## Comparison of snag and shoreline macroinvertebrate samples -

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## Introduction

The Great Rivers of North America, which account for a large proportion of the available water resources within the United States, are disproportionately impaired. Nonpoint source pollution from agriculture, alterations of hydrological patterns, reductions in floodplain quality and quantity, and invasive species are some of the many disturbances that threaten the integrity of the Great River Ecosystems (GRE) (Benke, 1990; Karr and Chu, 2000; Justic et al., 2003). Relative to other aquatic habitats such as wadeable streams and lakes, limited effort has been directed towards the development of bioassessment tools for GREs. Consequently, effective bioassessment tools must first be developed for these important ecosystems before local, regional, or national assessment programs can be designed and implemented (McDonald et al., 2004).

To address this, the USEPA coordinated the National Great Rivers Survey (www.epa.gov/emap/greatriver/index.html) in which state and federal agencies sampled the Upper Mississippi, Missouri, and Ohio Rivers using the Environmental Monitoring and Assessment Program Great River Ecosystems (EMAP-GRE) protocols (Angradi 2006). The Central Plains Center for Bioassessment (CPCB) took part in this effort by sampling 8 Lower Missouri River sites (Table 1, Figure 1). The EMAP-GRE protocols included sampling macroinvertebrates in two habitats by two methodologies: 1) near-shore littoral areas with a kick net and 2) main channel snags by boat with a modified kick net. Angradi et al. 2009 details these methods and statistical results.

To assess the variability in samples collected using the two methodologies, CPCB collected three additional replicates of each method at each of the 8 sites. Snags were present at 4 sites, while littoral samples were collected at all 8 sites, and with three samples of each method this totaled 36 samples. In addition to examining insite variability at the 8 CPCB sites, we compared these macroinvertebrate faunages with those found in the national study.

Table 1. Location of eight study sites on the lower Missouri River, with codes used in graphs and nearby city if applicable. Collection of kick and/or snag samples is indicated, along with date sampled.

| site \# | code | latitude | longitude | city | state | kick | snag | date |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1257 | 1 | 39.5803 | -95.0566 |  |  | Yes | No | 28-Aug-2006 |
| 1241 | 2 | 39.6196 | -95.0553 |  |  | Yes | No | 28-Aug-2006 |
| 1281 | 3 | 39.8756 | -95.0255 | St. Joseph | MO | Yes | Yes | 1-Aug-2006 |
| 1249 | 4 | 39.8629 | -95.0667 |  |  | Yes | Yes | 30-Jul-2006 |
| 1265 | 5 | 39.8670 | -95.0988 |  |  | Yes | Yes | 30-Jul-2006 |
| 1253 | 6 | 40.0773 | -95.4087 | Rulo | NE | Yes | No | 3-Aug-2006 |
| 1297 | 7 | 40.1302 | -95.4106 | Rulo | NE | Yes | No | 2-Aug-2006 |
| 1285 | 8 | 40.5246 | -95.7502 | Peru | NE | Yes | Yes | 12-Aug-2006 |



Figure 1. Map of the Missouri River and sampling sites. Black square on United States inset indicates sampling area. Black circles indicate sites in which both littoral and snag samples were collected. Hollow circles indicates site in which only littoral samples were collected.

## Methods

See Angradi et al. 2009 for site location methods and sampling details. Each site consisted of a 500 m river segment that was divided into 11 transects. At each transect, two shoreline 30 -second kick benthic samples were collected using a $500 \mu \mathrm{~m}$ mesh kick net. All 22 samples ( 11 transects x two 30 -second samples) were combined into one composite sample. This process was repeated two more times at each transect to derive three composited samples for each site. Also within each river segment we attempted to collect a $1-\mathrm{m}$ long sample from each of three snags using a $500 \mu \mathrm{~m}$ mesh snag net. Snag samples were not combined. Only four sites contained snags. Thus from four sites we collected three kick samples and three snag samples ( 24 samples), and from four sites we collected only the three kick samples ( 12 samples).

