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Comparison of Macroinvertebrate Colonization of Different Substrates in Artificial Substrate Basket Samplers to Determine Stream Quality

Hannah-Beth Griffis
Earth Science/Geography
Joliet Junior College
1215 Houbolt Road
Joliet, Illinois 60431USA

Faculty Advisor: Dr. John Griffis

Abstract

Many different sampling techniques are used to collect macroinvertebrates to determine stream quality, and artificial substrate samplers are becoming a more popular choice as a sampling technique. Three basket samplers, one filled with limestone bank rock, one filled with brick, and one filled with oak leaves, were placed in a creek for 20 days. The rock substrate resulted with the highest total abundance, with the brick substrate resulting in the least. All three substrates had similar Macroinvertebrate Biotic Indices (MBI). The leaf substrate resulted in significantly lower EPT specimens when compared to both rock and brick substrates ($p<0.001$, $p=0.0012$, respectively), and lower pollution intolerant/tolerant specimens when similarly compared ($p<0.001$, $p=0.015$. respectively). When the brick substrate was compared to the rock substrate, both EPT/Non-EPT and pollution intolerant/tolerant specimens were found to be not significantly different ($p=0.21$, $p=0.41$). Although the leaf pack was thought to be the most diverse habitat, it resulted in similar number for the Shannon Diversity Index as the rock and brick substrates. The rock and brick substrates had similar results in regard to EPT, pollution intolerant/tolerant taxa, and overall stream quality determination. Different substrates attracted different taxa, yet did not significantly alter conclusions regarding stream quality.

Keywords: Stream quality, macroinvertebrates, artificial substrates

1. Introduction

Stream quality is often observed by looking at the aquatic macroinvertebrates that inhabit the stream. There are many factors that can affect the stream quality, including pollutants, sediment, organic wastes, nutrient enrichment, temperature elevation, channelization, and toxic chemicals[1]. Aquatic macroinvertebrates are used to study the stream quality for many different reasons. They can reflect the whole ecological quality of a habitat, provide a measure for changing environmental conditions, and can be measured in an inexpensive way[2]. Some macroinvertebrates are also very susceptible to pollution (pollution intolerant) while others can survive in harsher conditions (pollution tolerant). For this reason, macroinvertebrates are good indicators for observing trends in stream quality. They are also easy for the researchers to work with. Sampling streams for macroinvertebrates is a simple procedure and effective because they do not move very fast[1]. There are many reasons for the use of macroinvertebrates as an indicator for water quality, but there is still debate over the best way to collect them.

Different sampling techniques are used in the collection of macroinvertebrates that provide varying results. A common technique that is used is kick net sampling. This type of sampling is conducted by holding a dip net against the bottom of the stream and disturbing the substrate in front of the net by kicking or shuffling[1]. Another technique of sampling is to use artificial substrates. These collection devices are usually placed in the stream for several weeks.

After removal of the devices, macroinvertebrates are then collected from them. One common type of artificial substrate is the Hester-Dendy. A Hester-Dendy is a device made of multiple plates with spacers in between each one. The plates are often attached to a cement block to hold them under water. Rock baskets are another type of artificial substrate that can be used to sample macroinvertebrates in streams. These baskets are usually some type of wire cage filled with different substrates. A wide range of substrates can be used inside of the baskets, such as, bricks, rocks, cement, etc.

The results obtained from different types of sampling have been compared by various researchers. One comparison is between kick net sampling and Hester-Dendy sampling. Letovsky *et al* compared kick net sampling and Hester-Dendy sampling and found a significant difference between the two techniques with the kick net sample having a higher taxa richness and Shannon Diversity Index (SDI)[3]. Another comparison made was done between kick net sampling and rock basket sampling by Crossman and Cairns, who compared a bag filled with bricks, similar to a rock basket, to a kick net sampling technique and determined them to not be significantly different[4]. Comparisons between Hester-Dendy sampling and rock basket sampling have also been made. Hall found the basket sampling resulted in higher macroinvertebrate density, biomass, and taxa number when compared to Hester-Dendy sampling[5]. It is important to know how the artificial substrate techniques compare to the standard kick net sampling to determine how to interpret results accurately in regard to stream quality. It is also important to compare the substrates within the artificial sampling techniques and these studies are much rarer.

