# Benthic Macro-Invertebrate population difference between sand and cobble substrates in the Arroyo Seco Watershed

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## Abstract

Watersheds are an integral and driving component of many terrestrial cycles. Fresh water resources are becoming increasingly valuable to local communities for urban development, agriculture, and recreation. Assessing environmental changes over time is important in evaluating impacts of human usage on our declining water resources. Benthic macro-invertebrates have been found to be an effective measure of changes in local waterways.  In the Arroyo Seco watershed, located east of Big Sur in Central California, a study was conducted to assess changes in the environment and their related changes in benthic macro-invertebrate diversities.  By comparing differences in substrate to the corresponding diversity levels of insect communities, a relationship between substrate and biological metrics can be formed.  To better understand this relationship, macro-invertebrate abundance levels, taxa richness, EPT taxa (number of taxa found in the orders: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddis flies)), EPT taxa composition and dominant taxa composition were analyzed given a cobble versus sand dominated substrate. EPT taxa, EPT composition, and taxa richness measures were higher in the upper site and decreased in the lower site. Dominant taxa composition was found to be higher in the sandy substrate than the cobble substrate.  Overall, less diversity was found in the sandy substrate (lower site) than in cobble substrate (upper site). A level II or III taxonomic effort would be necessary to further clarify EPT measures and associated ramifications found in both sites.

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**<u>Introduction</u>**

        As land management alters the biological and physical conditions of California streams, interactions between living organisms and their physical environments are increasingly important to understand. The loss of local diversity due to past management practices have had the potential to strongly affect ecosystem processes on both a local and global scale (Chapin, 1999). A quote from *Measuring the Health of California Streams and Rivers*; "….as we began to talk about the effects of physical manipulation on aquatic systems and how important habitat condition was to the health of streams and rivers---water quality could not be separated from habitat integrity." Disturbances that change natural cycles of nutrients and physical habitats have the ability to cause a shift in local biological processes within a river system (Harrington and Born, 2000). In an essay entitled *Macro-Invertebrate Instream flow studies after 20 years; A role in stream management and restoration*, Gore, Layzer, and Mead also support ideas of benthic macro-invertebrate monitoring to measure stream health (Gore et al., 2001).

        Bio-assessment of benthic macro-invertebrates (BMI) can serve as effective indicators to monitor disturbances.  Although physically small, these invertebrates can be important players in watershed monitoring and restoration. Benthic macro-invertebrate insects are at least 0.5mm in size and live primarily on the bottom substrate of streams and rivers. They are sedentary in nature, sensitive to perturbation, have variable life cycles, and many common species have known responses to pollution (Harrington and Born, 2000). These factors make aquatic insects feasible tools for monitoring changes over time.

        In aquatic ecosystems invertebrates also have been found to mix sediment, cycle nutrients, and functionally contribute to local food webs (Covich, 1999; Lehmkuhl, 1979). Soil and sediment biota are important because they process a variety of water, soil, and air pollutants including pesticides, industrial compounds and excess nutrients (Groffman, 1999; Chapin, 1997). In benthic communities, food resources are utilized differently by primary producers, herbivores, predators, or detritivores (Covich, 1999; Merritt and Cummins, 1996).

The sensitivity level of known aquatic species and their presence or absence can provide information about the local state of a particular river system. Particularly, taxa found within the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT taxa) can imply specific conditions within the benthic habitat. Generally, habitats with higher counts of EPT insects have cleaner, cooler, and more oxygenated water. Specific families within these three EPT orders have been found to identify various levels of disturbance such as excess nutrients or sediment (Harrington and Born, 2000). In our study, nine major orders of insects were used to compare how substrate type affects insect taxa richness and composition measures within the watershed. Benthic insect samples were desiccated in the field, counted by order, and statistically analyzed.

## Methods

### Site Location

The Arroyo Seco River is in central California and runs east from Los Padres National Forest into the Salinas valley along Arroyo Seco Road. Upper and lower watershed site locations were used to sample the two different substrates. The upper site was located approximately one mile upstream from the Los Padres National Forest entrance. The site has complexity within the channel dominated by large cobble substrate and bedrock with highly vegetated riparian corridors. The sampling location was a randomly chosen riffle from the first four riffle/pool sequences upstream from the picnic area just inside the park entrance. The lower site was located approximately a half mile downstream from the Elm St. bridge. This site has a much less complex channel, absent of riffle/pool sequences, and the dominant substrate is sand. This sample location was a randomly chosen cross-section area from a one hundred meter, uniform stretch below the bridge.

A non-point source sampling design was used for macro-invertebrate data collection at both sites (Krebs, 1989; Harrington and Born, 2000). Field and laboratory procedures were according to Harrington and Born's methods (2000).

*Field Procedure*

One riffle was randomly chosen from three riffle/pool sequences (or a uniform one hundred meter stretch) in each sampling site. A cross-section was randomly chosen out of the top third of each sampling riffle. A sample was taken from each margin and once in the thalweg of the chosen x-section for a total of three samples per site. Sample location within each cross-section corresponds with a sample number as shown below (Figure I).

