ARTIFICIAL SUBSTRATA AS QUANTITATIVE SAMPLING DEVICES OF BENTHIC MACROINVERTEBRATES IN FLOWING WATER HABITATS

Alberta
ENVIRONMENTAL CENTRE

ARTIFICIAL SUBSTRATA AS QUANTITATIVE SAMPLING DEVICES OF BENTHIC MACROINVERTEBRATES IN FLOWING WATER HABITATS

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ABSTRACT

This study was initiated to assess the use of artificial substrata for the quantitative sampling of benthic macroinvertebrates in flowing water (lotic) habitats and to prepare Standard Operating Procedures (SOP) for using artificial substrata in the field and for sample processing in the laboratory.

An artificial substratum is used to obtain quantitative samples of the organisms in an aquatic habitat. The sample consists of the organisms that colonize the artificial substratum after it is placed in the habitat and retrieved after a predetermined time. The use of artificial substrata to sample aquatic macroinvertebrates has increased substantially in the 1970s and 1980s, especially for the biomonitoring of lotic habitats in North America. In Alberta, artificial substrata have been used for biomonitoring and in experiments designed to examine the effect of specific contaminants and substratum characteristics on colonization by macroinvertebrates. A direct sampler, the modified Neill cylinder sampler used by the Environmental Assessment Division, Department of Environmental Protection, is routinely used to sample macroinvertebrates for the monitoring and assessment of lotic habitats.

Field experiments were conducted in the McLeod and Battle rivers to compare the use of a range of artificial substrata (single natural rock particle, single brick particle, and basket of the natural substratum) to sample the benthic macroinvertebrates. Two modifications to the procedures of using the artificial substrata were evaluated experimentally by examining the macroinvertebrate colonization on the artificial substrata either with or without a natural benthic cover of fine sediment and algae, and on the artificial substrata that were either embedded or placed on the natural substratum. The macroinvertebrates on the natural substratum were also sampled using the Neill cylinder sampler to compare this fauna with the organisms colonizing the artificial substrata.

The densities of most of the macroinvertebrate taxa, number of taxa, and quantity of organic material were greatest in the substratum baskets, in contrast to the single rock and brick particles. Most of the taxa showed an increase in their density after the longer colonization periods than after one day. It was not clear, however, if the fauna had reached an equilibrium level after the longest colonization period of 29 days. The presence of the natural benthic cover of fine sediment and algae on the artificial substratum or the position of the artificial substrata

relative to the surface of the natural substratum did not affect the density of most of the macroinvertebrate taxa.

The densities of the macroinvertebrate taxa, number of taxa, and quantity of organic material were greatest on the natural substratum than on the different types of artificial substrata at both study sites. And in general at both study sites, the taxonomic composition of the fauna (or relative abundances of the taxa) on the artificial substrata, with the exception of the substratum baskets, was different from the natural substratum. The taxonomic composition of the fauna among the artificial substrata was generally the same.

Of the three artificial substrata, the physical heterogeneity and the quantity of organic material in the substratum baskets were the most similar to the natural substratum. In addition, the relative abundances of the taxa on the substratum baskets and natural substratum were similar. There were statistical differences between the densities of most macroinvertebrate taxa on the substratum baskets and on the two types of single substratum particles compared to the natural substratum. This indicated that the macroinvertebrate assemblage on the artificial substrata might not represent the fauna on the natural substratum.

In conclusion, the type of sampler used in a study will depend on the objectives of the study. A direct sampler (such as the Neill cylinder) compared with artificial substrata is the most effective method for sampling the natural substratum. However, artificial substrata can be used in many studies. For example, artificial substrata can be used to sample deep-water and fast-flowing habitats when it is not possible to sample the natural substratum with a direct sampler or in experimental studies, to determine the effect of physical, chemical, and biological characteristics of the substrata on macroinvertebrate colonization.

1   INTRODUCTION

This study was initiated to assess the use of artificial substrata for the quantitative sampling of benthic macroinvertebrates in flowing water (lotic) habitats and to prepare Standard Operating Procedures (SOP) for using artificial substrata in the field and for sample processing in the laboratory.

An artificial substratum is used to obtain quantitative samples of the organisms (e.g., macroinvertebrates, algae, and microorganisms) in an aquatic habitat. The artificial substratum is placed in the habitat and retrieved after a predetermined time. Organisms that have colonized the artificial substratum represent the sample. Artificial substrata have been used to sample aquatic macroinvertebrates since 1935, and their use increased in the 1970s and 1980s, especially for the biomonitoring of lotic habitats in North America (Rosenberg and Resh 1982, Cairns and Pratt 1993, Resh and McElravy 1993). Artificial substrata have also been used in toxicity studies, for example, to examine the recolonization of the substratum after an environmental disturbance has occurred (Voshell et al. 1989, Buikema and Voshell 1993).

Two extensive reviews examined the types of artificial substrata and their advantages and disadvantages for sampling benthic macroinvertebrates in freshwater ecosystems (Flannagan and Rosenberg 1982, Rosenberg and Resh 1982). Artificial substrata were grouped into two main types: (1) those that resemble the natural substratum of the habitat in which they are placed (e.g., baskets of natural substratum and mesh bags of leaves) and (2) those that do not resemble but have similar physical and chemical characteristics to the natural substratum (e.g., clay tiles and bricks, plastic sheets, and rope).

There are several advantages to using artificial substrata compared to other sampling methods (Rosenberg and Resh 1982, Voshell et al. 1989, Cairns and Pratt 1993). Artificial substrata can be used (1) to sample habitats that are difficult to sample by other methods, (2) to permit non-destructive sampling of the habitat, (3) to reduce the variation in samples taken by different operators, (4) to increase the precision of samples by reducing the physical and chemical heterogeneity of the substratum, and (5) to reduce the sample processing time because there is less extraneous material in the sample. The disadvantages of using artificial substrata are that (1) the samples may not represent the taxonomic composition of the fauna on the natural substratum, (2) there is incomplete knowledge of the time required to obtain an equilibrium population or assemblage of organisms, (3) the artificial substrata do not provide a measure of the condition

of the natural substratum, (4) two visits to the study site (to place and retrieve the substrata) are required to obtain one sample, and (5) the artificial substrata are subject to loss (e.g., due to vandalism or water level fluctuations).

As noted above, there are many types of artificial substrata. Also, there are many different procedures for using artificial substrata. For example, the natural substratum that has been used in substratum baskets has been prepared in two main ways. Some investigators (Lake and Doeg 1985) have removed the natural benthic cover (including algae, bacteria, fungi, detritus, sand, silt, and clay) from the natural substratum particles, while others (Ciborowski and Clifford 1984) have not removed this material. The presence of the benthic cover on the substratum particles can affect colonization of the substratum by macroinvertebrates (Mackay 1991). Different positions of the artificial substratum relative to the natural substratum have also been used in field studies. In general, the artificial substrata have been placed on the natural substratum or embedded flush with the surface of the natural substratum in lotic habitats (e.g., Table VII of Rosenberg and Resh 1982).

In Alberta, artificial substrata have been used for biomonitoring and in experiments designed to examine the effect of specific contaminants and substratum characteristics on colonization by macroinvertebrates (e.g., Lock et al. 1981a, 1981b, Clifford et al. 1992). A direct sampler, such as the modified Neill cylinder sampler (Alberta Environment 1990), is routinely used by the Environmental Assessment Division (Department of Environmental Protection) to sample macroinvertebrates for the monitoring of lotic habitats. For this study, it was decided to determine if the fauna of artificial substrata and the natural substratum, using the Neill cylinder sampler, are the same.

This field study was designed to compare the use of a range (three types) of artificial substrata for the quantitative sampling of benthic macroinvertebrates in lotic habitats with a coarse substratum. Two modifications to the procedures of using artificial substrata were evaluated experimentally by examining macroinvertebrate colonization on artificial substrata, with or without a natural benthic cover of fine sediment and algae, and on artificial substrata that were either embedded or placed on the natural substratum. Macroinvertebrates of the natural substratum were also sampled to compare these organisms with the fauna of the artificial substrata. Standard Operating Procedures for the methods used in the study were prepared in separate documents.

2 DESCRIPTION OF STUDY SITES

Two study sites with similar natural substratum, water flow and depth were chosen on the McLeod River (54° 01' N, 115° 50' W) and Battle River (53° 01' N, 110° 49' W). Percent composition of the substratum was 66% and 74% cobble (6-26 cm) at the McLeod and Battle river sites, respectively; the remaining substratum was mostly a mixture of pebble and gravel (2-60 mm). Mean water velocity (n = 2) ranged from 0.32 to 0.63 m/s and 0.23 to 0.63 m/s and mean water depth (n = 2) ranged from 17 to 39 cm and 22 to 34 cm at the McLeod and Battle rivers, respectively.

The main difference between the substratum at the study sites was that an aquatic moss (Amblystegium riparium, Hypnaceae) covered most of the upper surface of the large substratum (cobble and pebble) particles at the Battle River. In contrast, the large substratum at the McLeod River was covered with a thin layer of sand and silt, and occasional patches of algae.

3 METHODS AND STUDY DESIGN

3.1 Methods

Standard Operating Procedures for the sampling of benthic macroinvertebrates using the artificial substrata and the modified Neill cylinder sampler, and for the laboratory processing of the benthic macroinvertebrate samples are in Appendix 1. Additional methods are described below.

The three artificial substrata used in this study were (1) single clay brick particles, (2) single natural rock particles, and (3) baskets of the natural substratum (Table 1). The modified Neill cylinder sampler was used to sample the natural substratum (Alberta Environment 1990). The macroinvertebrate samples were obtained with the Neill cylinder sampler by removing the organisms from the substratum enclosed by the cylinder (sample area = 0.0908 m^2). The pore size of the collection net on the Neill cylinder sampler and the smallest pore size of sieves used for the laboratory processing of all macroinvertebrate samples was 0.21 mm. Identifications of the macroinvertebrate fauna were determined using taxonomic keys for Alberta (Clifford 1991) and North America (Baumann et al. 1977, Edmunds et al. 1976, Merritt and Cummins 1984, Pennak 1978, Wiggins 1977). The organisms were identified using the taxonomic groups in Appendix 2.

The composition of the natural substratum at the study sites was measured by visually determining the approximate percentage of the mean substratum sizes in five 1 x 1 m quadrats of the substratum. The substratum sizes were boulder (> 26 cm), cobble (6-26 cm), pebble-gravel (2-60 mm), and sand (< 2mm). Water velocity was measured (at 0.6 x water depth) with a Price AA current meter. The water velocity and depth were measured at the start and end of each colonization period; measurements were taken at two points about 2 m upstream and downstream of each experimental area in the McLeod and Battle rivers (see section 3.2).

Table 1.   Physical characteristics of the artificial substrata used in the McLeod and Battle rivers.