Magoulick performed an experiment that compares the colonization of wood with different hardness, condition, texture, and substrate type. Different taxa preferred different wood hardness, condition, and substrate type but did not prefer one texture over another[6]. Another study, conducted by Schmude *et al*, compares macroinvertebrate collection by a basket filled with cement balls, imitating a riprap shoreline, to cement patio tiles, imitating a vertical retaining wall. They found the two substrates to be significantly different in total abundance and in taxa richness[7]. Saliu and Ovuorie compared macroinvertebrate colonization of four different substrates; Kakaban, gravel bucket, glass, and wood. Their experiment resulted with the gravel bucket collecting the highest number of macroinvertebrates while the glass substrate did not collect any macroinvertebrates. Their findings show great selectivity for different substrates by macroinvertebrates[8]. Studies like these are important to take into account when using artificial substrates. The main objective of this research was to compare the results of three rock baskets filled with different substrates; rock, brick, and leaves, to see if the different substrates are colonized by different macroinvertebrate taxa, and result in different stream quality determination.

2. Methods

The study site was located at Van Horn Woods East in Plainfield, IL. Van Horn Woods East is just South of US route 30 and can be accessed from US 55 West Frontage Road. The study site (or reach) consisted of a 61-meter section of Will County Lily Cache Creek. The sampling was done by using three rock baskets with different substrates (Figure 1). The rock baskets are made out of plastic coated wire 25 cm in length and 19 cm in diameter with 2.5 cm openings. One rock basket was filled with 33 domestic limestone bank rocks. The rocks varied in size, the smallest being 5 x 5 x 2.5cm in size and the largest being to 20.3 by 8.9 by 5cm. The majority of the rocks were around the same size, 7.62 x 7.62cm by 2.5-5cm. The total surface area of the rocks was 0.837 m^2. The second basket was filled with 8 Autumn Blend Belgian Reversible bricks. The bricks were all the same size, 5.7 x 7.5 x 14.9cm. The total surface area of the bricks was 0.383 m2. The third rock basket was filled a mixture of white and black oak leaves found on the ground surrounding the sample site. The leaves were placed in a mesh bag approximately 45 x 36 x 30cm. The leaves filled about half of the bag. The mesh bag was placed in the basket in between two 30 x 8.5 x 3.5cm boards to prevent movement. A 17.5 x 8.5 x 9cm rock was placed inside the basket to weigh it down. The total surface area of the leaves could not be determined.

All three baskets were placed adjacent to each other in the middle of the creek, mid-reach, within a riffle. The baskets were held down by multiple aluminum landscaping stakes. The baskets were placed in the creek on 19 May 2016 and were removed on 8 June 2016, providing about 3 weeks (20 days) to collect macroinvertebrates. To remove the rock baskets, a sieve bucket and two D-frame kick nets (500 micron), one on each side of the bucket, were placed downstream from the baskets to collect any organisms that fell off during the removal. Each basket was removed one at time and placed into its own bucket containing several liters of creek water. After each removal, the sieve bucket and D-frame kick nets were emptied into the bucket. Each bucket was then sorted through separately to find organisms. Organisms were collected from each artificial substrate and placed in sample jars containing 91% isopropanol. The organisms were then brought back to the lab to be classified under a dissecting microscope and to be counted.

