Colonization of Substrate in Lotic Ecosystems on the Macro and Micro Scale

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Introduction:

The colonization of macro invertebrate in a lotic ecosystem is an important aspect when looking into the health of a stream. Certain species are considered important indicators and are taken into consideration when performing macro invertebrate surveys. The physical environment also plays a crucial role in stream fitness with regards to chemical and physical tests, sediment composition, and bank buffers. The experimentation and observation of projects revolving around the colonization of aquatic organisms is a great way to broaden our knowledge on the effects of human interaction and possible ways to facilitate stream rehabilitation programs. Understanding how and what organisms colonize greatly increases the chance for success.

Our initial survey of Cedar Creek at PCCI revealed a stream that is fed by Brewster Lake and flows below a bridge on Cloverdale Road. Brewster Lake is a slightly basic lentic environment which is also warmer than the ground water that feeds it. All physical and chemical aspects of Brewster Lake affect the stream's attributes and were taken into consideration. With a basic knowledge of what the project entailed, we hypothesized that the diversity and abundance of organisms at the macro and micro scale will change with the changing physical stream environment, and that the colonization of both communities with change over time.

Methods:

Stream Analysis:

39 sampling sites were established 10 meters apart and marked using bamboo stakes and biodegradable tape. Those sites were used for collecting samples, characterization and measurements. Chemical and physical tests were performed at each sampling site during initial survey. Site #1 was located next to a bridge on the blue trail and site #39 was next to Cloverdale road which was also closest to Brewster Lake. Physical tests such as temperature, pH, conductivity and dissolved oxygen were measured once a week at each sampling site. Additional measurements, such as air temperature and general weather conditions were also recorded. Chemical tests were performed on water samples at each sampling site when the natural substrate was being inspected for organisms. The chemical tests include nitrate (NO_3^-), phosphate (PO_4^{2-}) and carbon dioxide (CO^2). This initial survey of the stream was completed prior to introducing artificial substrate.

Each sampling site was characterized based on available light, bank buffers, substrate abundance and sediment composition. A sediment sample was collected from each site, dried and then percent sand, silt, clay, gravel and organic matter was calculated based on weight. The sediment of the first fifteen sampling sites was predominantly decomposed organic debris, the middle of the stream was a mixture of organic and sandy sediment where as the last five sites were mostly rocky sedimentation. Based on this analysis, the sampling sites were divided into five different groups in which the artificial substrate was introduced. Each group consisted of three sampling sites with one piece of timber, one cement block and the third sampling site with both timber and a cement block. The groups were placed along the stream based on the sediment composition with group 1 in the organic sediment, group 3 in the mixed sediment, group 5 in the rocky sediment and groups 2 and 4 in between groups 1 and 3 and groups 3 and 5, respectively.

Substrate Analysis:

Natural substrate, or the predominant substrate found at each site, was also collected during the initial survey along with the physical and chemical tests. Macroinvertebrates were collected and counted in the lab for each site from the natural substrate as well as the sediment bed. Before analysis, the natural substrate was swabbed for bacteria and grown on Tryptic Soy Agar (TSA) plate. Using the streak plate method the bacteria grew in an incubator at 37^{o}C for 24 hours. Artificial substrate of two types was introduced to 15 different sampling sites along the stream which were then divided into 5 groups. Each of the 5 groups consisted of 3 sampling sites; one with timber, one with a cement block and then one with both timber and a cement block (Group #1 located near bridge and group #5 located closest to Brewster Lake). Group 1 was introduced first and checked after 2 hours for colonization. It was then checked twice a day for 1 week. Groups 2, 3, 4, and 5 were introduced a week after group 1 and checked after 2 hours for colonization. All groups were checked once a day for colonization for 2 additional weeks. Non treated manufactured timber was used for the artificial woody substrate and a cement block was used for the artificial rocky substrate. Surface area was calculated from the artificial substrate which allowed for statistical analysis. The artificial timber substrate was 2'' long and secured to the sampling sites using a 10'' metal stake nailed through the center. Before introducing the artificial substrate, it was swabbed for bacteria to be used as a control. This method was repeated for more accurate data. After the artificial substrate was introduced into the stream, each artificial was swabbed for bacteria and grown using the above method. In total there were 10 pieces of timber and 10 cement blocks introduced into the stream. In this experiment, the initial survey is used as a control.

