

## **Macro-Invertebrate Colonization of Manmade Litter in Aquatic Ecosystems**

### **Abstract**

The purpose of our study was to determine if aquatic macro-invertebrates prefer to colonize any of three types of manmade litter. We wanted to know if macro-invertebrate species showed any preference settling on aluminum, glass, and plastic containers commonly found in aquatic ecosystems. We also looked at two rock substrates, natural bio-film and bio-film removed, for a natural substrate comparison. We placed three containers of each material and one of each rock at three different sites along the Maple River in Cheboygan County, Michigan. Our experiment was divided into three five-day intervals during which we removed substrates and measured the abundance and type of organisms that had settled on them. There was not a consistent significant difference in organism abundance or species richness to conclude that anthropogenic material has a greater impact on macro-invertebrate substrate colonization.

### **Intro**

There is an ongoing concern about discarded inorganic materials being introduced into aquatic ecosystems. Manmade litter present in such ecosystems can be attributed to onshore recreational sources (Cundell 1973) and shipping vessels (Dixon and Cooke 1977; Merrell 1984). Studies have attempted to determine what can be done to reduce manmade litter in the environment (Earll et al. 2000) and to identify what impact it has in a system (Barnes and Milner 2005; Croxall et al.

1990), but regardless of this, litter is still an existing entity interacting in ecosystems and it is therefore necessary to look at its interactions with species.

Many species spend all or part of their life cycles in an aquatic ecosystem. Rivers and streams are habitat for macro-invertebrates such as Diptera and Gammarus that inhabit different substrates, which are in part colonized by light, texture of substrate, current, and depth (Hugh et al. 1992; Rittschof et al. 1998). For instance, light heavily determines organism frequency at a site by providing energy to a system. This promotes photosynthesis and productivity, while also maintaining thermal control. Direct sunlight deters some species of macro-invertebrates from warmer, sunnier spots, while others prefer them (Mackay, 1992). A different study by Winnel and Jude found that substrates with finer, consistent sized sand particles support a wider range of macro-invertebrates than coarser, less fine ones (1984). This research shows that organisms exhibit substrate preference. There has been surplus research on natural substrate colonization, but very little about the colonization on anthropogenic litter.

The purpose of our study was to examine if aquatic macro-invertebrates have a preference of settling on three different types of manmade litter. We hypothesized that macro-invertebrates would not show a preference between manmade materials. We predicted this because the materials are foreign to the environment and therefore would not yield different habitat benefits from each other. Our design involved examining organisms settled on common aquatic litter such as aluminum, plastic, and glass and comparing them to two rock substrates.

## Methods

Our study was conducted near the University of Michigan Biological Station Stream Lab on the Maple River in Cheboygan County, Michigan. It was run from July 19 to August 3, 2013. Macro-invertebrates were the organisms of focus because of their frequencies in aquatic ecosystems and their differing habits of colonizing substrates.

We selected three study sites along the west branch of the Maple River: upstream, midstream, and downstream. Each site was chosen based on similar depth, current, and sunlight measurements prior to the study. These were measured using a photometer, flowmeter, and hydrolab, respectively. Because the sites were all in close proximity, pH, dissolved oxygen, and conductivity remained mostly constant between sites. All three sites had a sandy bottom, minimizing colonizing preference resulting from surrounding bed substrate. The study was done in three intervals of five days. The experiment lasted for 15 days. The three intervals allowed us to analyze how long it took species to colonize and in what abundance.

We placed three replicates of different anthropogenic materials at each site: aluminum cans (volume: 354.84 cm<sup>3</sup>), glass bottles (volume: 354.84 cm<sup>3</sup>), and plastic bottles (volume: 591.40 cm<sup>3</sup>). For each container, we removed all labels and attached them to metal rebar with plastic zip ties. We oriented with the mouths facing downstream. The containers were attached in the alternating order, plastic, aluminum, and glass, respectively. All three materials were designated to each five-day interval per site. There were a total nine containers per rebar. A cement cinder block was placed in the center of the bar to prevent movement. We also used three

same-sized rocks as controls that were introduced on day 10 to show the natural five-day colonization by macro invertebrates. Rocks were scrubbed thoroughly with a soft bristled brush to remove all previously established macro or microorganisms before being placed in the stream.