Physical Characteristic	Artificial Substratum		
	Brick	Rock	Substratum Basket
Description	Rectangular clay brick (18.9 x 9.1 x 5.6 cm) with three circular holes (3.5 x 5.6 cm) through the brick.	Oval-shaped cobble (maximum length = 5-15 cm).	Square baskets (25.5 x 25.5 x 10 cm) made of 1.3 cm galvanized wire screen. Oval-shaped cobble (maximum length = 5-15 cm) arranged in a pattern similar to natural substratum on top of 3-5 cm of sand and gravel.
Surface Area/ Sample Area	Surface Area: 0.0785 m^2	Mean Surface Area (Standard Deviation): McLeod River: 0.0539 m^2 (0.0068 m^2) Battle River: 0.0470 m^2 (0.0077 m^2)	Sample Area: 0.0650 m^2

3.2   Experimental Design

3.2.1   Summary of Experiments

Experiments were conducted to determine the density of the most abundant taxa (1) on three and two types of artificial substrata at the McLeod and Battle rivers, respectively, (2) on artificial substrata that either had a natural benthic cover (of fine sediment and algae) or were cleaned of the benthic cover at the McLeod River, and (3) on artificial substrata that were either embedded flush with the surface of the natural substratum or placed on the natural substratum (i.e., not embedded) at the Battle River. In addition, the macroinvertebrate fauna of the artificial substrata were compared to the natural substratum of the McLeod and Battle rivers. The design of the experiments at each study site was influenced by the type and abundance of natural substratum, and the size of suitable habitat (see sections 3.2.3 and 5).

3.2.2   McLeod River

The study site was marked into two experimental areas (Areas A and B) of equal size and having similar substratum composition. The natural substratum was 62% and 69% cobble, and 25% and 26% pebble-gravel in Areas A and B, respectively; the remaining substratum was boulder and sand. Areas A and B were subdivided into a 4 x 4 matrix and 15 units of each of the three artificial substrata were arranged randomly within 15 of the 16 matrix cells; no substrata were placed in the 16th matrix cell (Figure 1). In Area A, the benthic cover (fine sediment and algae) was cleaned from the natural substratum particles that were used as the artificial substrata (single rock particles and substratum baskets). In Area B, the benthic cover was not cleaned from these substrata. Five replicates of each artificial substratum and for each of the three colonization periods (see below) were embedded flush with the surface of the natural substratum (Figure 1).

Macroinvertebrate colonization was determined on the three artificial substrata (single brick and natural rock particles, and baskets of the natural substratum) after the substrata were in situ for three colonization periods (1-, 8-, and 29-day) in Areas A and B. The colonization was determined on the two artificial substrata (single rock particles and substratum baskets) with the benthic cover (Area A) and on the same substrata without the benthic cover (Area B). In addition, the composition of the macroinvertebrate fauna on the artificial substrata was compared

to the natural substratum. Five Neill cylinder samples of the undisturbed natural substratum were chosen randomly in Areas A and B after the 1-, 8-, and 29-day colonization periods (Figure 1).
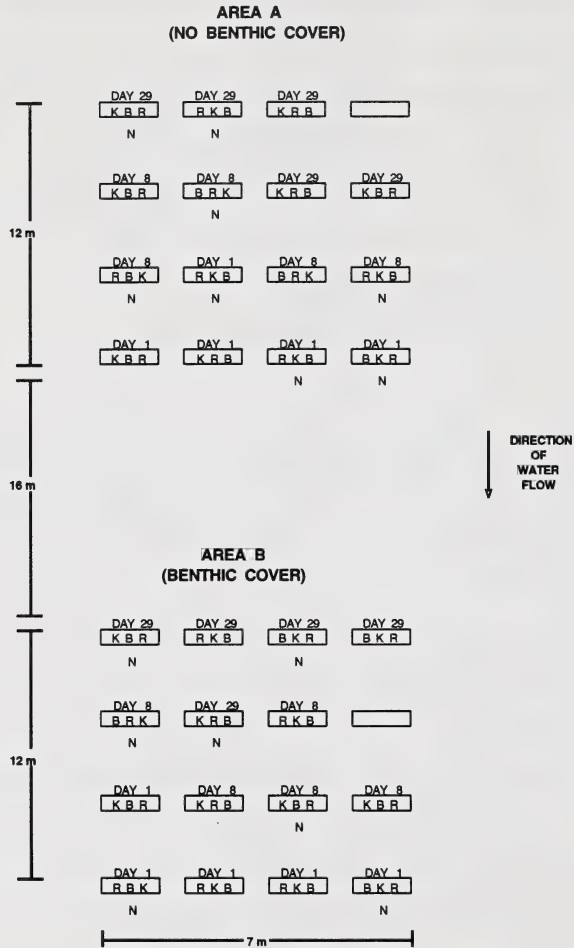
Figure 1.   Position and colonization period (1-, 8-, 29-day) of the single brick (K) and rock (R) particles, substratum baskets (B), and Neill cylinder (N) samples in Areas A and B of the McLeod River. The figure is not drawn to scale.

3.2.3   Battle River

In contrast to the McLeod River, the natural substratum in the experimental areas at the Battle River was physically disturbed to reduce the number of macroinvertebrates on the substratum. Consequently, the macroinvertebrate colonization of both the artificial and the disturbed natural substrata would occur over the same colonization period. It was not possible to physically disturb the substratum at the McLeod River because of the large size of the experimental area.

Ten transects were marked in two riffles (Riffles 1 and 2) at the study site (Figure 2). The substratum in each transect was physically disturbed using a hand-held hoe. The operator stood downstream of the transect and moved the hoe in an upstream-downstream direction with as much force as possible for about 10 minutes. The transects furthest upstream were disturbed first. A visual inspection of the substratum showed that most of the attached macrophytes were removed from the substratum, or that the substratum with the attached macrophytes was displaced from the transects. The percent composition of the substratum in the undisturbed and disturbed transects was similar although there was an increase in the percent composition of the finer (sand) substratum in the disturbed transects. The mean percent composition of the substratum in the undisturbed and disturbed transects (n = 5) was 5% and 1% boulder, 74% and 70% cobble, 18% and 18% pebble-gravel, and 3% and 13% sand, respectively.

Macroinvertebrate samples were taken less than 2 h after the substratum was physically disturbed to determine if the abundance of organisms was affected by the disturbance. Five Neill cylinder samples were taken in randomly selected transects of Riffles 1 and 2, and in the undisturbed natural substratum upstream of Riffle 1.

The macroinvertebrate colonization was determined on two artificial substrata (single natural rock and brick particles) that were left in situ for 21 days. Substratum baskets were not used at the Battle River because of insufficient quantities of suitable substratum at this site. The artificial substrata were placed on the natural substratum about 2 h after the substratum was physically disturbed.

3.2.3.1   Position of Artificial Substrata Relative to Natural Substratum

Macroinvertebrate colonization was determined on the artificial substrata that were embedded flush with the surface of the natural substratum and on artificial substrata that were

placed on top of the natural substratum. Clean natural rock particles (range in maximum length = 5-15 cm) and clay bricks were placed in the transects of Riffle 2 and retrieved after 20 days (Figure 2).
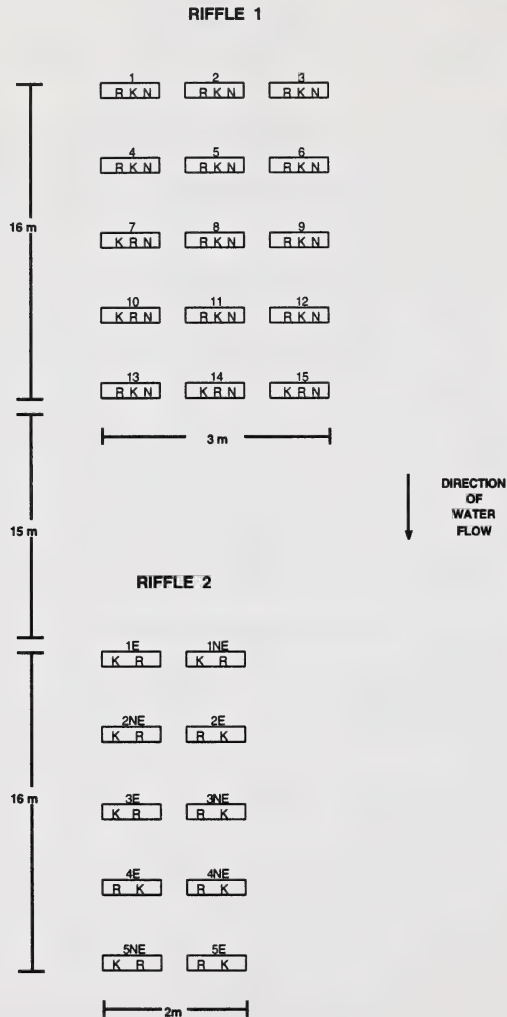
Figure 2.   Position of the single brick (K) and rock (R) particles, and Neill cylinder (N) samples in Riffles 1 and 2 of the Battle River. In Riffle 2, the artificial substrata were embedded (E) or not embedded (NE) flush with the surface of the natural substratum. The figure is not drawn to scale.

3.2.3.2   Artificial Substrata Versus Natural Substratum

Macroinvertebrate colonization on the artificial substrata was compared to that on the natural substratum. Fifteen replicates of each of the single natural rock and brick particles were arranged randomly in the transects of Riffle 1 (Figure 2). After 21 days, the artificial substrata were retrieved and 15 Neill cylinder samples were taken on the substratum beside the artificial substrata in the transects (Figure 2).

3.3   Statistical Analyses

The macroinvertebrate data (no. of organisms/sample) were converted to density data (no. of organisms/m^2) using the surface area of the substratum particles or the surface area sampled for the substratum baskets and the Neill cylinder sampler (Table 1, Appendix 1). The density data were transformed (log $(x + 1)$) to obtain equal variances; plots of the mean and variance demonstrated that the dependence of the mean on the variance was eliminated or reduced (Elliott 1977). The probability level of $p \leq 0.05$ was used as the level of statistical significance in the statistical analyses.

Differences between the densities of the most abundant taxa in the samples were determined using the analysis of variance (ANOVA) fixed effects model and the unpaired t-test. Significant interactions ($p \leq 0.05$) were determined in the ANOVA tests with more than one treatment factor. If the ANOVA test showed a statistical difference between more than two levels of a factor, the Student-Newman-Keuls multiple comparison test was used to determine the levels that were statistically different ($p = 0.05$). All of the analyses were performed using the SAS/STAT statistical software package (SAS Institute Inc. 1988).

3.3.1   McLeod River

The three-way ANOVA was used to determine whether the benthic cover (present or absent), substratum type (single rock particle and substratum basket), and the three colonization periods (1-, 8- and 29-day) affected the densities of each taxon. The two-way ANOVA was used to determine the differences in the densities of taxa on the three artificial substrata after the three colonization periods in each experimental area (A and B). The two-way ANOVA was used to determine the differences in the densities of taxa on the three artificial substrata and on the

natural substratum (Neill cylinder samples) after the three colonization periods in each experimental area.

3.3.2   Battle River

The effect of the physical disturbance on the densities of the most abundant macroinvertebrate taxa on the natural substratum was determined using the unpaired t-test. The two-way ANOVA was used to determine whether the position (embedding or not embedding) of the artificial substrata relative to the natural substratum and the type of artificial substratum (single rock and brick particles) affected the densities of taxa. The one-way ANOVA was used to determine the differences between the densities of taxa on the artificial substrata (single rock and brick particles) and the natural substratum (Neill cylinder samples).