Macroinvertebrate Collection:

Macroinvertebrates were counted on substrate as well as from the sediment bed. Substrate and samples were brought back to the lab to be counted and identified to the family level using dichotomous keys and a variety of texts. Natural substrate, water samples and macroinvertebrates were brought back to the field after identification.

Bacteria Collection:

All substrate was swabbed with a sterile Q-tip which was transferred to a TSA plate and was streaked and incubated for 1 day at 37. Each colony was then separated via quadrant steak and incubated again for 1 day at 37. Once a colony was separated it was identified using a variety of stains (Gram and Endospore stain), metabolic tests (Catalase and Oxidase tests), and cell and colony morphologies. Most colonies were identified to the genus level and some to the species.

Statistical Analysis:

After transferring all data onto spreadsheets in Excel, regression data analysis was utilized to determine significant relationships between aspects of the physical and chemical environment and location on the stream, as well as associations involving the physical and chemical tests with species richness and organism abundance. Significant relationships were determined based on a p-value of less than 0.05. Any p-value above the numerical mark were considered not significant.

Results:

Physical Environment:

Looking at the physical and chemical environment of the stream based on location, distance from Brewster Lake, we were able to obtain five relationships that can be deemed significant. Dissolved oxygen, pH, and temperature all showed a similar trend being that the environment closest to Brewster Lake contains the highest measurements of each. With p-values of <0.01, which are considerably less than 0.05, each test can be considered significant. Phosphate and conductivity revealed p-values of 0.0037 and <0.01, respectively, and demonstrate an inverse relationship with location. As our sample sites approach Brewster Lake, the measurements of both aspects decreases. Carbon dioxide and nitrate were both found to have non-significant relationships with p-values of 0.082531 and 0.996851, both larger than the necessary 0.05.

Upon looking at relationships between environmental properties and species diversity, we found three that were significant. Phosphate had a p-value of 0.0027, conductivity and temperature with p-values of <0.01. Nitrate, carbon dioxide, pH, and dissolved oxygen all had p-values greater than 0.05 and were therefore not significant.

Diversity/Abundance and Time (Colonization Rate):

After initially introducing our substrate into the stream, we waited about two hours before returning to the site to observe macro invertebrate that had colonized it. Eighteen of the twenty substrate were colonized after this two hour time period, with colonization most likely occurring before the two hour mark. The average colonization rate we observed was 2 hours and 49 minutes, with the fastest being 1 hour and 59 minutes.

Bacterial Diversity:

Below lists all organisms identified to the genus level on both natural and artificial substrates. It total there were 53 total genera identified.

Bacterial Genera List		
Acetobacter	*Chrysemonas luteola*	*Moraxella*
Acetobacterium	*Clavibacter*	*Morococcus*
Acidothermus	*Clostridium*	*Neisseria elongate*
Acidovorax	*Coriobacterium*	*Neisseria sicca*
Acinetobacter	*Corynebacterium*	*Oligella*
Alcaligenes	*Cupriavidus*	*Paracoccus*
Amphibacillus	*Curtobacterium*	*Peptococcus*
Arachnia	*Desulfotomaculum*	*Pimelobacter*
Arcanobacterium	*Ensifer*	*Propionbacterium*
Argobacterium	*Eubacterium*	*Rhizomonas*
Arthrobacter	*Flavobacterium*	*Sinorhizobium*
Aureobacterium	*Francisella*	*Sphaerobacter*
Azomonas	*Hydrogenophage*	*Sporolactobacillus*
Bacillus	*Kurthia*	*Sporosarcina*
Bifidobacterium	*Lactobacillus*	*Staphylococcus*
Brochothrix	*Listeria*	*Syntrophospora*
Carnobacterium	*Microbacterium*	*Vagococcus*
Caryophanon	*Micrococcus*	*Xanthobacter*
Cellulomonas	*Micrococcus luteus*	

Macroinvertebrate Diversity:

Below lists all organisms identified to the family level on both natural and artificial substrate. In total 53 different families were identified.