All benthic samples were preserved in buffered formalin with rose Bengal, returned to the laboratory, and transferred to $95 \%$ ethanol. Samples were processed following EMAP methods which included picking specimens from random grids in a Canton tray until specimen counts reached $500+20 \%$ (excluding "large and rare" taxa which were retained from other grids). Specimens were identified to genus where possible, and data entered into an MSAccess database. If total count in a sample exceeded $500+20 \%$ specimens, data were randomly removed to bring the total count down to 600 . See www.cpcb.ku.edu/datalibrary/assets/library/protocols/BenthicLabSOP2009.pdf for detailed benthic lab protocols. From the final macroinvertebrate dataset the following community characteristics and metrics were calculated for each sample:

Taxonomic composition: Taxa richness (number of taxa in the sample), \% Ephemeroptera taxa (i.e. number Ephemeroptera taxa divided by total number of taxa in the sample), \% Trichoptera taxa, \% EPT taxa, \% Chironomidae taxa.

Abundance composition: Total abundance (number of specimens in the sample), \% Ephemeroptera abundance (i.e. number Ephemeroptera specimens divided by total number of specimens in the sample), \% Trichoptera abundance, \% EPT abundance, \% Chironomidae abundance, Dominant taxa.

Diversity indices: Metrics measuring abundance (evenness), Evenness, Shannon's Index, Brillouin's Index.

Similarity indices: Jaccard Coefficient, Bray-Curtis Similarity, Bray-Curtis Distance.
Diversity and similarity indices were calculated using EcoMeas 1.6 (2005). Statistical analyses were performed in NCSS (Hintze 2004). Data that were not normal were log+1 transformed prior to analyses. If the data of both factors of habitat (littoral or snag) and site were normal, we looked at habitat x site interactions with 2-way GLM ANOVA. If there was not an interaction then we examined data with 1way GLM ANOVA. Significance was reported at $\mathrm{p} \leq 0.05$.

## Results and Discussion

In littoral samples, 11764 specimens comprised 177 taxa, while on snags 136 specimens comprised 22 taxa (Table 2). All taxa found on snags were also found in littoral samples. See Appendix 1 for the three dominant taxa of each sample. The most dominant taxa by sample type were similar to the most dominant taxa found in the national GRE study (Angradi et al. 2009) in littoral and snag locations on the lower Missouri River (Table 3). Four of the six most abundant taxa found in littoral samples, Oligochaeta (24.5\%), Pseudocloeon (9.3\%), Corixidae (all Trichocorixa, 8.0\%), and Caenis (6.2\%) comprised the four most abundant littoral taxa found by Angradi et al. (2009): immature Tubificidae without capilliform chaetae ( $12.3 \%$ ), Pseudocloeon ( $8.8 \%$ ), Caenis ( $5.2 \%$ ), and Corixidae (4.4\%). Three of the four most abundant taxa on snags, Pseudocloeon (25.4\%), Rheotanytarsus (16.4\%), and Tanytarsus ( $10.7 \%$ ) also comprised three of the four most abundant snag taxa ( $13.8 \%, 12.2 \%$, and 6.5 respectively) found by Angradi et al. (2009).

Table 2. Number of specimens and taxa in each type of sample.

|  | \# specimens |  | \# taxa |  |
| :---: | :---: | :---: | :---: | :---: |
| Taxon | littoral | snag | littoral | snag |
| Ephemeroptera | 2613 | 57 | 21 | 4 |
| Plecoptera | 7 | 0 | 4 | 0 |
| Trichoptera | 490 | 14 | 15 | 6 |
| Chironomidae | 4086 | 83 | 30 | 7 |
| other | 4568 | 23 | 66 | 5 |
| total | 11764 | 177 | 136 | 22 |

Table 3. The 22 most abundance macroinvertebrate taxa in littoral kick samples and mid-channel snags collected from eight Missouri River sites. This list includes all taxa collected from snags.