After each interval, we collected all accumulated matter on the inside and outside surfaces of the materials using a soft bristled brush. Macro-invertebrates from the surfaces were identified by species. Data were organized by site, interval, material, surface, and species (ie. Site 1, day 5, plastic, outside, x diptera). ANOVA tests were used to compare variables. Algal growth was not studied.

## Results

The plastic bottle had a volume of 591.40 cm<sup>3</sup>. The glass and aluminum containers both had volumes of 354.84 cm<sup>3</sup>.

There was not a significant difference between the frequencies of organisms on the outside of each material ( $F=1.444$ ,  $df=2$ ,  $p=.256$ ; Fig. 1). There was a significant difference between the outside surface of plastic and both rock substrates (Fig. 1).

There was a significant difference between the frequencies of organisms on the inside of each material ( $F=9.702$ ,  $df=2$ ,  $p=.001$ ; Fig. 2). Inside plastic was significantly different than the other two manmade materials (Fig. 2). Frequencies inside glass and aluminum were not significantly different (Fig. 2).

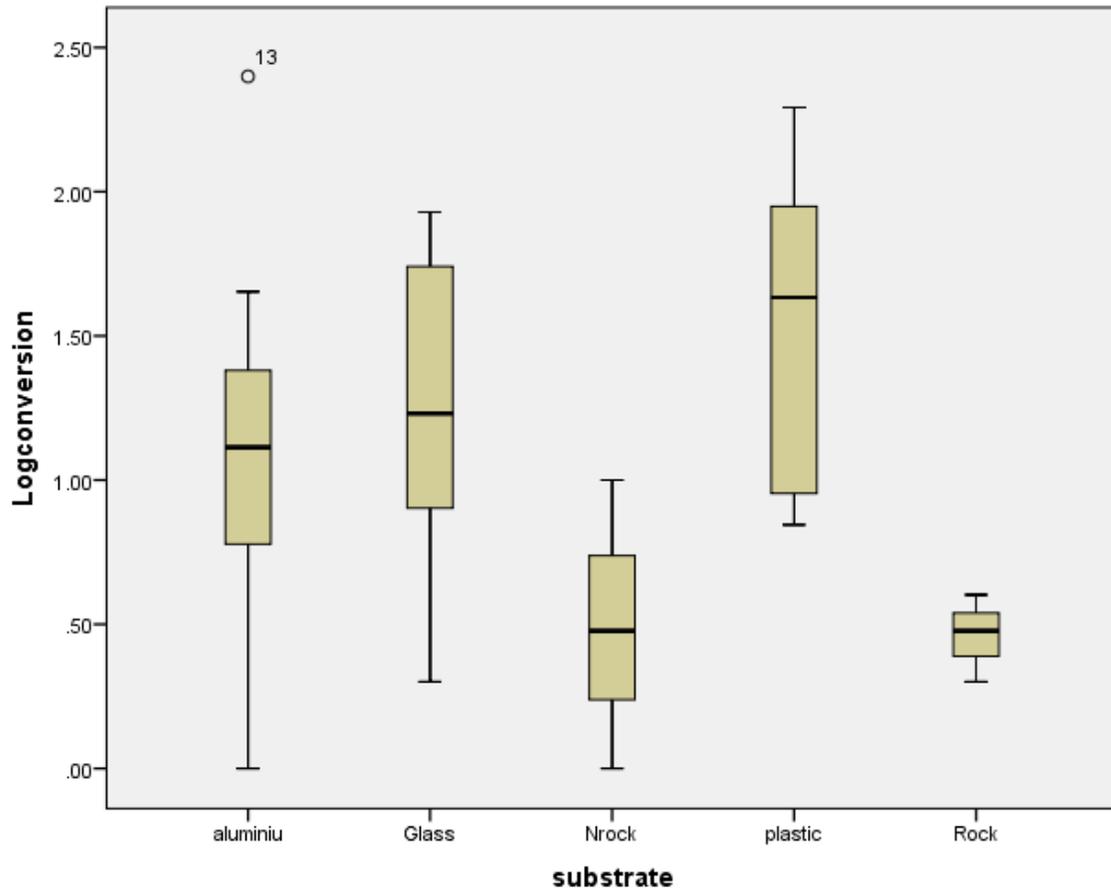
There was significant difference between the inside surfaces during the three intervals ( $F=7.759$ ,  $df=2$ ,  $p=.002$ ). There was also significance between the outside surfaces over the three intervals ( $F=6.399$ ,  $df=2$ ,  $p=.008$ ). Between interval one and three, no significance existed ( $p=.929$ ). Significance existed between interval one and two ( $p=.001$ ), and between interval two and three ( $p=.001$ ).