Organisms List		
Natural Substrate	Artificial Substrate	
	Wood	Cement
Annelida (Aquatic Earthworm, leech) Arachnidae (Water Mite) Astacidae (Crayfish) Belostomatidae (Giant Waterbug) Calopterygidae (Broadwinged Damselfly) Corixidae (Water Boatman) Corydalidae (Fishfly) Diptera (True Flies) Ceratopogonidae (Biting Midge) Chironomidae (Non-biting Midge) Empididae (Dance Fly) Simuliidae (Blackfly) Stratiomyidea (Soldierfly) Tipulidae (Crane Fly) Dytiscidae (Predacious Diving Beetle) Elmidae (Riffle Beetle) Ephemeroptera (Mayfly) Ameletidae (Ameletid) Baetidae (Small Minnow) Caenidae (Small Square Gill) Hepatageniidae (Flathead) Leptophlebiidae (Pronggill) Gomphidae (Progomphos) Gordiacea (Horsehair Worm) Hyalellidae (Scuds) Hydrophilidae (Water Scavenger Beetle) Nematode (Phylum) Odonata (Dragonfly) Aeshnidae (Darner) Gomphidae (Club Tail) Plecoptera (Stonefly) Chloroperlidae (Green) Perlidae (Common Stonefly) Perlodidae (Perlodid) Pulmonata (Snails) Bithyniidae (Bithyniid) Lymnaeidae (Lymnaeid) Physidae (Physid) Planorbidae (Planorbid) Viviparidae (Viviparid) Sialidae (Alderfly) Trichoptera (Caddisfly) Brachycentridae (Humpless Case Maker) Glossosomatidae (Saddle Case Maker) Hydroptilidae (Micro) Hydropsychidae (Common Netspinner) Limnephilidae (Northern Case Maker) Lepidostomatidae (Case Maker) Odontoceridae (Strong Case Maker) Phryganeidae (Giant Case Maker) Veneroida (Clams) Corbiculidae (Asian) Sphaeriidae (Pea)	Annelida (leech) Arachnidae (Water Mite) Calopterygidae (Broadwinged Damselfly) Diptera (True Flies) Chironomidae (Non-biting Midge) Empididae (Dance Fly) Simuliidae (Blackfly) Stratiomyidea (Soldierfly) Elmidae (Riffle Beetle) Ephemeroptera (Mayfly) Ameletidae (Ameletid) Baetidae (Small Minnow) Caenidae (Small Square Gill) Hepatageniidae (Flathead) Leptophlebiidae (Pronggill) Gordiacea (Horsehair Worm) Hyalellidae (Scuds) Odonata (Dragonfly) Aeshnidae (Darner) Plecoptera (Stonefly) Chloroperlidae (Green) Perlidae (Common Stonefly) Pulmonata (Snails) Lymnaeidae (Lymnaeid) Physidae (Physid) Planorbidae (Planorbid) Trichoptera (Caddisfly) Glossosomatidae (Saddle Case Maker) Hydroptilidae (Micro) Hydropsychidae (Common Netspinner) Limnephilidae (Northern Case Maker) Veneroida (Clams) Sphaeriidae (Pea)	Annelida (Aquatic Earthworm, leech) Astacidae (Crayfish) Calopterygidae (Broadwinged Damselfly) Diptera (True Flies) Chironomidae (Non-biting Midge) Simuliidae (Blackfly) Stratiomyidea (Soldierfly) Elmidae (Riffle Beetle) Ephemeroptera (Mayfly) Ameletidae (Ameletid) Baetidae (Small Minnow) Caenidae (Small Square Gill) Hepatageniidae (Flathead) Leptophlebiidae (Pronggill) Gordiacea (Horsehair Worm) Hyalellidae (Scuds) Isopoda (Sow Bug) Odonata (Dragonfly) Aeshnidae (Darner) Plecoptera (Stonefly) Chloroperlidae (Green) Perlidae (Common Stonefly) Pulmonata (Snails) Lymnaeidae (Lymnaeid) Physidae (Physid) Planorbidae (Planorbid) Trichoptera (Caddisfly) Glossosomatidae (Saddle Case Maker) Hydroptilidae (Micro) Hydropsychidae (Common Netspinner) Limnephilidae (Northern Case Maker) Lepidostomatidae (Case Maker) Veneroida (Clams) Sphaeriidae (Pea)