Figure I – X-Section view of sampling areas in each riffle
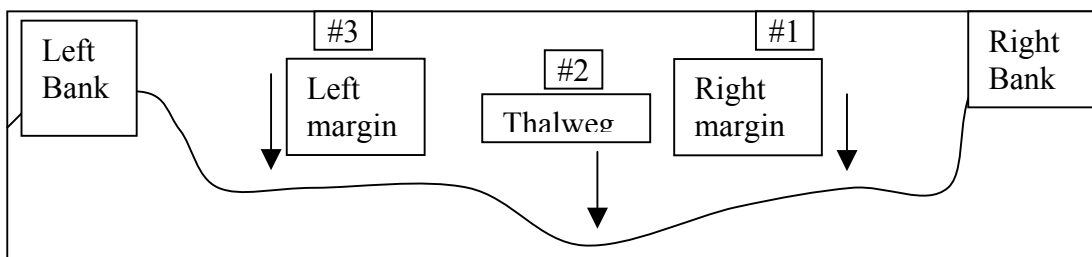


A 0.5mm D-shaped kick net was used to catch downstream flowing debris. A 0.305m by 0.610m area immediately downstream from the net was disturbed three to six inches deep for three minutes in each x-section location (Harrington 2000). All contents of the net from each sampling location were placed in a 0.5mm sieve to sort out large organic matter, with care not to lose any organisms. Each sample was preserved with 95% ethanol. There were six field samples in total.

*Lab Procedure*

Each sample was placed in a 0.5mm sieve, and rinsed thoroughly. Large organic matter was discarded and all macro- invertebrates were separated from each field sample. A deviation from the cited protocol was due to low numbers of insects found; all complete and identifiable invertebrates were used instead of sub-samples. Each insect was grouped and identified to the order and other invertebrate groups were identified to phylum. In addition, Ephemeroptera, Plecoptera, and Trichoptera (EPT taxa) were further divided into similar groupings likely to represent different families. During identification, nine major orders of insects were used for reference (five orders were found): Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddis flies), Diptera

(aquatic flies), Megaloptera (alderflies), Coleoptera (aquatic beetles), Odonata (dragonflies and damselflies), Hemiptera (true bugs), and Lepidoptera (aquatic moths) (Harrington and Born 2000).

Statistical analysis between sites was performed on calculated biometrics from results using standard t-tests. Each sample was kept separate for the statistical purpose of making n as large as possible, six samples in total (Watt 2001). According to the limited biometrics produced from a level one taxonomic effort, five general parameters were analyzed. Taxa richness gives a measure for how many different taxa were found, a broad level of diversity or variability within the population. The EPT taxa are the number of taxa found in the orders: Ephemeroptera (mayflies), Plecoptera (stonefly), and Trichoptera (caddisfly). These three orders have been found to have specific sensitivities and generally low tolerance (to pollution) levels. The EPT composition measure gives an index for what percentage of the total population is made up of these three orders. The percent dominant taxa can be found by dividing the number of organisms for the most abundant taxon by the total number of organisms. This gives a measure for the complexity of the population.

**Results**

Taxa richness, EPT taxa, and EPT composition values for the upper sample site were consistently higher than values found for the lower site.  Single dominant taxa percentage values were higher for the lower site than the upper site. Abundance levels were variable between sites ranging from four to thirty organisms per sample (Table I).

The null hypothesis was that biometrics are equal for both sites. Two tailed t-tests for taxa richness, EPT taxa, and EPT percent composition give p-values $< 0.05$, the upper site values are different from the lower. Abundance levels have a p-value $> 0.05$, showing no significant difference between sites. The percent dominant taxa also has a p-value $> 0.05$, although close to the cut-off with a p-value of 0.051(Table II). The dominant taxa found in the upper site were of the order Trichoptera, and the lower site were of the order Diptera. The most significant difference found between sites was in EPT percent composition values ($P = 0.002$), values clearly decrease from the upper site to the lower site (Figure II).

**Table I** – Biometric results by number of taxa or number of organisms found within each parameter between sites

|  | Sample # | Upper Site | Lower Site |
|---|---|---|---|
| **Taxa Richness** | 1 | 5 | 2 |
| Total # of individual taxa | 2 | 5 | 3 |
|  | 3 | 9 | 2 |
| **EPT Taxa** | 1 | 4 | 1 |
| Number of taxa in the Orders: | 2 | 3 | 2 |
| Ephemeroptera, Plecoptera, Trichoptera | 3 | 6 | 0 |
| **EPT % Composition** | 1 | 83 | 16 |
| Percent composition of EPT taxa | 2 | 67 | 7 |
|  | 3 | 57 | 0 |
| **% Dominant Taxa** | 1 | 66 | 86 |
| Percent composition of the single most | 2 | 33 | 94 |
| Abundant taxon | 3 | 35 | 95 |
| **Abundance** | 1 | 14.5 | 11 |
| Number of organisms in the total sample | 2 | 4.5 | 15.5 |
|  | 3 | 31.5 | 9.5 |