There was no significant difference in the total outside-surface organisms between sites ( $F=1.639$ ,  $df=2$ ,  $p=.211$ ). The total inside-surface organisms were also not significantly different between sites ( $F=1.444$ ,  $df=2$ ,  $p=.256$ ).

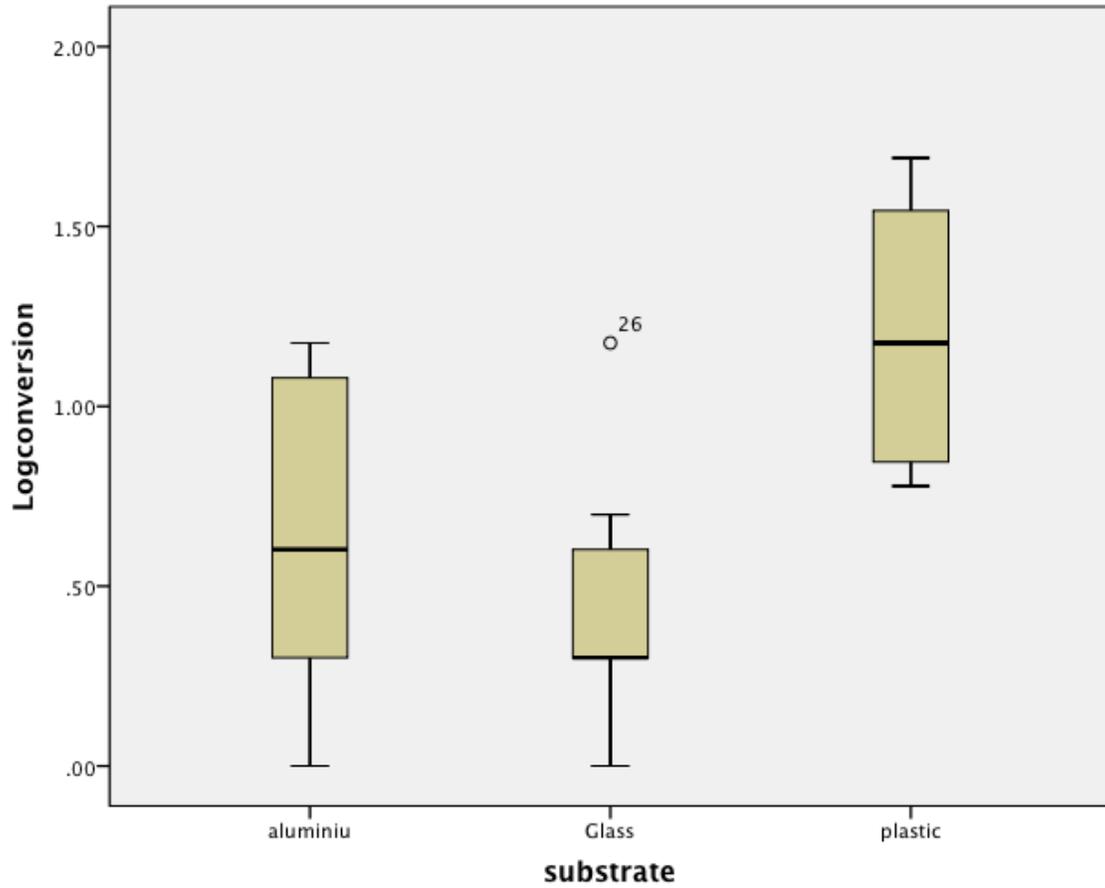
Species richness for the outside surface of each material was not significantly different ( $F=1.811$ ,  $df=4$ ,  $p=1.55$ ; Fig. 3). Species richness on the inside surface of the materials was significant ( $F=8.393$ ,  $df=2$ ,  $p=.002$ ; Fig. 4) because there was a significant preference for plastic. Four macro-invertebrate species were present on