Conclusion & Discussion: do together

Upon reviewing our data and the significant relationships, we were able to conclude that some aspects of a stream environment do affect the colonization of a stream. Significant relationships were found between species richness and the physical components temperature, conductivity, and phosphate content. Conductivity and phosphate both showed trends that we expected. As conductivity decreases, richness should increase, and as phosphate increases, richness should increase as well. Temperature however did not show a trend we expected. Temperature increased as we neared Brewster Lake, and so did species richness. Upon reviewing the substance available throughout the stream, it became apparent that as the location neared Brewster Lake (towards site 39), substrate became much more available. This leads us to conclude that species richness is affected much more by substance abundance than by temperature.

After analyzing the data on bacterial diversity with the physical environment, the results suggest that there is not a significant relationship present. This conclusion was based on the p-values being greater than 0.05. However, when comparing bacterial and macro invertebrate diversity and colonization, a weak relationship was found. This conclusion was based on comparing the diversity and abundance at each sampling site. For example, at sites where the macro invertebrate midge was found, so was genus *Bacillus*. Similarly, where midge was not found, neither was *Bacillus*. The relationship was found with a few other organisms, but this one was the most prominent. This analysis cannot be concluded without taking other environmental aspects into consideration. Also, the family Midge and genus *Bacilli* were some of the most abundant species found at most sampling sites. Based on this information, the only conclusion that can be made is that both species are abundant in the tributary of Cedar Creek at Pierce Cedar Creek Institute.

We also looked at the colonization rate of the artificial substrate we introduced. We noticed an average rate of 2 hours 49 minutes, with 18 of our 20 sites being colonized when we checked it at the first two hour interval. With our method of allowing the substrate to remain in the water for about two hours before the first check, it is more than likely that colonization occurred before this two hour mark, however, we are not able to determine exactly when. We recorded the fastest colonization time at 1 hour 59 minutes, although this time also could have been affected by our method.

Along with colonization rate, we observed the first and last species that colonized the artificial substrate. At the initial check, the species most frequently found on timber were scuds, physid snails, ameletid minnow mayflies, black fly larva, and leeches. When we looked at the cement, the most common species found were scuds, physid snails, ameletid minnow mayflies, netspinner caddisflies, and red non-biting midges. As we compared these two lists, we noticed that scuds, physids, and ameletids were common on both substrates. These organisms are all great indicators of a healthy stream and are easily found throughout the tributary at PCCI. When we removed the substrate, we found a similar pattern with the species found upon removal. Both timber and cement were colonized by micro caddisflies, planorbid snails, and non-biting midges. Timber also held pronggill mayflies and small minnow mayflies, and cement had leeches and flathead mayflies utilizing it.

This is an appropriate set up for future research that could be performed at Pierce Cedar Creek Institute or other biological stations. Because the Cedar Creek tributary is a relatively uninhabited lotic ecosystem, it is a great example of a natural stream environment. This data can be used to observe the effects of human interactions in urban environments. Additionally, this

data can be used to help further enhance the research exploring invertebrate sensitivity to changes in their environment.