3.3.3   Taxonomic Composition on the Artificial and Natural Substrata

The non-parametric Spearman rank correlation test was used to determine the similarity between the taxonomic composition of the 10 most abundant taxa on each of the artificial substrata with the fauna of the natural substratum (Neill cylinder samples) at the McLeod and Battle rivers. The density data were ranked to reduce the effect of the taxa that were extremely abundant or rare in the correlation coefficient. The number of taxa, using the taxonomic groups in Appendix 2, were calculated on each of the artificial substrata and the natural substratum at the McLeod and Battle rivers. The lowest taxonomic level for related organisms in Appendix 2 was used to calculate the number of taxa at each study site. For example, some organisms were identified to the family level or to genera belonging to the same family. But only the genus, lowest taxonomic level, was used to calculate the number of taxa.

4   RESULTS

4.1   McLeod River

4.1.1   Presence of Benthic Cover on Artificial Substrata

The 10 most abundant taxa made up 89.7% of the 12,705 organisms in the samples. The densities of Chironomidae (Diptera) and Ameletus (Ephemeroptera) larvae and the total

number of organisms, but none of the other taxa, were affected by the presence of the benthic cover on the artificial substratum (Table 2). Only three of 44 statistical interactions tested were significant; one interaction was significant for the benthic cover (i.e., for Ameletus larvae: benthic cover x colonization period, $p = 0.04$). This indicated that the effect of the three factors (benthic cover, substratum type and colonization period) on the densities of most taxa were independent of each other.

Table 2.   Percent composition and results of the three-way (benthic cover, substratum type, and colonization period) analysis of variance (* = $p \leq 0.05$) and Student-Newman-Keuls (SNK) tests for the densities (n=5) of the ten most abundant taxa and the total number of organisms on the substratum baskets (B) and single rock particles (R) in Areas A and B of the McLeod River. Statistical differences ($p = 0.05$) in the SNK test are separated into groups from the greatest to least (High-Low) density.

Taxonomic Group	Benthic Cover (Present/Absent) Probability Level	Substratum (B,R) Probability Level	Colonization Period (1,8,29 day)	SNK Colonization Period (High-Low)	Percent Composition
Chironomidae	0.0155*	0.0001*	0.0002*	29-8,1	68.697
Baetis	0.1934	0.0205*	0.0001*	8,29-1	5.446
Cheumatopsyche	0.0916	0.0001*	0.2328		3.384
Hydropsychye	0.4089	0.0010*	0.0954		3.124
Acari	0.0685	0.3343	0.0462*	29,8-8,1	3.109
Oecetis	0.8574	0.7211	0.0137*	8,29-29,1	2.180
Ameletus	0.0001*	0.0001*	0.0001*	29,8-1	1.778
Empididae	1.0000	0.1638	0.6097		0.716
Stenonema	0.6843	0.0001*	0.0700		0.669
Capniidae	0.3223	0.3223	0.3754		0.637
Total no. of organisms	0.0005*	0.0001*	0.0001*	29-8-1	100

4.1.2   Comparison Among Artificial Substrata

The seven most abundant taxa made up 90.4% and 84.5% of the organisms in Areas A and B, respectively (Table 3). The total number of organisms, and three and five of the most abundant taxa were at statistically different densities on the three artificial substrata in Areas A and B, respectively (Table 3, Figure 3). Three taxa (Chironomidae, Cheumatopsyche (Trichoptera), and Ameletus) and the total number of organisms were at statistically different

densities on the artificial substrata in both Areas A and B (Table 3). Only one of 16 statistical interactions (substratum type x colonization period) was significant (Ameletus, p = 0.04). This indicated that the same trend in the densities of taxa on the artificial substrata occurred after each of the three colonization periods. The Student-Newman-Keuls test showed that the taxonomic groups were always at the greatest densities in the substratum baskets in contrast to the single rock and brick particles (Table 3, Figure 3).

Table 3.   Percent composition and results of the two-way (substratum type and colonization period) analysis of variance (* = p ≤ 0.05) and Student-Newman-Keuls (SNK) tests for the densities (n=5) of the seven most abundant taxa and the total number of organisms on the substratum baskets (B), and single rock (R) and brick (K) particles in Areas A and B of the McLeod River. Statistical differences (p = 0.05) in the SNK test are separated into groups from the greatest to least (High-Low) density.

AREA A					
Taxonomic Group	Substratum (B,R,K) Probability Level	SNK Substratum (High-Low)	Colonization Period (1,8,29 day) Probability Level	SNK Colonization Period (High-Low)	Percent Composition
Chironomidae	0.0059*	B-KR	0.0130*	29-8,1	75.709
Baetis	0.3618		0.0043*	8,29-1	4.760
Hydropsychye	0.0621		0.9180		2.633
Cheumatopsyche	0.0083*	B-KR	0.2650		2.170
Oecetis	0.1754		0.6524		2.011
Acari	0.1801		0.0433*	29,8-8,1	1.997
Ameletus	0.0001*	B-KR	0.0186*	8,29-1	1.128
Total no. of organisms	0.0001*	B-KR	0.0008*	29,8-1	100
AREA B					
Chironomidae	0.0007*	B-RK	0.0010*	29-8,1	60.331
Baetis	0.0028*	B-RK	0.0001*	8,29-1	6.266
Cheumatopsyche	0.0006*	B-RK	0.0407*	29,1-1,8	4.833
Acari	0.3506		0.2115		4.436
Hydropsychye	0.0092*	B-KR	0.0595		3.711
Ameletus	0.0002*	B-RK	0.0024*	29-8,1	2.555
Oecetis	0.2008		0.0724		2.382
Total no. of organisms	0.0001*	B-RK	0.0001*	29-8-1	100

The greatest number of taxa was found in the substratum baskets in contrast to the other artificial substrata in Areas A and B (Table 4). In Areas A and B, respectively, the mean dry weight of organic material for all colonization periods together (n = 15) was greatest in the substratum baskets (mean = 3.21, standard deviation = 1.93 and mean = 4.66, standard deviation = 2.67 mg/sample) than on the single rocks (mean = 0.13, standard deviation = 0.10 and mean = 0.36, standard deviation = 0.24 mg/sample) or single bricks (mean = 0.30, standard deviation = 0.31 and mean = 0.19, standard deviation = 0.16 mg/sample).

Table 4.   Number of taxa in each sample type and all of the sample types together for the McLeod River and Riffle 1 in the Battle River. Percent composition of taxa in each sample type relative to the total number of taxa in all samples is given in parentheses.

Sample Type	No. of Taxa (Percent Composition)		
	McLeod River		Battle River
	Area A	Area B	Riffle 1
Brick	20 (39%)	18 (35%)	30 (63%)
Rock	14 (27%)	20 (39%)	25 (52%)
Substratum Basket	27 (52%)	28 (54%)	-
Neill Cylinder	Area A and Area B: 33 (64%)		36 (75%)
All Samplers	Area A and Area B: 52 (100%)		48 (100%)

4.1.3   Comparison Among Colonization Periods

The total number of organisms and four of the seven most abundant taxa were at statistically different densities on the artificial substrata after the 1-, 8-, and 29-day colonization periods in Areas A and B (Table 3, Figure 3). Three taxa (Chironomidae, Baetis (Ephemeroptera), and Ameletus) and the total number of organisms were at statistically different densities on the artificial substrata in both Areas A and B (Table 3). Because only one statistical interaction was significant (section 4.1.2), the effect of colonization period was independent of the type of artificial substratum. The Student-Newman-Keuls test indicated that the densities of the taxa were generally greatest after the longest colonization periods (8 and 29 days) in contrast to the 1-day colonization period (Table 3, Figure 3).
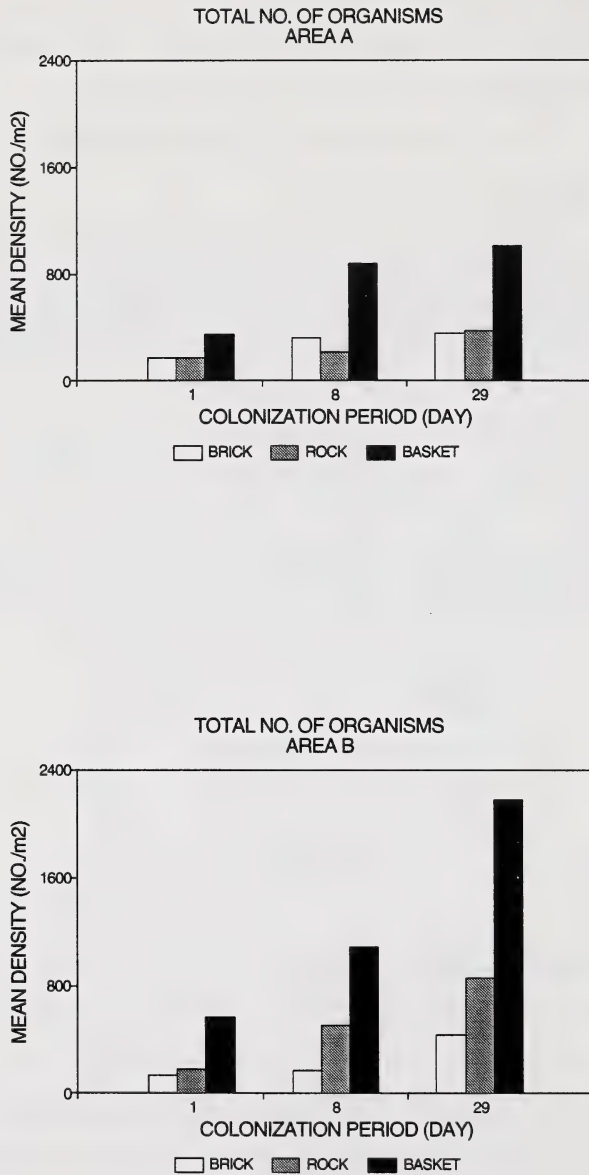
Figure 3.   Mean density (n=5) of the total number of organisms on the single brick and rock particles, and the substratum baskets after each colonization period in Areas A and B of the McLeod River.

4.2 Battle River

4.2.1 Physically Disturbed Versus Undisturbed Natural Substratum

The 10 most abundant taxa made up 99.4 % of the 22,026 organisms in the samples (Table 5). Four of the most abundant taxa and the total number of organisms were at greater densities on the undisturbed in contrast to the disturbed substratum; these four taxa accounted for 97.1% of the total number of organisms (Table 5, Figure 4). For example, the abundance of Hyalella azteca (Amphipoda) and Leptophlebia (Ephemeroptera) larvae were clearly different between the disturbed and undisturbed substrata, in contrast to the other taxa such as Optioservus (Coleoptera) and Hydropsyche (Trichoptera) larvae (Figure 5). The mean dry weight (n = 5) of organic material was greater in the undisturbed (mean = 16.43, standard deviation = 3.52 mg/sample) than in the disturbed substratum (mean = 3.74, standard deviation = 1.10 mg/sample).

Table 5.   Percent composition and results of the unpaired t-test (* = $p \leq 0.05$) for the densities (n=5) of the ten most abundant taxa and the total number of organisms in the Neill cylinder samples on the natural substratum that was physically disturbed (D) and not disturbed (ND) in the transects of the Battle River.

