22.2)	0.047	35.6 (±5.2)	38.8 (±14.8)	0.424
<i>Helobdella stagnalis</i>	2.2 (±1.6)	45.2 (±7.9)	<0.001	9.6 (±3.8)	1.9 (±1.3)	0.023
Hydrachnidia	5.0 (±1.0)	29.1 (±6.8)	<0.001	7.0 (±2.0)	26.5 (±9.9)	0.01
Daphniidae	18.0 (±4.2)	14.4 (±5.0)	0.328	56.8 (±17.6)	63.4 (±15.8)	0.414
<i>Hyaella azteca</i>	150.8 (±22.6)	158.3 (±24.8)	0.405	125.4 (±23.5)	44.9 (±11.9)	0.003
Ephemeroptera	0.6 (±0.3)	30.2 (±6.2)	<0.001	2.8 (±2.4)	70.6 (±18.1)	<0.001
<i>Caenis simulans</i>	23.8 (±4.7)	8.6 (±1.7)	0.003	5.0 (±1.9)	0.3 (±0.3)	0.008
Chironomidae	412.0 (±60.7)	395.4 (±43.4)	0.435	256.6 (±47.2)	971.2 (±110.8)	<0.001

using sweep-net and rock-bag sampling techniques, also recognised that physical characteristics of the site would influence the taxonomic richness, diversity and relative abundance within the collected sample making interpretation of the data difficult (Colbo et al., 1997). Because rock-bag samplers provide abundant interstitial spaces they can be colonised by this fauna (Flannagan & Rosenberg, 1982; Rosenberg & Resh, 1982; Way et al., 1995) and therefore this technique may better characterize this community. However, the rate of colonisation of an artificial substrate sampler is an important factor affecting estimates obtained by such samplers (Clements et al., 1989; Clements, 1991). Colonisation dynamics of artificial substrates are complex and generally not well understood (Rosenberg &

Resh, 1982). The present study did not investigate the colonisation dynamics but made the assumption that similar changes were occurring in the rock-bags as in the natural substrate upon which they rested. Another factor that needs investigation is the rock size used in rock-bags as this influences both amount of interstitial space and total surface area within the rock bag sampler, adding further to the biases involved (Rosenberg & Resh, 1982; Francis & Kane, 1995). The use of density (number of individuals per unit area) as a measure of the abundance of benthic macroinvertebrates is a common practice (e.g. Franquet, 1999; Lemly & Hilderbrand, 2000; Murphy & Giller, 2000; Robinson et al., 2000), but often one that completely disregards the interstitial spaces

Table 4. Comparison of indices characterizing the community structure of macroinvertebrate taxa collected by the two sampling techniques within each pond. Numbers in parentheses represent one standard error calculated by bootstrap estimates using 1000 randomizations. *P* values are based on comparisons of mean densities based on 1000 randomizations

Community measures	Pond 1			Pond 2		
	Sweeps (<i>N</i> =15)	Rock bags (<i>N</i> =15)	<i>P</i> value	Sweeps (<i>N</i> =15)	Rock bags (<i>N</i> =15)	<i>P</i> value
Richness of taxa	14.3 (±0.7)	15.4 (±0.7)	0.142	15.2 (±0.9)	12.5 (±0.7)	0.013
Shannon's index	2.2 (±0.1)	2.4 (±0.1)	0.041	2.4 (±0.1)	1.4 (±0.1)	<0.001
No. of EPT taxa	2.4 (±0.3)	2.3 (±0.2)	0.371	3.3 (±0.6)	2.2 (±0.3)	0.053
No. of insect families	6.4 (±0.4)	5.9 (±0.4)	0.147	7.3 (±0.6)	3.6 (±0.4)	<0.001
% Chironomidae	0.55 (±0.02)	0.49 (±0.02)	0.050	0.35 (±0.03)	0.76 (±0.02)	<0.001
% EPT	0.05 (±0.01)	0.05 (±0.01)	0.323	0.04 (±0.01)	0.07 (±0.01)	0.039
EPT/Chironomidae	0.10 (±0.01)	0.11 (±0.02)	0.398	0.14 (±0.06)	0.09 (±0.02)	0.384
% Non-insect invertebrates	0.34 (±0.02)	0.41 (±0.03)	0.038	0.57 (±0.03)	0.16 (±0.02)	<0.001