| taxa | abundance \% | taxa | abundance \% |
| :---: | :---: | :---: | :---: |
| littoral |  | snag |  |
| Oligochaeta | 24.53 | Pseudocloeon | 25.42 |
| Tanytarsus | 10.10 | Rheotanytarsus | 16.38 |
| Pseudocloeon | 9.27 | Polypedilum | 15.82 |
| Trichocorixa | 8.02 | Tanytarsus | 10.73 |
| Rheotanytarsus | 7.28 | Atrichopogon | 5.08 |
| Caenis | 6.15 | Caenis | 4.52 |
| Polypedilum | 4.76 | Rhagovelia | 3.95 |
| Chironomus | 4.28 | Hydropsychidae | 2.82 |
| Maccaffertium | 3.49 | Hemerodromia | 2.26 |
| Thienemannimyia group | 3.43 | Cricotopus/Orthocladius | 1.69 |
| Potamyia | 1.41 | Cheumatopsyche | 1.69 |
| Telopelopia | 1.25 | Mayatrichia | 1.13 |
| Hemerodromia | 1.18 | Oligochaeta | 1.13 |
| Cryptochironomus | 1.05 | Hydropsyche | 1.13 |
| Musculium | 0.91 | Maccaffertium | 1.13 |
| Isonychia | 0.77 | Stenochironomus | 1.13 |
| Cladotanytarsus | 0.77 | Amercaenis | 1.13 |
| Nectopsyche | 0.60 | Thienemannimyia group | 0.56 |
| Amercaenis | 0.60 | Gomphidae | 0.56 |
| Argia | 0.58 | Ceraclea | 0.56 |
| Physa | 0.55 | Dicrotendipes | 0.56 |
| Hydroptila | 0.54 | Nectopsyche | 0.56 |

The objective of this study was to determine if samples collected using the same method varied within sites, and if samples varied between sites. We expected samples to vary between sites, but methods should not lend themselves to variability within a site. Ideal metrics should have high discriminate ability and low sensitivity to sample size (Table 4). For metrics (both real and log+1 transformed) in which both snag and littoral data were normally distributed, significant ( $\mathrm{p}<0.05$ ) habitat x site interactions (GLM ANOVA) existed for taxa richness, abundance, Brillouin's, Margalef's Index, Shannon's Index, dominance ( 3 taxa), and abundance and richness of EPT, Ephemeroptera, and Chironomidae (Table 5). Thus, these metrics are ideal for detecting differences among snag and littoral samples. However, Stepenuck et al. (2008) cautions against intermixing metrics collected by different sampling methods.

Table 4. Ability of some macroinvertebrate metrics to discriminate among samples and relative sensitivity to sample size (references).

| Metric | Discriminate ability | Sensitivity to sample size |
| :--- | :---: | :---: |
| richness | good | high |
| Brillouin's Index | moderate | moderate |
| Margalef's Index | good | high |
| McIntosh's Index | poor | moderate |
| Shannon's Index (H') | moderate | moderate |
| Simpson's Index | moderate | low |

Table 5. Results of GLM AOVA on real or log 1+transformed metrics of four sites at which both snag and littoral samples were collected. Transforming did not normalize all variables. 2-Way GLM ANOVA was used unless there was not a significant habitat x site interaction, in which case 1-Way GLM ANOVA was used. ${ }^{*} \mathrm{p} \leq 0.05$.

| metric | habitat x site | habitat | site |
| :---: | :---: | :---: | :---: |
| Taxa richness | * | * | * |
| Abundance (total count) | * | * | * |
| Brillouin's Index | * | * |  |
| Margalef's Index | * | * | * |
| McIntosh's Index (not normal) |  |  |  |
| Richness/Abundance (not normal) |  | * |  |
| Shannon's Index (H') (not normal) | * | * |  |
| Simpson's Index (not normal) |  |  |  |
| EPT rich | * |  | * |
| E rich | * |  | * |
| T rich (transformed) | * |  | * |
| C rich (transformed) | * | * | * |
| EPT abundance | * |  | * |
| E abundance | * |  |  |
| T abundance (not normal) | * |  | * |
| C abundance | * | * | * |
| Dominance 3 taxa | * | * |  |

To compare within and between site variability among samples, we examined the standard deviations by creating error bar charts in which standard deviations are shown as lines extending zero (Figure 2). To center the standard deviations on zero, the average of the three samples of a given method collected at each site was subtracted from the metric value of each sample. Error bar charts for all metrics are presented in Appendix 2, with sample sizes in Table 2.


Figure 2. Error bar charts for EPT abundance in which standard deviations are shown as lines extending from zero, for eight littoral kick samples and four mid-channel snag samples. Sites are coded from downstream (site 1) to upstream. See Table 1 for site localities.