Taxonomic Group	Substratum Disturbance (D,ND) Probability Level	Percent Composition
Chironomidae	0.0059*	81.770
Hyalella azteca	0.0000*	13.650
Optioservus	0.3245	1.048
Stenonema	0.0201*	0.844
Leptophlebia	0.0000*	0.817
Acari	0.6315	0.454
Hydropsyche	0.2465	0.377
Cheumatopsyche	0.2545	0.300
Oecetis	0.5075	0.095
Physa	0.5951	0.059
Total no. of organisms	0.0001*	100
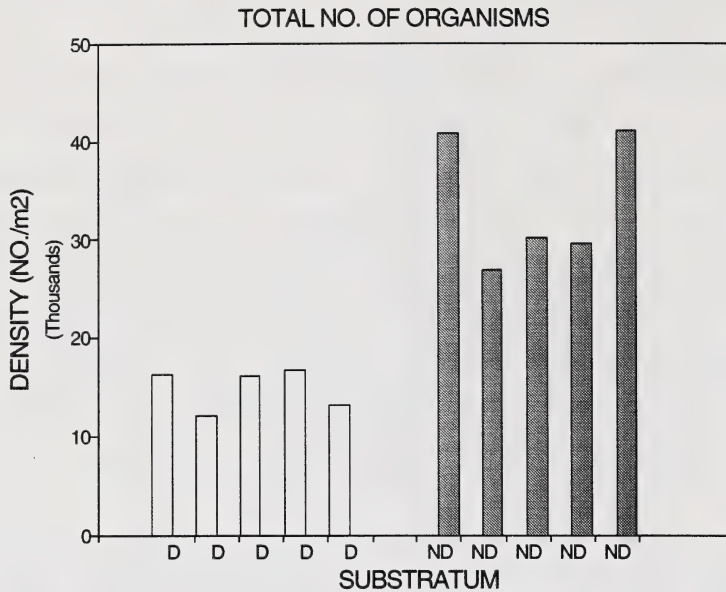
Figure 4.   Density of the total number of organisms in the samples of the natural substratum that was physically disturbed (D) or not disturbed (ND) in the Battle River.

4.2.2   Position of Artificial Substrata Relative to Natural Substratum

The 10 most abundant taxa made up 98.4 % of the 3,541 organisms on the artificial substrata (Table 6). There was no effect of the position of the artificial substrata relative to the natural substratum on the densities of the total number of organisms and the 10 most abundant taxa; Chironomidae larvae and the total number of organisms, however, were close to the statistical level of significance (Table 6). There were no differences between the densities of all taxa and the total number of organisms on the single brick and rock substrata (Table 6). The effect of substratum type and substratum position were independent of each other because there were no significant interactions between these factors in the ANOVA test.
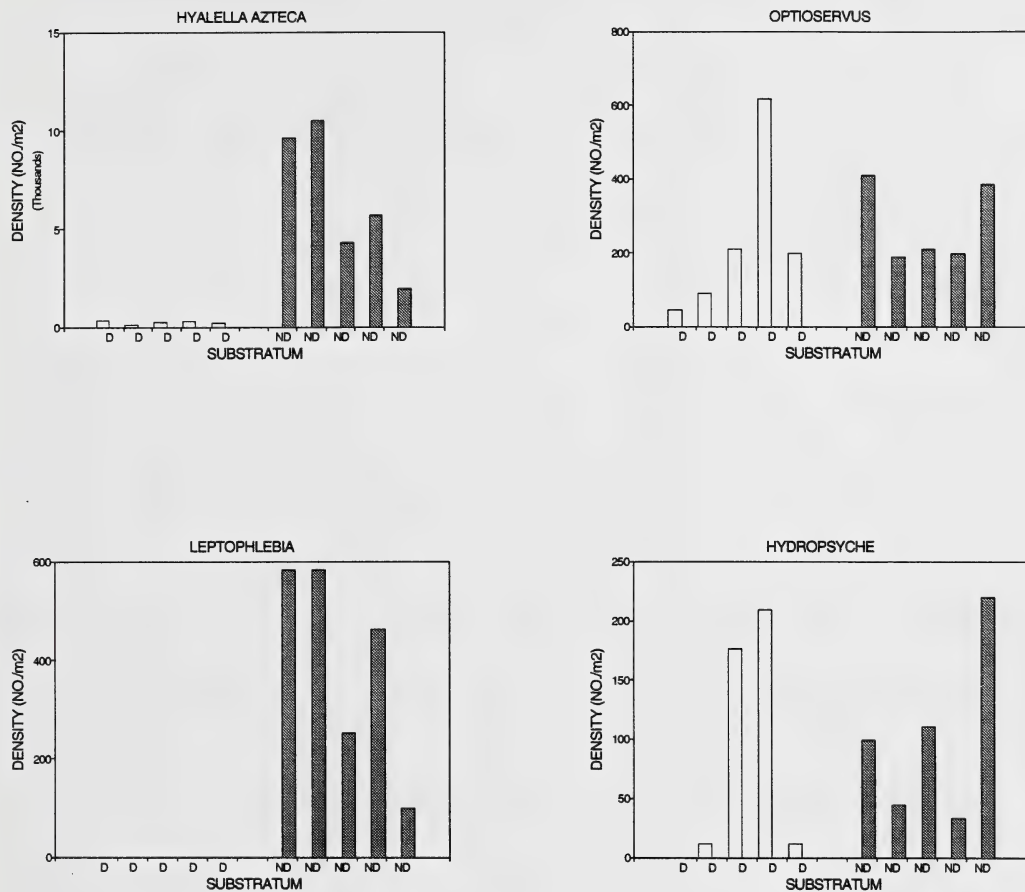
Figure 5. Density of <u>Hyalella azteca</u>, <u>Leptophlebia</u>, <u>Optioservus</u>, and <u>Hydropsyche</u> in the natural substratum that was physically disturbed (D) or not disturbed (ND) samples at the Battle River. Note that the "Density" scale is different for each taxon.

Table 6.   Percent composition and results of the two-way (substratum type and substratum position) analysis of variance (* = p ≤ 0.05) for the densities (n=5) of the ten most abundant taxa and the total number of organisms on the single brick (K) and rock (R) particles that were embedded (E) or not embedded (NE) flush with the surface of the natural substratum in Riffle 2 of the Battle River.

Taxonomic Group	Substratum Type (K,R) Probability Level	Substratum Position (E,NE) Probability Level	Percent Composition
Chironomidae	0.0506	0.1263	83.479
Hyalella azteca	0.9728	0.0513	3.163
Simuliidae	0.4001	0.1579	2.513
Stenonema	0.2751	0.7143	2.372
Acari	0.1283	0.3892	2.316
Hydropsyche	0.6179	0.5518	1.327
Cyclopoida	0.5900	0.4387	1.017
Calanoida	0.8003	0.4878	0.875
Collembola	0.4961	0.5967	0.875
Leptophlebia	0.1367	0.9295	0.452
Total no. of Organisms	0.0541	0.1929	100

4.3   Comparison Among Artificial Substrata And Natural Substratum

4.3.1   McLeod River

The densities of the total number of organisms and the seven most abundant taxa were statistically different between the three artificial substrata and the natural substratum of Areas A and B (Table 7, Figure 6). Three of 16 statistical interactions (substratum type x colonization period) were significant (Area A: Baetis - p = 0.04, Ameletus - p = 0.03; Area B: Ameletus - p = 0.03), indicating the effect of the substratum type and colonization period were independent of each other for most taxa. The Student-Newman-Keuls test indicated that the greatest densities of all taxa, with the exception of Ameletus, were on the natural substratum of Areas A and B (Table 7). The exception of Ameletus might have been caused by the significant interactions in the ANOVA test.

Table 7.   Percent composition and results of the two-way (substratum type and colonization period) analysis of variance (* = p ≤ 0.05) and Student-Newman-Keuls (SNK) tests for the densities (n=5) of the seven most abundant taxa and the total number of organisms on the substratum baskets (B), single rock (R) and brick (K) particles, and the Neill cylinder samples (N) in Areas A and B of the McLeod River. Statistical differences (p = 0.05) in the SNK test are separated into groups from the greatest to least (High-Low) density.

Taxonomic Group	Substratum (B,R,K,N) Probability Level	SNK Substratum (High-Low)	Colonization Period (1,8,29 day) Probability Level	SNK Colonization Period (High-Low)	Percent Composition
AREA A					
Chironomidae	0.0001*	N-BKR	0.2209		75.709
Baetis	0.0001*	N-BRK	0.0027*	8,29-1	4.760
Hydropsyche	0.0001*	N-BKR	0.9334		2.633
Cheumatopsyche	0.0001*	N-B-KR	0.6416		2.170
Oecetis	0.0001*	N-BRK	0.1111		2.011
Acari	0.0001*	N-BRK	0.1014		1.997
Ameletus	0.0001*	BN-NK-KR	0.0071*	29,8-1	1.128
Total no. of Organisms	0.0001*	N-B-KR	0.0008*	29,8-1	100
AREA B					
Chironomidae	0.0002*	N-BRK	0.1603		60.331
Baetis	0.0001*	N-BR-K	0.0001*	8,29-1	6.266
Cheumatopsyche	0.0001*	N-B-RK	0.0770		4.833
Acari	0.0001*	N-BRK	0.1946		4.436
Hydropsyche	0.0001*	N-B-KR	0.0977		3.711
Ameletus	0.0016*	BN-NRK	0.0057*	29,8-1	2.555
Oecetis	0.0001*	N-RBK	0.0106*	8-1,29	2.382
Total no. of Organisms	0.0001*	N-B-R-K	0.0001*	29-8-1	100
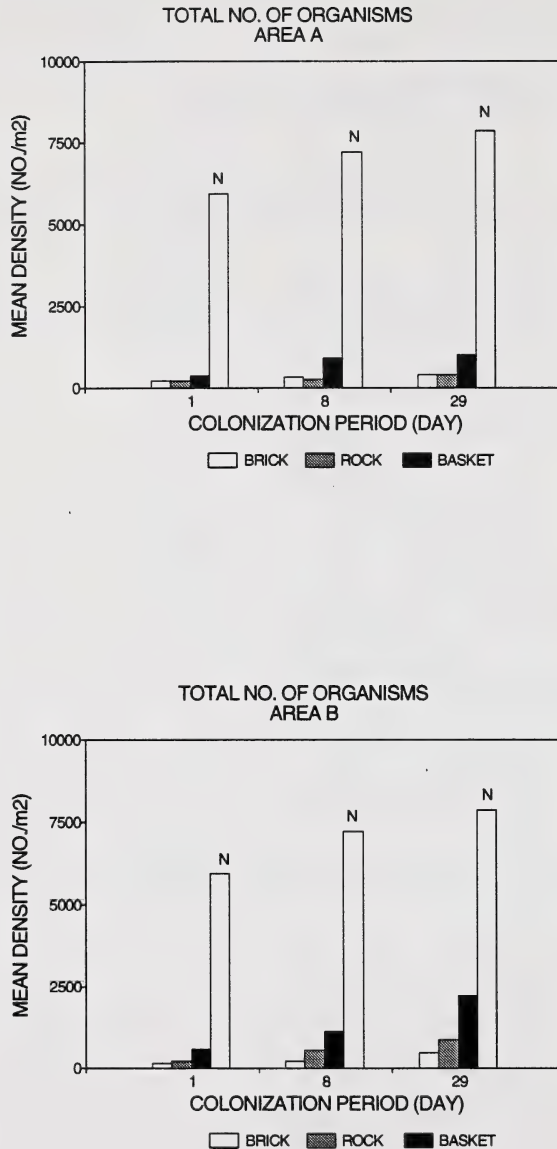
Figure 6.   Mean density (n=5) of the total number of organisms on the single brick and rock particles, substratum baskets, and the Neill cylinder (N) samples after each colonization period in Areas A and B of the McLeod River.