(e.g. Clements et al., 1989; Robinson et al., 2000). The measurement of the actual surface area available for colonisation by macroinvertebrates in the rock-bag sampler of our study attempts both to better assess the true density for comparison with the sweep-net sampler and to show the complications associated with comparing even these density measurements. Monitoring studies need to cautiously interpret community structure based on density data from any one technique because of the selective sampling of the community by each technique. For example, the EPT index differs between the sweep and rock-bag data (Table 3) as *Caenis simulans* is abundant in the sweeps and not the rock-bags while the early instar Ephemeroptera (Table 2), which were the very young nymphs of other genera, have the opposite pattern of occurrence.

In addition, Tables 2 and 3 suggest that in the exposed rock-grazing community, certain interstitial dwellers, such as *Chimarra* sp., several other Trichoptera taxa (Wiggins, 1996), and some swimmers like *Haliphus* sp. (beetles) (Thorp & Covich, 1991) were not adequately sampled by the rock-bags. Differences in measures of taxonomic richness and community diversity could hence be attributed partly to differential colonization of rock-bag samplers.

Table 5. Variation in family biotic indices (FBI) based on the two sampling techniques. The associated evaluations of water quality are based on tolerance values of insect taxa provided in Hilsenholf (1988)

Pond and sample type	FBI	Water quality	Degree of organic pollution
Pond 1			
Sweeps	4.28	Good	Some organic pollution probable
Rock-bags	3.62	Excellent	Organic pollution unlikely
Pond 2			
Sweeps	2.56	Excellent	Organic pollution unlikely
Rock-bags	5.41	Fair	Fairly substantial organic pollution

Clearly, the characteristics of the pond itself affected the community of macroinvertebrates present on the same substrate at similar depths. Here the two ponds were within a 1 km of each other at approximately the same elevation and in the same forest ecosystem, but Pond 2 was about twice as deep at 2 m with less emergent vegetation. Measures of taxonomic richness, diversity and relative abundance derived from the two sampling techniques (Tables 2 and 3) varied between the two ponds, with 24 taxa not shared between them. This study shows caution is needed

when ascribing differences between sites to human disturbance because like Colbo et al. (1997), we found confounding natural factors have a major affect on inter-site comparisons of benthic macroinvertebrate communities.

However, each method can be used to compare sites and ponds provided the same protocol is followed but may not provide an accurate characterisation of the real macrobenthic community. It is therefore critical that these biases be acknowledged because they can affect assessment of impacts, ecological processes, survival and other phenomena. For example, rock-bags had a higher number of early instar Ephemeroptera. If older nymphs of these taxa avoided the sampler (Buffagni et al., 1995), high mortality might be implied when little had occurred. If this were used in an assessment, a negative impact could be erroneously indicated.

Another example of bias is shown by the indices used to assess water quality and the impact of pollution on benthic organisms derived from the two techniques (Tables 4 and 5). Substrate preferences of benthic macroinvertebrates differ, which causes the proportions of various families to differ which results in different indices calculated from that data. In the present study, sweeps were probably collecting more macroinvertebrates from the surface of the substrate and adjacent surface areas unlike the rock-bags which might have collected more interstitial fauna, yielding different FBI values (Mackay et al., 1984).

The rock-bags have two advantages for collecting macroinvertebrates on coarse substrates in shallow ponds compared to sweep samples. First they can sample the fauna dwelling within interstitial spaces, and second the amount of debris from a rock-bag sample is much lower than that in a sweep-sample (Table 1) permitting quick sorting. Sweep-nets also have the inherent disadvantage of collecting more epibenthic taxa than truly benthic ones. However, an analysis of sampling devices carried out by Clements (1991) suggests similar outcomes from artificial substrate-filled trays and more conventional devices in the rapid assessment of benthic communities. Our conclusion is that all sampling techniques have problems, which are often paid scant attention or disregarded. The effect of this error should be explored further if accurate assessment of water quality by such techniques is to be achieved.

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