all five substrates. Seven total species were shared on all three manmade substrates.

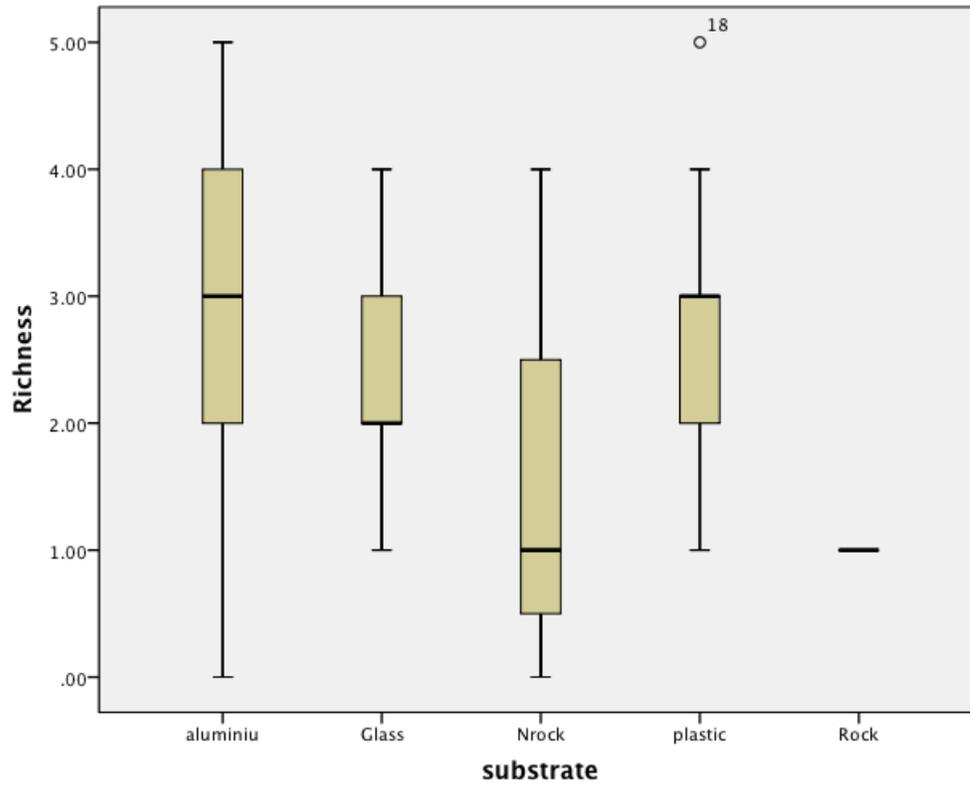
Aluminum had one species that had not colonized any other substrate.