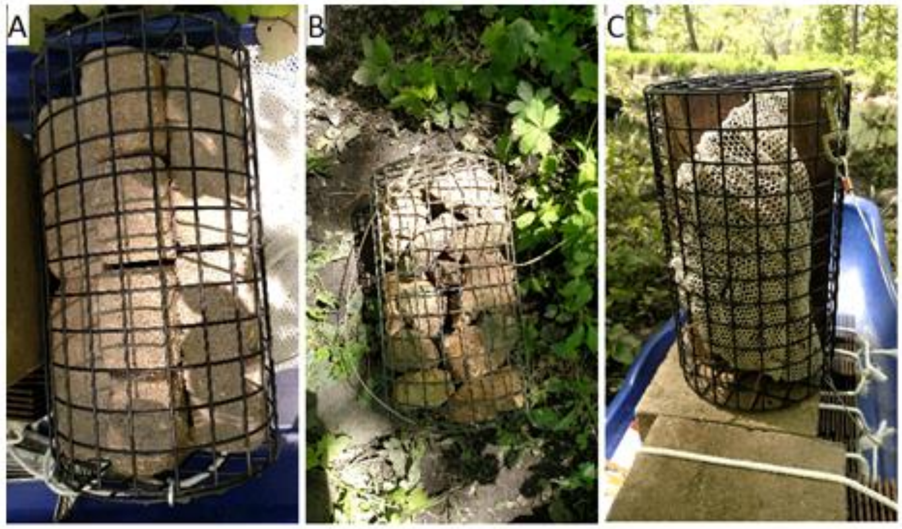

Figure 1. Artificial rock basket samplers with different substrates to collect macroinvertebrates.

Figure 1a shows a wire basket filled with brick. Figure 1b shows a wire basket filled with limestone bank rock. Figure 1c shows a wire basket with a mesh bag filled with oak leaves, wood planks to hold bag in place, and a large rock was added after picture to weigh down basket.

The macroinvertebrates were identified to order/family following Illinois Riverwatch protocol[1]. Total taxa and EPT taxa were determined. MBI (Macroinvertebrate Bioassessment Index) was determined using equation (1). The Shannon Diversity Indexes were calculated for each basket using equation (2).

$$MBI = \frac{\sum n_i \times a_i}{N} \quad (1)$$

where n_i = number of specimens in taxa $_I$, a = tolerance value of taxa $_I$; N = total number of specimens in sample.

$$SDI = \sum_{i=1}^{s} -(P_i \times lnP_i) \quad (2)$$

where P_i = the frequency of the entire sample made up of taxon $_I$, S = the total number of taxa in sample.

The number of EPT/Non-EPT individuals and pollution tolerant/intolerant individuals that colonized each substrate were also calculated. Pollution intolerant was determined to be any taxon with a tolerance value (TV) less than or equal to 5.5 and pollution tolerant was any taxon greater than 5.5, as determined by Illinois Riverwatch protocol. Quality ratings were also determined by Illinois Riverwatch protocol (Table 1)[1]. Chi-square analysis was performed on EPT/non-EPT and pollution tolerant/intolerant data.

Table 1. Stream Quality Ratings as determined by Illinois Riverwatch

	Taxa Richness	EPT Taxa Richness	MBI
Excellent	≥14	≥5	≤4.35
Good	12-13	4	≥4.36 - ≤5.00
Fair	9-11	3	≥5.01 - ≤5.70
Poor	7-8	2	≥5.71 - ≤6.25
Very Poor	≤6	0-1	≥6.26

3. Results

The basket with the limestone bank rocks collected the highest total abundance (747 specimens), the basket with the leaves had the second highest (519 specimens), and the basket with the bricks had the lowest total abundance(487 specimens). The limestone bank rocks and the leaves resulted in the same taxa richness, while the bricks resulted in a slightly lower taxa richness (Table 2). All three substrates resulted in a taxa richness that is considered 'excellent' according to RiverWatch quality rating[1]. The percentage of EPT macroinvertebrates was the highest for the rock substrate (79.4%), followed by the brick substrate (76.4%), and the leaf substrate had the lowest percent (67.4%); (Fig. 1).