The taxonomic composition of the fauna on either the single rock or brick particles were not the same as on the natural substratum in Areas A and B (Table 8). However, the fauna of the substratum baskets and the natural substratum, and between the pairs of the three artificial substrata were statistically correlated or showed strong positive correlations (Table 8). The greatest number of taxa was on the natural substratum in contrast to the artificial substrata (Table 4). The mean dry weight of organic material for all colonization periods and Areas A and B together (n = 15) was greatest in the natural substratum (mean = 7.16, standard deviation = 1.74 mg/sample) than on the three artificial substrata (for three artificial substrata, range of means = 4.66 to 0.13 and range of standard deviations = 2.67 to 0.10 mg/sample).

Table 8.　　Results of the Spearman rank correlation two-tailed test for the similarity of the 10 most abundant taxa between pairs of each artificial substratum (brick: B, rock: R, and substratum basket: B) and the Neill cylinder sampler (N), and pairs of the artificial substrata at McLeod and Battle rivers. Statistically significant correlations are indicated by an asterisk where $r_s \geq 0.648$, $p = 0.05$, n=10.

Study Site	Artificial Substratum Comparison (B,R,K,N)	Benthic Cover On Rock (R) and Brick (K) Substrata	Spearman Rank Correlation(r_s) Probability Level
McLeod River	K-N	-	0.404
	K-N	-	0.583
	R-N	Absent	0.445
	R-N	Present	0.620
	B-N	Absent	0.689*
	B-N	Present	0.602
	K-R	Absent	0.619
	K-R	Present	0.905*
	K-B	Absent	0.656*
	K-B	Present	0.979*

Table 8 (Cont.)

Study Site	Artificial Substratum Comparison (B,R,K,N)	Benthic Cover On Rock (R) and Brick (K) Substrata	Spearman Rank Correlation(r_s) Probability Level
	R-B	Absent	0.859*
	R-B	Present	0.854*
Battle River	K-N	-	0.669*
	R-N	-	0.430
	K-R	-	0.841*

4.3.2   Battle River

The 10 most abundant taxa made up 95.9 % of the 32,326 organisms in the samples (Table 9). The densities of nine of the 10 most abundant taxa and the total number of organisms were different on the single rock and brick particles and the natural substratum (Table 9). Eight of the nine taxa and total number of organisms were greatest on the natural substratum in contrast to the two artificial substrata (Table 9). For all samples, the densities of organisms on the natural substratum were 10-fold greater than on the artificial substrata. For example, the mean density (n = 15) of the total number of organisms was 1851 (standard deviation = 550) on the natural substratum, in contrast to 188 (standard deviation = 98) and 117 (standard deviation = 42) organisms on the single brick and rock particles, respectively.

Table 9.   Percent composition and results of the one-way analysis of variance (* = p ≤ 0.05) and Student-Newman-Keuls (SNK) tests for the densities (n=15) of the ten most abundant taxa and the total number of organisms in the Neill cylinder (N), and single rock (R) and brick (K) particles in Riffle 1 of the Battle River. Statistical differences (p = 0.05) in the SNK test are separated into groups from the greatest to least (High-Low) density.

Taxonomic Group	Sample Type (N,R,K) Probability Level	Sample Type SNK (High-Low)	Percent Composition
Chrionomidae	0.0001*	N-RK	85.448
Stenonema	0.0001*	N-KR	4.393
Hyalella azteca	0.2731		2.100
Hydropsyche	0.0001*	N-KR	1.179
Cheumatopsyche	0.0001*	N-KR	0.931
Acari	0.0001*	N-RK	0.909
Optioservus	0.0001*	N-RK	0.408
Calanoida	0.0056*	R-KN	0.210
Taeniopteryx	0.0001*	N-RK	0.204
Cyclopoida	0.0059*	K-RN	0.158
Total no. of Organisms	0.0001*	N-RK	100

There were no differences in the taxonomic composition of the fauna on the two artificial substrata, and on the single brick particles and natural substratum, in contrast to the single rock particles and natural substratum (Table 8). The greatest number of taxa was on the natural substratum than on the two artificial substrata (Table 4). The mean dry weight (n = 15) of organic material was greatest on the natural substratum (mean = 4.57, standard deviation = 2.00 mg/sample) than on the single brick (mean = 0.85, standard deviation = 1.34 mg/sample) and rock (mean = 0.32, standard deviation = 0.30 mg/sample) particles.

4.4   Summary of Results

The densities of most of the macroinvertebrate taxa, number of taxa, and quantity of organic material were greatest in the substratum baskets in contrast to the single rock and brick particles. Most of the taxa showed an increase in their density after the longer colonization periods than after 1 day, but it was not clear if the fauna had reached an equilibrium level after the longest colonization period of 29 days. The presence of the benthic cover on the artificial substratum or the position of the artificial substrata relative to the surface of the natural substratum did not affect the density of most of the macroinvertebrate taxa.

The densities of the macroinvertebrate taxa, number of taxa, and quantity of organic material were greatest on the natural substratum than on the different types of artificial substrata at both study sites. And in general at both study sites, the taxonomic composition of the fauna (or relative abundances of the taxa) on the types of artificial substrata, with the exception of the substratum baskets, was different from the natural substratum. The taxonomic composition of the fauna among the artificial substrata was generally the same.

5   DISCUSSION

The comparisons between the artificial substrata evaluated in this study indicated that the greatest densities of macroinvertebrate taxa, number of taxa, and quantity of organic material occurred in the substratum baskets than on the single brick or rock particles. The differences between the fauna on the artificial substrata were probably caused by several factors, including the method of estimating the density of organisms, the structural complexity (or physical heterogeneity) of the substrata and the quantity of organic material on the artificial substrata.

The densities of organisms on the single brick and rock particles were based on the surface area of each particle. But the densities of the macroinvertebrates in the substratum baskets were based on the sample area of the basket unit and not the surface area of each substratum particle in the basket. Thus, the densities of organisms in the substratum baskets reported here overestimate the number of organisms per surface area of the substratum. Other studies that have used substratum baskets have also reported the number of organisms per basket as the density of organisms on the artificial substratum (e.g., Ciborowski and Clifford 1984, Peckarsky 1986, Doeg et al. 1989, Clements 1991). Investigators of these studies probably did

not measure the surface area of each substratum particle because of the considerable time and resources that would be required to measure the surface area. However, the number of organisms in the studies using substratum baskets are generally comparable because the baskets are prepared with predetermined quantities of the substratum with similar physical characteristics.

The physical heterogeneity of the microhabitat in the substratum baskets was clearly different from that on the single brick and rock particles. The substratum in the baskets was made up of more than one particle and particle size, which provided a greater diversity of habitat. Other studies on the colonization of substratum by macroinvertebrates showed changes in the abundance of organisms due to substratum size and particle size mixtures (Rabeni and Minshall 1977, Shelly 1979, Williams 1980, Erman and Erman 1984), and the surface area and shape of particles (Minshall and Minshall 1977, Khalaf and Tachet 1980, Hart 1978). No studies were found in the literature that compared the densities of the fauna on the substratum baskets and single substratum particles. The comparisons between the single brick and rock particles in the McLeod and Battle rivers showed no differences between the densities of the macroinvertebrates on these substrata. These particles had similar physical heterogeneity. Lamberti and Resh (1985) also found similar densities of macroinvertebrates on single clay tiles and natural rock particles.

The greatest quantities of organic material in the substratum baskets (about 10-fold increase) in contrast to the single brick and rock particles in the McLeod River was probably caused by the greater surface area and the number and diversity of spaces where the organic material could accumulate. This organic material probably affected the macroinvertebrate colonization of the artificial substrata. Macroinvertebrates can use organic material as a suitable habitat, for case-building material, and as a source of food. Other experimental studies have shown the numbers of organisms to increase with the quantity of detritus in substratum containers (Culp et al. 1983, Culp and Davies 1985).

The densities of macroinvertebrate taxa and the quantity of organic material were clearly greater in the substratum baskets than on the single substratum particles. However, the taxonomic composition of the fauna (or relative abundances of the taxa) on the substratum baskets and single brick and rock particles were similar. Thus, at the taxonomic level of classification used in this study, the relative abundances of the taxa, in contrast to the densities of the macroinvertebrate taxa colonizing the artificial substrata, were not affected by the physical heterogeneity of the

artificial substrata. More precise identification of the fauna to lower taxonomic levels might affect the similarity of the taxonomic composition of the fauna on the substrata in this study.

In the McLeod River, the densities of the macroinvertebrate taxa after the three colonization periods did not appear to have reached an equilibrium or stable level even after the longest colonization period of 29 days. In their review of the studies using artificial substrata, Rosenberg and Resh (1984) reported that the approximate time for macroinvertebrate taxa to reach an equilibrium level ranged from 9 to 35 days. However, they found much inconsistency in the measures of the equilibrium levels (e.g., density or biomass of organisms) used by the investigators. In this study, more frequent colonization periods and colonization periods greater than 29 days would have better represented the macroinvertebrate colonization dynamics on the artificial substrata.

Most of the macroinvertebrate taxa were not affected by the presence of the benthic cover on the artificial substrata (single rock particles and substratum baskets) at the McLeod River. The benthic cover on the substratum was mostly fine inorganic material (sand and silt), 1-2 mm thick, and occasional patches of algae. Reviews of macroinvertebrate colonization of substratum have shown that silt and algae have different effects on macroinvertebrate taxa (Minshall 1984, Mackay 1992). In one study, Rabeni and Minshall (1977) found a thin layer (about 1 mm) of silt reduced the number of organisms on the substratum. But Cummins and Lauff (1969) found no effect or an increase in the abundance of organisms on the substratum after a thin layer of silt was added to the substratum. In another study, Lamberti and Resh (1985) found different densities of macroinvertebrates on sterilized rocks than on natural rocks after five colonization periods. Algae, because it is a food, can attract macroinvertebrates. However, algae on the substratum of this study probably did not affect the abundance of the macroinvertebrates because it was rare on the substratum.

The position of the artificial substrata relative to the natural substratum (embedded or placed on the surface) at the Battle River indicated no effect on the macroinvertebrate colonization of the substrata. As noted above, identification of the fauna to lower taxonomic levels might affect this result. Researchers of other studies have either embedded or placed the substratum on the natural substratum (see Table VII of Rosenberg and Resh 1982), but these investigators did not examine the effect of the substratum position on zoobenthos colonization.

Results of these studies were not comparable to the Battle River data because of differences between the type of artificial substrata and the fauna.

The greatest densities of macroinvertebrate taxa, number of taxa, and quantity of organic material were on the natural substratum rather than on the artificial substrata. This trend was evident at the McLeod River and even at Battle River after the densities of the macroinvertebrate taxa and the quantity of organic material were substantially reduced by physically disturbing the natural substratum. Clement (1991) found a greater number of taxa on the natural substratum than in substratum trays (10 x 10 x 6 cm) of the natural substratum in five of six streams. In contrast to the McLeod River results, Clement (1991) found densities of macroinvertebrate taxa in six streams were similar, or 5 to 13 times greater, in the substratum trays than on the natural substratum. The natural substratum samples in Clement's study were obtained with a direct sampler (sample area = 0.1 m^2) that was similar to the Neill cylinder sampler (sample area = 0.09 m^2) of this study.