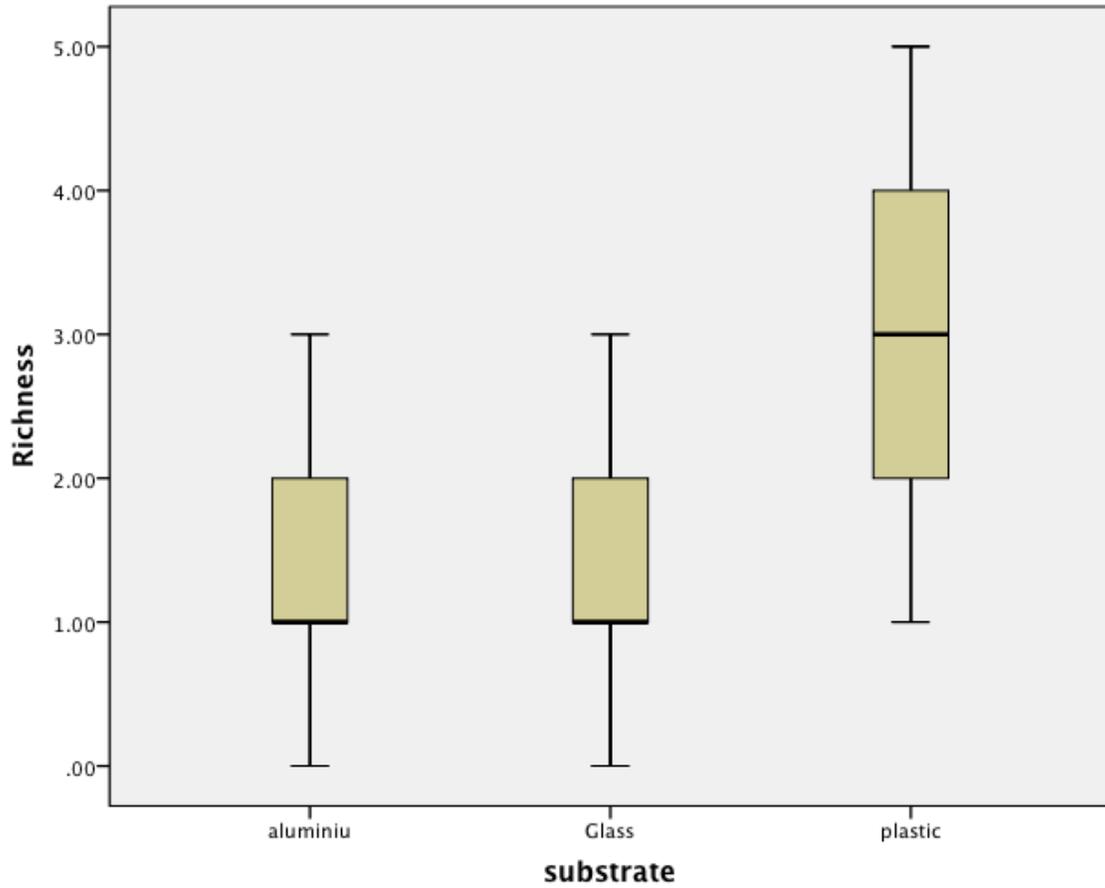
**Figure 1:** Log Conversion of Organism Frequency on Outside Surfaces by Substrate (F=1.444, df=2, p=.256, aluminium median ( $\tilde{x}$ )=1.11, Glass  $\tilde{x}$ =1.23, Nrock  $\tilde{x}$ =0.477, plastic  $\tilde{x}$ =1.63)



**Figure 2:** Log Conversion of Organism Colonization on Inside Surfaces by Substrate (F=9.702, df=2, p=. 001, aluminum  $\bar{x}$ =0.602, Glass  $\bar{x}$ =0.301, Plastic  $\bar{x}$ =1.18 )



**Figure 3:** Species Richness on Inside Surfaces by Substrate ( $F=1.811$ ,  $df=4$ ,  $p=1.55$ , aluminium  $\bar{x}=3.00$ , glass  $\bar{x}=2.00$ , Nrock  $\bar{x}=1.00$ , plastic  $\bar{x}=3.00$ , rock  $\bar{x}=1.00$ )