Table 2. Results comparing three rock baskets filled with different substrates that were used to sample of Lily Cache Creek, Will Co., IL at Van Horn Woods

Substrates	**Bank Rock**	**Brick**	**Leaf**
Organisms Sampled	747	487	519
Taxa Richness	19	18	19
EPT Taxa Richness	7	7	6
MBI	4.71	5.08	5.18
SDI	2.075	1.889	2.127
# EPT Individuals	593	372	350
# Non-EPT Individuals	154	115	169
# Pollution Intolerant	643	411	407
# Pollution Tolerant	104	76	112

The ratio of the number of EPT/Non-EPT specimens differed the most between the limestone bank rocks and the leaves ($p<0.001$). The ratio of the number of EPT/Non-EPT also differed between the bricks and the leaves ($p<0.01$) but it did not vary significantly between the rocks and the bricks ($p=0.212$). The ratio of the number of pollution intolerant/tolerant specimens followed a similar pattern. The rock substrate had the highest percentage of pollution intolerant (86.1%), followed by the brick substrate (84.4%), and the leaf substrate resulted in the lowest percentage (78.4%); (Fig. 2). The limestone bank rocks and the leaves varied the most ($p<0.001$), followed by the bricks and the leaves ($p<0.05$), and the limestone bank rocks and the bricks did not vary significantly ($p=0.413$). All three substrates were compared together and showed a significant difference between EPT/Non-EPT specimens ($p<0.001$) and between pollution intolerant/tolerant specimens ($p<0.01$).

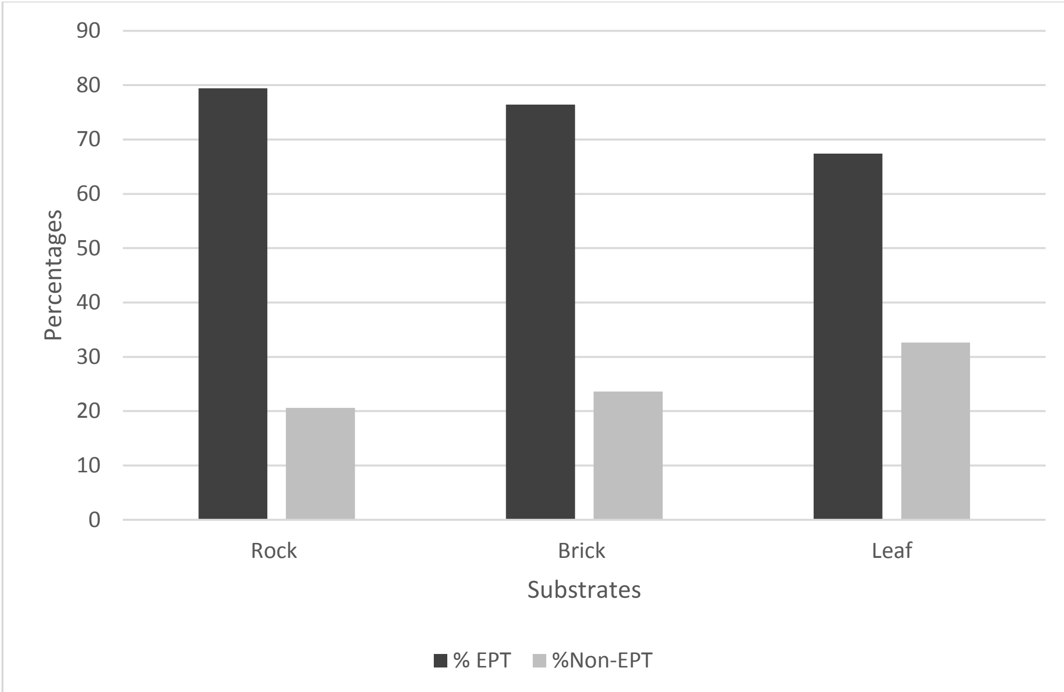

Figure 2. Comparison of EPT/Non-EPT macroinvertebrates that inhabited rock baskets with different substrates over a 3-week period in Lily Cache Creek, Will. Co., IL, May 2016