Of the three artificial substrata used in this study, the physical heterogeneity and the quantity of organic material in the substratum baskets were the most similar to the natural substratum. In addition, the relative abundances of the taxa on the substratum baskets and natural substratum were similar. Clements (1991) also found similarities between the abundances of the most dominant macroinvertebrate taxa on the substratum trays and natural substratum. But in the McLeod River, there were statistical differences between the densities of most macroinvertebrate taxa on the substratum baskets and natural substratum. This indicated that the macroinvertebrate assemblage on the substratum baskets might not represent the fauna on the natural substratum. This was also the case for the single substratum particles in the McLeod and Battle rivers where the densities of most of the macroinvertebrate taxa on these artificial substrata were statistically different from the natural substratum.

The results of this study show clear trends for the macroinvertebrate colonization of the artificial and natural substrata. But these results should be treated with caution since the study was conceived and conducted within a two-month period (September and October, 1992) before freeze-up. Because of this time constraint, it was not possible to replicate the experiments, and the experimental designs were affected by the availability and physical characteristics (size and quantity) of the natural substratum at each site. Also, at this time of the year, some macroinvertebrate taxa are present in the egg stage, and some taxa might be less abundant on the

substratum because the organisms move deeper into the substratum, or to deep-water habitat, to avoid being frozen to the substratum.

In conclusion, the type of sampler used in a study will depend on the objectives of the study. A direct sampler (such as the Neill cylinder) compared with artificial substrata is the most effective method for sampling the natural substratum. However, artificial substrata can be used for many studies. For example, artificial substrata can be used to sample deep-water and fast-flowing habitats when it is not possible to sample the natural substratum with a direct sampler or in experimental studies, to determine the effect of the physical, chemical, and biological characteristics of the substrata on macroinvertebrate colonization.

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APPENDIX 1

STANDARD OPERATING PROCEDURES THAT WERE DEVELOPED
AND USED IN THE FIELD AND LABORATORY FOR THIS STUDY:
(1) QUANTITATIVE SAMPLING OF BENTHIC MACROINVERTEBRATES
IN LOTIC HABITATS, AND
(2) LABORATORY PROCESSING OF BENTHIC MACROINVERTEBRATE SAMPLES

QUANTITATIVE SAMPLING OF BENTHIC MACROINVERTEBRATES IN LOTIC HABITATS

2350-AJ4/FLD/4/93

by

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Alberta Environmental Centre
Vegreville, Alberta

March 21, 1994

STANDARD OPERATING PROCEDURE

QUANTITATIVE SAMPLING OF BENTHIC MACROINVERTEBRATES IN LOTIC HABITATS

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STANDARD OPERATING PROCEDURE

QUANTITATIVE SAMPLING OF BENTHIC MACROINVERTEBRATES IN LOTIC HABITATS

1   TITLE

        Quantitative sampling of benthic macroinvertebrates in lotic habitats

2   SOP NUMBER

        2350-AJ4/FLD/4/93

3   AUTHOR

        Richard J. Casey, Laboratory Scientist, Environmental Enhancement Program

4   EFFECTIVE DATE

        September, 1993

5   SUCCESSION

        New

6   PURPOSE

        Quantitative samples of benthic macroinvertebrates in flowing water (lotic) habitats are
        sampled with a wide range of samplers, including direct samplers and artificial substrata.
        This Standard Operating Procedure (SOP) was prepared for a field study on the use of
        artificial substrata and a direct sampler, the modified Neill cylinder sampler (Alberta
        Environment 1990), for the quantitative sampling of benthic macroinvertebrates on a
        coarse substratum (mostly small cobble, pebble and gravel, approximate size range = 0.2-
        15 cm).

        The artificial substratum sampler is used to obtain quantitative samples of the organisms
        (e.g., macroinvertebrates, algae, microorganisms) in an aquatic habitat. The artificial
        substratum is placed in the habitat and retrieved after a predetermined time. The
        organisms that have colonized the artificial substratum represent the sample. The choice
        of the substratum will depend on the objectives and design of the study. Artificial
        substrata can be grouped into two main types: (1) representative artificial substrata that
        closely resemble the natural substratum of the habitat in which they are placed (e.g.,
        baskets of the natural substratum and mesh bags of leaves) and (2) standardized artificial
        substrata that are different from the natural substratum, but have identical physical and
        chemical characteristics (e.g., clay tiles and bricks, plastic sheets, and rope) (Flannagan

and Rosenberg 1982). The advantages and disadvantages of using artificial substrata are extensively reviewed by Rosenberg and Resh (1982).

The modified Neill cylinder sampler is used to sample the benthic macroinvertebrates on the coarse substratum of lotic habitats (Alberta Environment 1990). Briefly, the sampler is a stainless steel cylinder (height = 65 cm, inner diameter = 34 cm) with two circular openings that are opposite each other, i.e., the upstream-opening (diameter = 14 cm) and downstream-opening (diameter = 20 cm). A coarse mesh (1.3 cm) is attached to the upstream-opening to prevent material from entering the cylinder. The sample net (length = 85 cm) and a removable sample bottle (1 L) are attached to a flange on the downstream opening of the cylinder. The pore size of the sample net is 0.21 mm. The sample is taken by placing the Neill cylinder on the natural substratum and forcing the cylinder into the substratum to obtain a seal between the bottom-edge of the sampler and the substratum. The macroinvertebrates on the substratum, enclosed by the Neill cylinder (sample area = 0.09 m^2), are removed from the large substratum particles and the remaining substratum is vigorously disturbed to a specific depth (e.g., 10 cm). The sample material is collected in the sample net and bottle attached to the downstream-opening of the sampler. The Neill cylinder sampler can be used on mixtures of small cobble (about 6-15 cm), pebble (2-6 cm), gravel (2-20 mm), and finer substratum (<2 mm). The Neill cylinder sampler is typically used in water depth of about 30-50 cm and moderate water velocity (e.g., <80 cm/s).

7   SCOPE

The SOP can be used for experimental protocols and the monitoring and assessment of lotic habitats.

8   DISTRIBUTION

Author: R. Casey

9   SAFETY PRECAUTIONS

The safety of the operator(s) is paramount in all cases when using these sampling techniques. The operators should not sample in habitats (e.g., fast water velocities) that are likely to endanger the safety of the operators. Preservatives should be handled with caution following all pertinent safety procedures including the Material Safety Data Sheets.

10   EQUIPMENT/MATERIALS

Equipment and materials are given in the procedure.

11   PROCEDURE

11.1   Artificial Substratum Samplers

The method described here is for the use of (1) single natural rock particles, (2) baskets of the natural substratum, and (3) single clay bricks. The same method or modifications to the method can be used for other artificial substrata (e.g., single tile particles).

11.1.1   Selection of the substratum

11.1.1.1   Natural substratum

Select the chemical and physical characteristics of the substratum. For example, sandstone rocks that are oval-shaped with a maximum length of 10 to 15 cm and a maximum width of 5 to 10 cm (the width measurement is taken perpendicular to the axis of the length measurement). Choose a suitable location where there is sufficient quantity of the substratum.

The proportions and quantities of substratum types in the substratum baskets can be determined based on the objectives of the study. Fine substratum, such as gravel and sand, can be placed in the baskets beneath the larger substrata. Using the fine substratum with the larger rocks often provides a substratum that is typical of the natural substratum.

11.1.1.2   Clay bricks

The substrata should be identical in all physical and chemical characteristics. Thus, the bricks should be purchased from the same supplier and from the same batch to reduce differences between the bricks.

11.1.2   Preparation of the artificial substratum

11.1.2.1   Natural substratum

The substratum should be thoroughly cleaned to remove inorganic (e.g., fine sediments) and organic material (e.g., algae, microorganisms) that is not part of the substratum particle. Thus, colonization of the substratum will only be influenced by the characteristics of the clean substratum.

Thoroughly clean the substratum in containers of suitable size. Clean water from the study site can be used to scrub and wash the surface of the substratum. A nail brush with stiff fibres or a wire brush can be used to clean the rocks. If necessary, a sharp object can be used to remove material that is firmly attached to the substratum. Weak solutions of acid can be used to clean the substratum, but this may not be practical in many field studies.

If fine substratum (sand and gravel) is used as the artificial substratum, wash and rinse the substratum in water to remove the organisms and lighter organic material (see SOP 2350-AJ4/FLD/5/993). Discard substratum with attached material (e.g., algae) that is difficult to remove from the substratum.

For the substratum baskets, place all baskets in a convenient group so that the physical characteristics and the arrangement of the substratum particles can be compared as the baskets are being filled.

Place equal amounts of the substratum types, such as sand and gravel, and cobble and pebble in each basket. Arrange the substratum in the baskets so that it is similar to the substratum at the study site (e.g., with the coarse substratum on top of the fine substratum). The substratum can be partitioned into size classes using a series of sieves.

For the single rocks, choose rocks with similar physical characteristics (e.g., size, shape and surface area).

11.1.2.2   Clay bricks

Thoroughly clean the bricks to remove loose inorganic material. Wash and rinse the bricks with tap water and allow them to dry. If necessary, a nail brush with stiff fibres or a wire brush can be used to clean the bricks.

11.1.3   Selection of the study site and installation of the artificial substratum

Selection of the study site will depend on the objectives of the study. In general, study sites that are being compared should have uniform habitat characteristics (such as the natural substratum, and the water velocity and depth).

Mark the study site (e.g., with metal pegs and flagging tape).

Disturbance to the natural substratum of the marked area should be minimized by eliminating or reducing activity within the area. Install the substrata, working from upstream to downstream sites.

Artificial substrata can be embedded flush with the surface of the natural substratum or placed directly on the natural substratum.

To embed the substratum, remove an area of the natural substratum that is greater than the artificial substratum. Place the artificial substratum in the excavation. Then, arrange the natural substratum around the artificial substratum to hold it in place.

If the water flow is likely to disturb the artificial substratum, secure the substratum to the stream bed.

11.1.4   <u>Retrieval of the artificial substratum</u>

11.1.4.1   Natural substratum and clay bricks

Remove the artificial substratum with minimum disturbance to the natural substratum and the surrounding habitat. Approach the artificial substratum from downstream to upstream sites. Retrieve the substrata immediately, spending about the same amount of time at each sample site.

Place the retrieval net on the natural substratum immediately downstream of the artificial substratum. The retrieval net is used to collect organisms and other sample material that are displaced when the artificial substratum is removed from the stream bed and to transport the artificial substratum. The retrieval net (e.g., pore size = 0.21 mm) is secured in a metal frame (e.g., 30 x 30 cm) that is greater than the diameter of the artificial substratum.

For the individual rock and brick particles, lift the substratum and move the net under the artificial substratum.

Lift the substratum and net from the water. Carry the substratum in the net to the stream bank for sample processing.

For the substratum baskets, hold the net downstream of the basket and place the three-sided shovel at the downstream edge of the basket. The three-sided shovel can be constructed from a square piece of aluminum sheet that is cut and formed into the shape of the substratum basket. The frame of the net should surround the downstream edge of the three-sided shovel.

Lift the downstream edge of the basket into the shovel and slide the shovel underneath the basket.

Lift the net and the three-sided shovel containing the basket from the water so that excess water and other sample material from the basket and the three-sided shovel drains into the net. Carry the substratum and shovel in the net to the stream bank for sample processing.