**Figure 4:** Species Richness on Inside Surfaces by Substrate ( $F=8.393$ ,  $df=2$ ,  $p=.002$ , aluminium  $\tilde{x}=1.00$ , glass  $\tilde{x}= 1.00$ , plastic  $\tilde{x}=3.00$ )

## **Discussion**

The results of our study showed that plastic was the only manmade material that macro-invertebrates had a preference toward. This is contrary to our original hypothesis that organisms would have no preferential settlement. This trend may have emerged as a result of resource availability. The plastic container had a greater volume than the other two and, therefore, a larger surface area, making space more available to for organisms to settle on. Additionally, the plastic we used was colorless increasing penetration by light. This may have promoted algal growth and bio-film accumulation, which is defined as any group of cells that stick together on a surface (Whitchurch et al., 2002). Light restriction on non-plastic surfaces may have reduced or eliminated algae and bio-film growth. Algal and bio-film accumulation encourages the settlement of aquatic grazing species by providing them a food source (Barlocker and Murdock, 1989). Furthermore, bio-film makes surfaces easier for sessile macro-invertebrates to colonize. Early settlers like diptera facilitate later succession predators into the habitat by offering them prey (Burns and Ryder, 2001). If bio-film accumulation were restricted by substrate material, it would prevent primary succession that must occur to promote species richness. Resources alone cannot explain the difference in colonization, though. One would not expect to see a significant richness and frequency difference between the inside surface of plastic and all the outside surfaces if resources were the same. This is because the surfaces would have the same available sunlight and opportunity for algal growth as a result.

A second possible explanation for the trend towards plastic could be that the bottle provided a microhabitat for organisms. A microhabitat is a small habitat within a larger one that offers different resources and conditions than the surrounding habitat (Baldes and Vincent, 2011). This micro-habitat may have been created due to the orientation of the containers away from the current and the supplemental protection from larger predators too big to fit into the mouth of the container. This theory is insufficient to stand alone because by it, it would be expected that all inside surfaces be roughly equivalent in species richness and frequency. Because the insides were significantly different, we must assume that the results are attributed to a combination of the algal and bio-film accumulation in conjunction with an available microhabitat.

Significance existed in the comparison of time intervals one and three to the second interval. The second interval had significantly more organisms than the other two, which may be because of a hatching event occurring near our second day of measurement. Diptera was in a much greater abundance on that day than any other interval, showing that a diptera hatch may have occurred.

This trend may exist because organisms were unable to survive on manmade substrates after their initial colonization. This theory would suggest a negative preference towards manmade substrate settling. The organisms also may have somehow determined that natural substrates were simply more preferable than manmade ones. Macro-invertebrates live on several surfaces throughout their lives (Blindow and Hargeby, 2001), giving them opportunity to make such a

determination. If such organisms were unable to survive on substrate or otherwise wouldn't remain on the substrates, it would support our original hypothesis.

Additional study would be needed to distinguish between our explanations. A study that controlled surface area would provide further data on whether the larger plastic container significantly impacted our results. The microhabitat theory could be investigated by examining the difference between inside and outside surfaces. Also, a study focusing on algal and bio-film growth on anthropogenic materials would also be helpful in determining its effect on macro-invertebrates' colonization. Lastly, further tests should be conducted to compare the two rock substrates and the manmade ones. This would give evidence on the theory that organisms died on or moved away from manmade substrates.

In conclusion, our study shows little correlation between macro-invertebrate abundance and anthropogenic substrate. Significance emerged with plastic, but it cannot be determined from the data whether this was directly a result of the substrate. Factors beyond substrate such as interval and inside vs. outside surface showed equal significance, so it cannot be said that substrate alone influenced organism abundance. We must therefore assume that our original hypothesis was correct that macro-invertebrates do not show a preference towards manmade substrates.

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