**Table II -** Statistical results for biometrics comparison between sites

| Biological Metrics |  |  | P-Values |
|---|---|---|---|
| Taxa Richness |  |  | 0.044 |
| EPT Taxa |  |  | 0.034 |
| EPT % Composition |  |  | 0.002 |
| % Dominant Taxa |  |  | 0.051 |
| Abundance |  |  | 0.611 |

**Figure II –** Average +/- Standard Deviation of % EPT composition measurements for the upper (cobble) and lower (sand) site locations
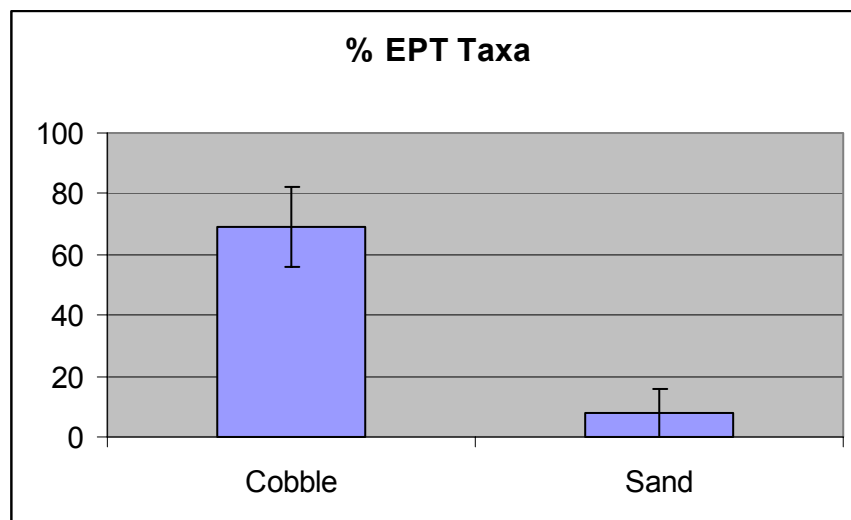
Discussion

   Taxa richness in the cobble substrate was higher than in the sandy substrate (Table I). The p-value of 0.04 is within 0.01 of the cut-off value (0.05), showing 96% confidence in a significant difference between sites. EPT taxa richness was also found to be higher in the cobble substrate than in the sandy substrate. The p-value of 0.03 gives a 97% confidence level, sufficient to conclude a significant difference in EPT taxa between sites.

   EPT composition values show the most profound deviation between sites. Of the total organisms found, environmentally sensitive orders (EPT taxa) were more present in the upper site than in the lower site. The highest percentage of dominant taxa were found in the lower site (Table I). Dominant taxa is a measure of tolerance reflecting the communities' sensitivity to aquatic disturbances, therefore the lower site shows a response to some form of environmental impact. Naturally, we would expect the downstream site to have more sedimentation than in the upper watershed. The question then becomes how much sediment is natural and to what capacity will there be negative biological effects.

   Possible sources of error include human error during sub-sampling of organisms from organic matter and bottom materials. There was also the possibility of error due to amateur insect identification. Lastly, there was a possible loss of organisms during container and net transfers. The conclusions drawn above from EPT values have variable implications by species type particularly for mayflies and caddisflies. A level II or III taxonomic effort would be necessary to clarify EPT measures and the associated ramifications.

   Over all, BMI assemblage differs dramatically between upper and lower sites. Less complexity was found in the lower site, which had low EPT index values and high single dominant taxa values. The upper site provided a more diverse invertebrate population, with higher EPT values, and lower dominant taxa values. Broadly, these results imply a richer ecosystem higher up in the watershed than in the lower part of the Arroyo Seco river.

At this point it is unknown if the variability found was due to substrate difference, naturally existing conditions, or impacts from land-use between site locations. Further study is needed where two different substrates can be found in the same site location. Comparison with reference sites known to be unimpaired would also be useful.

Analysis of benthic macro-invertebrate communities to determine the physical, chemical, or biological conditions of a watershed may prove to be a useful tool in restoration and monitoring efforts. Land use planning and management of areas surrounding Arroyo Seco can use benthic insects to index long and short-term ecosystem impacts from management practices or naturally occurring variability.

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Appendix A

Taxonomic Effort Level 1 - Number counts of each insect identified to the order. Insects found in the orders of Ephemeroptera, Plecoptera, and Trichoptera were divided and recorded between different taxa.

| Insect Order | Upper #1 | Upper #2 | Upper #3 | Lower #1 | Lower #2 | Lower #3 |
|---|---|---|---|---|---|---|
| Ephemeroptera | 5 | 5 | 30 | 3 | 2 | 0 |
| # of different taxa | 3 | 2 | 4 | 1 | 2 | 0 |
| Plecoptera | 0 | 1 | 2 | 0 | 0 | 0 |
| # of different taxa | 0 | 1 | 1 | 0 | 0 | 0 |
| Trichoptera | 19 | 0 | 4 | 0 | 0 | 0 |
| # of different taxa | 1 | 0 | 1 | 0 | 0 | 0 |
| Coleoptera | 5 | 1 | 3 | 0 | 0 | 0 |
| Diptera | 0 | 2 | 22 | 19 | 29 | 18 |
| Odonata | 0 | 0 | 2 | 0 | 0 | 1 |