11.1.5   <u>Sample processing of the artificial substratum sample at the study site</u>

Whenever possible, avoid damaging the organisms in the sample. Identification of the organisms to an appropriate taxonomic level will be hindered if they are damaged.

Add clean river water to a large sample container (e.g., 12 L pail, henceforth called the sample pail) that will contain the sample material. Then, remove the artificial substratum from the net and carefully place the substratum particle(s) in the sample pail.

Use clean river water to concentrate the remaining sample material in the bottom of the net.

Use a wash bottle and forceps to remove the sample material and organisms from the net into the sample bottle of appropriate size or the sample pail.

Wash all surfaces of each large substratum particle (cobble, pebble and large gravel; approximate size range = 1-26 cm) into the sample pail with a wash bottle or similar apparatus (e.g., turkey baster).

Examine the entire surface of the large substratum particles for organic material (algae and detritus) and macroinvertebrates and place them in the sample pail.

Add water to the sample pail and agitate the pail in a circular motion to separate and suspend the lighter organic material (including many of the organisms) from the inorganic material. Heavy organisms, such as snails, bivalves, tube-dwelling chironomid and cased caddis fly larvae, will remain in the inorganic material.

Pour the water and suspended sample material from the sample pail through the sieve that is the same pore size as the retrieval net.

Continue to add water to the sample pail and pour the solution into the sieve until all of the organic material is separated from the heavier inorganic material of the sample. Carefully examine the remaining inorganic material for organisms (e.g., snails and caddis fly larvae) and add these organisms to the sample bottle.

Add the sample material in the sieve to the sample bottle.

Preserve and label the sample. A solution of rose bengal can be added to the sample to stain the organisms, thereby reducing the time to process the sample (see section 11.3).

11.2   Sampling Macroinvertebrates Using The Modified Neill Cylinder Sampler

11.2.1   Selection of sample site

Select the sample site with suitable substratum water velocity and water depth. A typical substratum might be composed of small cobble, pebble and gravel and finer substrata (sand and silt). The Neill cylinder is not an appropriate sampler for sampling a large cobble substratum.

11.2.2   Sampling

Sample from downstream to upstream sites to prevent disturbance to the organisms on the substratum to be sampled.

Prepare the Neill cylinder by attaching the clean net and sample bottle to the flange of the downstream-opening of the sampler.

Carry the Neill cylinder to the sample site with the net held on the outside of the cylinder and the sample bottle on the inside of the top opening of the cylinder. Approach from and stand downstream of the sample site.

Place the Neill cylinder on the substratum to be sampled. The operator should always stand downstream of the Neill cylinder so that material upstream of the sampler will not be disturbed and enter the upstream-opening of the cylinder.

Use the cylinder handles to apply pressure to the cylinder. Simultaneously rotate the cylinder in a circular motion in a clockwise and counter-clockwise direction to drive the sampler into the substratum. It may be necessary to use two operators to apply enough force to drive the sampler into the substratum. The footrests on the sampler can be used to hold the sampler firmly on the substratum.

Check the seal between the inside of the cylinder and the substratum by hand. There should be no space between the substratum and the cylinder. If a seal is not obtained, move the sampler to a new undisturbed sample site.

Submerge the net and sample bottle in the river water. Remove excess air from the net by submerging it in the water and allow the net and sample bottle to float between the operator's legs.

Remove the large rock particles (cobble and pebble) of the top layer of the substratum and brush off the organisms and benthic cover including attached algae, macrophytes and sediment. This can be done by hand. If necessary, a brush or a blade can be used to clean the rocks. Discard the clean rock particles outside the Neill cylinder.

Thoroughly disturb by hand the remaining substratum enclosed by the sampler. A narrow shovel or similar object can be used if the substratum is hard-packed and difficult to disturb by hand. The depth of disturbance to the substratum should be the same for all samples (e.g., 10 cm) and be reported in the study results. The same amount of time should be spent taking each sample.

Leave the Neill cylinder in place at the sample site until the water enclosed by the cylinder is clear, similar to the river water outside the cylinder. This will permit the sample material to leave the cylinder and collect in the net and sample bottle. Gently brush the net on the outside to displace the sample material into the sample bottle.

When sampling is complete, lift the upstream bottom edge of the Neill cylinder from the substratum allowing the water in the cylinder to flow through the downstream-opening of the cylinder into the net and sample bottle. Then, lift the cylinder from the water, permitting the remaining water to drain into the sample bottle and through the net.

Carry the cylinder to where the sample will be processed, usually close to the shoreline.

Remove the sample net with the sample bottle attached from the Neill cylinder.

Remove material attached to the downstream and upstream openings of the cylinder and add this material to the sample.

Use clean river water to wash the sample material from the net into the attached sample bottle. This can be done by partially submerging the net (with the net-opening above the water) several times in the river water.

Hold the sample bottle over a container that is capable of holding the entire sample (e.g., 12 L pail, henceforth called the sample pail) and carefully remove the sample bottle from the net. Excess water can be drained from the sample bottle into the sample pail.

Examine the net for organisms and other sample material and add to the sample bottle or sample pail.

Wash the sample material from the sample pail into a sieve that has the same pore size as the net of the Neill cylinder sample. Transfer this material to the sample bottle.

Preserve and label the sample. A solution of rose bengal can be added to the sample to stain the organisms, thereby reducing the time to process the sample (see section 11.3).

Thoroughly clean the Neill cylinder sampler and the net between sample sites.

11.3   Preservation, Staining and Labelling Macroinvertebrate Samples

Most benthic macroinvertebrate samples can be preserved by adding ethanol to obtain a sample solution of about 80% ethanol. If there is a large quantity of organic and/or inorganic material in the sample, the preservative should be replaced (e.g., when the samples are taken back to the laboratory) to maintain the correct ethanol concentration in the sample. A stock solution of about 90% ethanol can be used in the field.

For certain macroinvertebrate taxa, such as soft-bodied organisms (e.g., leeches, oligochaetes, flatworms), the specimens should be fixed in 10% formalin for about 10 minutes before they are preserved in ethanol. For more details on the procedures of fixing and preserving benthic macroinvertebrates, see the recent methods manuals of Environment Canada and the United States Environmental Protection Agency (Klemm et al. 1990, Gibbons et al. 1993).

The time spent sorting the sample can be reduced by adding a solution of rose bengal to the sample in the field. Staining the organisms will assist in separating the fauna from the remaining sample material. The rose bengal solution is made up of 250-500 mg of rose bengal dissolved in 1 L of the preservative.

Each sample container should have a label with the pertinent information, including a sample identification code, sampling date, study site and the name of the sample collector(s). Labels should be made from strong paper (e.g., waterproof or bond paper with a high rag content). Information on the label should be written using a lead pencil. When the samples are processed in the laboratory, new sample labels will include additional information such as the type of sample fraction (coarse, fine), taxonomic group, number of subsamples, etc.

12   QUALITY ASSURANCE/QUALITY CONTROL

R. Casey

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LABORATORY PROCESSING OF BENTHIC MACROINVERTEBRATE SAMPLES

2350-AJ4/FLD/5/93

by

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March 21, 1994

STANDARD OPERATING PROCEDURE

LABORATORY PROCESSING OF BENTHIC MACROINVERTEBRATE SAMPLES

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STANDARD OPERATING PROCEDURE

LABORATORY PROCESSING OF BENTHIC MACROINVERTEBRATE SAMPLES

1   TITLE

         Laboratory Processing of Benthic Macroinvertebrate Samples

2   SOP NUMBER

         2350-AJ4/FLD/5/93

3   AUTHOR

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4   EFFECTIVE DATE

         September, 1993

5   SUCCESSION

         New

6   PURPOSE

         The method of processing the sample will depend on the type and quantity of organic (algae, macrophytes, detritus) and inorganic (clay, silt, sand) material in the sample. For example, samples obtained on a coarse substratum (pebble and gravel) with little benthic cover will not be difficult to process in contrast to a sample of a fine substratum (sand and silt) with abundant macrophytes.

         The procedure described here is for the processing of macroinvertebrate samples obtained using a modified Neill cylinder sampler and artificial substrata (see SOP 2350-AJ4/FLD/4/93) in flowing water (lotic) habitats. The procedure includes methods for separating the coarse and fine fractions of the sample, subsampling the abundant taxa, separating the organic and inorganic material in the fine fraction, calculating the dry weight of organic material in the sample, and calculating the surface area of natural rock particles.

7   SCOPE

The SOP can be used for the laboratory processing of benthic macroinvertebrate samples that are collected in experimental protocols and the monitoring and assessment of lotic habitats.

8   DISTRIBUTION

Author: R. Casey and S. Kendall

9   SAFETY PRECAUTIONS

Preservatives should be handled with caution following the Material Safety Data Sheets.

10   EQUIPMENT/MATERIALS

Equipment and materials are given in the procedure.

11   PROCEDURE

11.1   Separating and Sorting the Coarse and Fine Fractions

Thoroughly rinse the preservative from the sample using a gentle flow of tap water. A strong flow of water will damage the fragile structures (e.g., the external gills of Ephemeroptera larvae) and organisms in the sample. Identification of the organisms can be hindered if the animals are damaged.

Rinse the sample with tap water through the 1.18 mm and 0.12 mm sieves to separate the material into coarse (> 1.18 mm) and fine (< 1.18 mm and > 0.21 mm) fractions. The pore size of the sieves used to separate the coarse and fine fractions can be determined based on the composition of the size particles in the sample. The smallest sieve size should be equal to the pore size of the net used to sample the organisms (e.g., 0.21 mm).

Use a fine-pointed forceps and a dissecting microscope at 60x or greater magnification to extract the organisms from the coarse and fine fractions of the sample.

Examine small portions of the sample in a petri dish (diameter = 9 cm, depth = 13 mm) containing about 3 mm depth of water. Divide the petri dish into equal sections (e.g., using a permanent marker) and examine each section twice in a systematic manner.

Preserve the organisms in the coarse and fine fractions in separate vials.

Organisms can be separated into taxonomic groups (e.g., orders or families) and placed in separate vials. This will be helpful for the subsequent identification and enumeration

of the organisms in the samples. Place labels with the appropriate information in each vial (see SOP 2350-AJ4/FLD/4/93).

Taxa that are present in large numbers in the fine fraction can be subsampled (see sections 6, 11.2).

11.1.1   Coarse fraction

Examine the contents of the coarse fraction under the dissecting microscope at 60x or greater magnification. Extract all organisms from the coarse fraction.

Firstly, extract the organisms from the large inorganic (e.g., gravel) and organic (e.g., twigs, leaves) material in the petri dish. Then, examine small amounts of the remaining sample material in the petri dish.

Separate the sample material, such as aquatic mosses and algae, to ensure that the organisms are not attached or concealed in the material.

11.1.2   Fine fraction

Examine the contents of the fine fraction under the dissecting microscope at 60x or greater magnification. Extract all of the organisms, except taxa that will be subsampled from the fine fraction (see section 11.2). Extraction of the organisms in the finer inorganic material can be expedited by separating the organic and inorganic material (see section 11.3).

Examine small amounts of the sample material in the petri dish. When the sample material is separated and dispersed in the petri dish, it should not conceal the entire bottom surface of the dish.