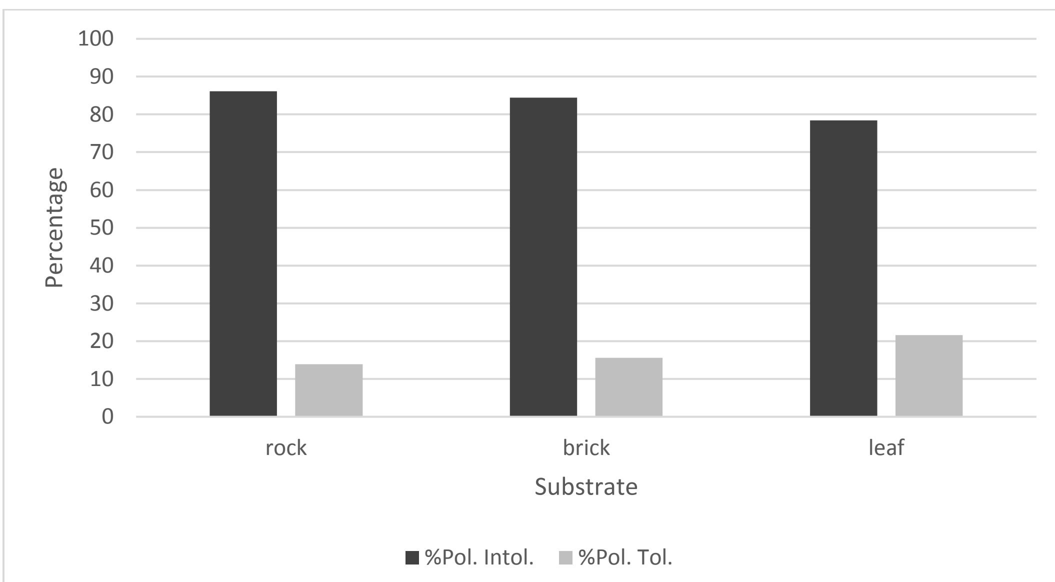

Figure 3. Comparison of Pollution intolerant/tolerant macroinvertebrates that inhabited rock baskets with different substrates over a 3-week period in Lily Cache Creek, Will. Co., IL, May 2016

The Shannon Diversity Indices varied between the three substrates, the leaves having the highest and the bricks having the lowest (Table 2). The bricks and leaves both resulted in MBI stream quality ratings of "fair" while the rocks resulted in a rating of "good" (Table 2). The three substrates attracted different taxa of macroinvertebrates. The rock basket consisted of approximately 50% hydropsychid caddisflies (O. Trichoptera F. Hydropsychide) while the brick and leaf substrates collected less (32.9%, 37.6% respectively). The brick substrate collected the highest percentage of clinging mayflies (O. Ephemeroptera F. Heptageniidae) and swimming mayflies (O. Ephemeroptera F. Saponaria) while the leaf substrate collected the highest percentage of crawling mayflies (O. Ephemeroptera F. Leptohyphidae); (Table 3).

Table 3. Comparison of taxa of macroinvertebrates collected using rock baskets with 3 different substrates over a 3-week period in Lily Cache Creek, Will. Co., IL, May 2016

Substrate	**Rock**	**Brick**	**Leaf**
Flatworm	1.74%	1.03%	3.66%
Aquatic Worm	0.00%	0.00%	0.19%
Leech	0.00%	0.00%	0.39%
Sow Bug	0.40%	1.23%	6.55%
Scud	4.95%	4.93%	7.13%
Broadwing Damselfly	0.27%	1.23%	0.19%
Narrowing Damselfly	0.54%	0.41%	0.39%
Swimming MF	17.80%	8.42%	11.95%
Clinging MF	17.94%	7.39%	2.50%
Crawling MF	3.75%	5.54%	11.18%
Stonefly	0.13%	0.21%	0.39%
Hydropsychid	32.93%	50.31%	37.57%
Snail case	3.88%	2.05%	0.00%
Other Caddisfly	2.95%	2.46%	3.85%
Riffle Beetle	0.54%	0.62%	3.08%
Water Penny	0.40%	0.82%	0.19%
Midge	5.76%	7.80%	6.74%
Black fly	3.35%	1.64%	2.50%
Left-hand	0.27%	0.21%	0.39%
Right-hand	0.54%	0.00%	0.00%
Operculate	1.87%	3.70%	1.16%