Care should be taken to separate all of the organisms from the sample material. Most of the small macroinvertebrates will be present in this sample fraction.

11.2   Subsampling of the Fine Fraction

Subsampling of a taxon is generally used when there is a large number of organisms in the sample. For example, more than 100 organisms for the taxon in the sample can be used as a general rule for deciding when to subsample. The subsampling method described here is based on the method of Wrona et al. (1982). The method is only suitable for the subsampling of taxa that will remain suspended in a mixture of the sample and water when it is agitated. Common taxa that can be subsampled using this method include Chironomidae, Ephemeroptera and Plecoptera. Heavier organisms such as the cased caddis fly larvae (Trichoptera) and Mollusca cannot be subsampled using this method. Discussion of other subsampling methods can be found in Klemm et al. (1990) and Gibbons et al. (1993).

The subsampling apparatus consists of a 1 L graduated Imhoff settling cone and an aquarium air stone, connected to an air supply, that is sealed with silicone in the bottom of the cone.

Place the sample in the Imhoff cone. Allow a small amount of air to bubble through the air stone to prevent liquid draining into the air stone and tubing at the base of the Imhoff cone.

Fill the Imhoff cone with tap water to the 1 L mark. Large clumps of the sample material, such as moss and algae, should be carefully separated.

Adjust the amount of air bubbling through the sample to minimize damage to the organisms. Excessive bubbling will damage delicate organisms or structures (e.g., the gills of Ephemeroptera nymphs). Identification of organisms will be hindered if they are damaged.

The physical disturbance caused by the air bubbles will separate the organic material and organisms from the remaining inorganic material to give a homogenized sample of the lighter material in suspension. Allow air to bubble through the sample for 3 to 5 minutes.

Take ten 50 ml subsamples from a mid-point in the sample mixture with a 50 ml vial attached to the end of a rod (e.g., length = 25 cm, width = 0.5 cm).

Place each subsample in a labelled petri dish (diameter = 9 cm, depth = 13 mm).

Use the dissecting microscope at 60x or greater magnification to extract all specimens of the subsampled taxon.

Extract the organisms from at least two of the 50 ml subsamples.

Continue to extract the organisms from each 50 ml subsample until the total number of the subsampled taxon is greater than 100 organisms.

Record the number of subsamples that are processed in this manner so that the number of organisms in the subsampled taxon can be calculated. Estimate the number of organisms per sample volume (1 L).

11.3   Separating the Organic and Inorganic Material in the Fine Fraction

The method described here is for the separation of the organic material (e.g., leaf and wood particles and algae) and organisms from the inorganic material (clay, silt and sand) in the fine fraction (< 1.18 mm, see section 11.1).

Place the sample in the sieve size that is the same as the pore size of the sampler net (e.g., 0.21 mm). Thoroughly rinse the preservative from the sample using a gentle flow of tap water.

Rinse the sample material into a 2 L beaker. Fill the beaker to about 1/3 of the volume. Use a gentle flow of tap water by pouring the water onto the edge of the beaker to avoid damaging the organisms. A strong flow of water will damage the fragile structures (e.g., the external gills of Ephemeroptera larvae) and organisms in the sample. Identification of the organisms can be hindered if the animals are damaged.

Agitate the sample by swirling the solution in a circular direction. If necessary, use a glass rod to separate the material. This action will separate the lighter organic material (including most of the organisms) from the heavier inorganic portion of the sample.

Pour the suspended lighter material into the sieve.

Continue to add water and pour off the lighter material until only the inorganic material remains in the 2 L beaker. Heavy organisms, such as cased caddis fly and tube-dwelling chironomid larvae and molluscs, will remain in the inorganic material.

Extract all organisms from the organic and inorganic fractions of the sample.

11.4   Dry Weight of the Organic Material in the Sample

The method described here is for measuring the dry weight of the organic material after the organisms have been extracted from the sample.

Separate the organic and inorganic material in the sample (see section 11.3). Preserve the organic material to make a solution of about 80% ethanol until the weight of the sample is measured.

Rinse the sample with tap water into the sieve of the same pore size as the sampler net (e.g., 0.21 mm). Thoroughly rinse the preservative from the sample.

Drain excess water from the sample material in the sieve. Place the sample in weigh boats that have been previously weighed.

Place the samples in the drying oven at 60°C.

Dry the samples to a constant weight. The time necessary to dry the sample to a constant weight can be determined from preliminary measurements on a test sample.

Place the samples in a desiccator after they have been removed from the oven and before they are weighed.

Measure the weight of the organic material and the weigh boat (e.g., to two numbers after the decimal point) until three successive weights are the same. Subtract the weight of the weigh boat from this measurement to obtain the weight of organic material.

11.5   <u>Surface Area of Natural Rock Particles</u>

The method described here is for measuring the surface area of the cobble-sized (diameter = 6-26 cm) natural substratum. The substrata are oval-shaped and laterally flattened.

The substratum volume is obtained by measuring the volume of water displaced by the substratum. The apparatus that can be used to measure the volume displacement consists of a 12 L pail with Tygon tubing (inner diameter = 1 cm) attached to a hole that is located (diameter = 1.5 cm) about 5 cm below the top rim of the pail.

Place the pail on a level surface. Fill the pail with water to the level of the opening in the side of the pail. Allow excess water to drain through the attached tubing.

Slowly place the substratum in the pail until the rock is completely submerged. Care should be taken to ensure that the water displaced from the pail is caused only by the addition of the substratum. If necessary, the volume of the pail can be reduced by placing an object in the bottom of the pail so that the substratum can be submerged just below the water surface.

Allow the water displaced by the substratum to drain into a graduated cylinder. The volume of the water in the graduated cylinder is equivalent to the substratum volume (cm^3).

The mean circumference (cm) of the substratum is calculated from four measurements that are made using a length of string. The number of circumference measurements can be increased for more accurate estimates of the mean circumference. This will be necessary, especially for irregular-shaped substrata.

Place the substratum on a flat surface and mark the mid-point on the upper and lower surfaces of the substratum. All the circumference measurements will be made through these two points.

Use an appropriate length of string and a ruler to measure the four circumferences that are equally spaced (i.e., 45°) apart. The mean circumference is calculated from the four measurements.

The surface area of the substratum particle is calculated using the following equation (derived from the formulae in Perry and Chilton 1992):

Surface Area of Substratum = (6/Diameter) x Volume Displacement of Substratum

where, Diameter = Mean Circumference of Substratum x 0.31831

12   QUALITY ASSURANCE/QUALITY CONTROL

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13   REFERENCES

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APPENDIX 2

MACROINVERTEBRATE TAXA PRESENT (*) IN THE ARTIFICIAL SUBSTRATUM
AND NEILL CYLINDER SAMPLES AT THE MCLEOD AND BATTLE RIVERS

Taxon			Study Site	
			McLeod River	Battle River
P	Arthropoda			
C	Arachnida			
SC	Acari		*	*
C	Crustacea			
	SC	Branchiopoda		
	O	Cladocera	*	*
	F	Daphnidae	*	*
	SC	Copepoda		*
	O	Calanoida		*
	O	Harpacticoida	*	*
	O	Cyclopoida		*
	SC	Malacostraca		
	O	Amphipoda		
		Gammarus lacustris		*
		Hyalella azteca		*
C	Insecta			
O	Collembola		*	*
O	Emphemeroptera			
	F	Baetidae	*	*
		Baetis	*	*
	F	Heptageniidae	*	*
		Stenonema	*	*
		Cinygma	*	*
		Heptagenia	*	*
		Rhithrogena	*	
	F	Emphemerellidae	*	
		Ephemerella	*	
	F	Siphlonuridae	*	
		Ameletus	*	
	F	Leptophlebiae		
		Leptophlebia	*	*
		Paraleptophlebia	*	*
	F	Tricorythidae		
		Tricorythodes	*	*
	F	Polymitarcyidae		
		Ephoron	*	*
	F	Ephemeridae		
		Hexagenia		*
		Ephemera	*	
	F	Caenidae		*
		Caenis		*
		Brachycercus	.	*

Taxon			Study Site	
			McLeod River	Battle River
O	Trichoptera			
	F	Hydropsychidae		*
		Hydropsyche	*	*
		Cheumatopsyche	*	*
	F	Rhyacophilidae		
		Rhyacophila	*	*
	F	Hydroptilidae	*	*
		Hydroptila	*	*
		Agraylea		*
		Ochrotrichia		*
	F	Lepidostomatidae		
		Lepidostoma	*	*
	F	Leptoceridae		
		Oecetis	*	*
	F	Brachycentridae		
		Brachycentrus	*	*
	F	Polycentropidae		*
		Neureclipsis	*	*
	F	Polycentropus		*
	F	Phryganeidae		
		Ptilostomis		*
	F	Limnephilidae	*	
O	Plecoptera			
	F	Pteronarcyidae		
		Pteronarcys	*	*
	F	Perlidae		
		Claassenia	*	*
		Acroneuria	*	
	F	Perlodidae	*	*
		Isogenoides	*	
		Isoperla		*
		Skwala	*	
		Cultus	*	
		Kogotus	*	
	F	Taeniopterygidae		
		Taeniopteryx		*
	F	Peltoperlidae		
		Yoraperla	*	
	F	Chloroperlidae	*	*
	F	Capniidae	*	
	F	Leuctridae	*	

Taxon			Study Site	
			McLeod River	Battle River
O	Diptera			
SO	Nematocera			
F		Chironomidae	*	*
F		Simuliidae	*	*
F		Ceratopogonidae	*	
SF		Ceratopogoninae		*
F		Tipulidae		
SF		Limoniinae	*	*
SO	Brachycera			
F		Anthomyiidae		
F		Empididae	*	*
O	Odonata			
SO		Anisoptera		
F		Corduliidae		
		Somatochlora		*
F		Gomphidae		
		Ophiogomphus	*	*
O	Coleoptera			
F		Elmidae(L)	*	*
		Optioservus	*	*
		Dubiraphia		*
		Narpus		*
F		Elmidae(A)	*	*
F		Hydrophilidae(A)	*	*
		Helophorus		*
F		Hydraenidae		
		Ochthebius		*
F		Dyticidae(A)		*
F		Corixidae(A)		*
F		Haliplidae(L)		*
		Haliplus		*

	Taxon		Study Site	
			McLeod River	Battle River
P	Annelida			
SC	Oligochaeta		*	
F		Tubificidae		*
F		Naididae	*	*
SC	Hirudinea			
O		Pharyngobdellida		
F		Erpobdellidae		
		Erpobdella punctata		*
O		Rhynchobdellida		
F		Glossiphoniidae		*
		Batrachobdella picta		*
		Placobdella parasitica		*
		Marvinmeyeria lucida		*
P	Nematoda		*	*
P	Mollusca			
C	Gastropoda			
SC		Pulmonata		
		Ferrissia	*	
		Bakerlymnaea	*	*
F		Physidae		
		Physa	*	*
F		Planorbidae		
		Promenetus umbilicatellus	*	*
SC		Prosobranchia		
F		Hydrobiidae		
		Probythinella lacustris	*	*
C	Pelecypoda			
F		Unionidae		
F		Sphaeriidae		
		Sphaerium	*	*
		Pisidium	*	*

P=PHYLUM
C=CLASS
SC=SUBCLASS
O=ORDER
SO=SUBORDER
F=FAMILY
SF=SUBFAMILY