**Flatworm (C. Turbellaria), aquatic worm (C. Oligochaeta), leech (C. Hurudinea), sow bug (O. Isopoda F. Asellidae), scud (O. Amphipoda F. Gammaridae), broadwing damselfly 9 O. Odonata F. Calopterygidae), narrowing damselfly (O. Odonata F. Coenagrionidae), swimming mayfly (O. Ephemeroptera F. Siphlonuridae), clinging mayfly (O. Ephemeroptera F. Heptageniidae), crawling mayfly (O. Ephemeroptera F. Leptohyphidae), stonefly (O. Plecoptera), hydropsychid caddisfly (O. Tricoptera F. Hydropsychide), snail case caddisfly (O. Tricoptera F. Helicopsychidae), other caddisflies (O. Tricoptera), riffle beetle (O. Coleoptera F. Elmidae), water penny beetle (O. Coleoptera F. Psephenidae), non-biting midge (O. Diptera F. Chironomidae), black fly (O. Diptera F. Simuliidae), left-handed snail (O. Gastropoda F. Physidae), right-handed snail (O. Gastropoda F. Lymnaeidae), operculate snail (O. Gastropoda F. Viviparidae)*

4. Discussion

The rock substrate had a higher total abundance of macroinvertebrates than the brick substrate, possibly because the rocks have more than twice the surface area of the bricks. However, the leaf substrate had the lowest total abundance and although the surface area of the leaves could not be calculated, it is likely that the leaves provided more surface area than the rocks. In Saliu and Ovuorie's study, the gravel substrate was found to have the highest total abundance, similar to the rock substrate in the Van Horn study. They suggest that bricks and rocks tend to collect more macroinvertebrates because they provide multiple microhabitats, collect organic material, and tend to be a more stable substrate[8].

The rock substrate and the brick substrate both resulted with similar EPT and pollution intolerant ratios based on the chi-square tests. The chi-square analysis also showed the leaf substrate to collect significantly different macroinvertebrates. The lower EPT and pollution intolerant ratios would suggest that the leaf pack would result in a lower water quality rating but the MBIs were similar between the three substrates. According to the RiverWatch manual, leaf packs are said to be a more diverse habitat, followed by snags (logs or branches), undercut banks, and sediment[1]. The leaf substrate in the Van Horn Woods study did result in the highest SDI but the other two substrates were not much lower. There are many factors that affect whether or not macroinvertebrates will inhabit substrates. These factors include the diversity of the substrate, the size and shape of spaces within the substrate, and surface complexity[6].

The leaf substrate had collected the most sow bugs (37); (O. Isopoda F. Asellidae) and had the highest percentage of sow bugs (6.55%); (Table 3). Sow bugs are the only taxa that was determined with certainty to be a shredder out of the taxa that we collected. The higher number of shredders found in the leaf substrate may because they are either only using it for food or using it for food and as a substrate. Landeiro *et al.* found a positive relationship between the rate of decomposition of the leaves and the shredders that inhabit them. They found that the highest number of shredders occurred around day 19[9]. The baskets were pulled out after 20 days and if it followed a similar rate of decay, the leaf substrate would be at its highest level of shredders. This could explain the higher number of sow bugs collected from the leaf substrate.

Macroinvertebrates provide important information about stream quality and therefore it is beneficial to develop the best way to collect and analyze them. Different substrates attract different macroinvertebrates and is an important topic to take into consideration when sampling. In this study, the leaf substrate resulted in the lowest percentages of EPT and pollution intolerant specimens. The leaf pack also collected the most mud and was very difficult to sort through after removal from the water. However, the rock and brick substrates provided similar results and were much easier to work with. There are few published studies comparing different substrates and the field would benefit from more related experiments.

5. Acknowledgements

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