In conclusion, taxa collected from multiple samples at each site in this study reflected the taxa collected from one sample at each site in the larger national GRE effort. Taxa richness, abundance, Brillouin's, Margalef's Index, Shannon's Index, dominance ( 3 taxa), and abundance and richness of EPT, Ephemeroptera, and Chironomidae which exhibited significant habitat x site interactions are ideal for determining if macroinvertebrate differences exist between habitats (snag vs. littoral) and between sites.

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Appendix 1. Abundance of the three dominant taxa in each sample collected from eight Missouri River sites, by littoral kick sample and mid-channel snag.

| site | habitat | sample | taxa | abundance \% | habitat | sample | taxa | abundance \% |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1241 | littoral | A | Rheotanytarsus | 14.10 |  |  |  |  |
|  |  | A | Tanytarsus | 13.36 |  |  |  |  |
|  |  | A | Oligochaeta | 12.62 |  |  |  |  |
|  |  | B | Tanytarsus | 17.13 |  |  |  |  |
|  |  | B | Rheotanytarsus | 13.22 |  |  |  |  |
|  |  | B | Oligochaeta | 10.24 |  |  |  |  |
|  |  | C | Oligochaeta | 13.52 |  |  |  |  |
|  |  | C | Tanytarsus | 13.30 |  |  |  |  |
|  |  | C | Rheotanytarsus | 12.23 |  |  |  |  |
| 1249 | littoral | A | Oligochaeta | 69.74 | snag | D | Caenis | 26.32 |
|  |  | A | Trichocorixa | 13.92 |  | D | Polypedilum | 26.32 |
|  |  | A | Chironomus | 3.78 |  | D | Pseudocloeon | 15.79 |
|  |  | B | Oligochaeta | 63.42 |  | E | Pseudocloeon | 23.91 |
|  |  | B | Trichocorixa | 14.44 |  | E | Polypedilum | 19.57 |
|  |  | B | Pseudocloeon | 6.12 |  | E | Tanytarsus | 15.22 |
|  |  | C | Oligochaeta | 44.07 |  | F | Polypedilum | 63.64 |
|  |  | C | Trichocorixa | 21.88 |  | F | Pseudocloeon | 18.18 |
|  |  | C | Pseudocloeon | 20.21 |  | F | Tanytarsus | 9.09 |
| 1253 | littoral | A | Chironomus | 34.22 |  |  |  |  |
|  |  | A | Trichocorixa | 30.67 |  |  |  |  |
|  |  | A | Oligochaeta | 20.04 |  |  |  |  |
|  |  | B | Trichocorixa | 47.91 |  |  |  |  |
|  |  | B | Chironomus | 26.13 |  |  |  |  |
|  |  | B | Oligochaeta | 11.98 |  |  |  |  |
|  |  | C | Tanytarsus | 18.83 |  |  |  |  |
|  |  | C | Rheotanytarsus | 16.95 |  |  |  |  |
|  |  | C | Trichocorixa | 14.23 |  |  |  |  |
| 1257 | littoral | A | Oligochaeta | 22.06 |  |  |  |  |
|  |  | A | Tanytarsus | 18.02 |  |  |  |  |
|  |  | A | Rheotanytarsus | 11.54 |  |  |  |  |
|  |  | B | Rheotanytarsus | 24.61 |  |  |  |  |
|  |  | B | Oligochaeta | 16.47 |  |  |  |  |
|  |  | B | Tanytarsus | 15.50 |  |  |  |  |
|  |  | C | Oligochaeta | 22.25 |  |  |  |  |
|  |  | C | Rheotanytarsus | 19.70 |  |  |  |  |
|  |  | C | Tanytarsus | 13.35 |  |  |  |  |
| 1265 | littoral | A | Caenis | 20.81 | snag | D | Rhagovelia | 54.55 |
|  |  | A | Oligochaeta | 18.38 |  | D | Hydropsyche | 18.18 |
|  |  | A | Tanytarsus | 13.94 |  | D | Cheumatopsyche | 9.09 |
|  |  | B | Oligochaeta | 40.98 |  | E | Pseudocloeon | 75.00 |
|  |  | B | Tanytarsus | 12.55 |  | E | Rheotanytarsus | 12.50 |
|  |  | B | Caenis | 11.18 |  | E | Cheumatopsyche | 6.25 |
|  |  | C | Oligochaeta | 38.48 |  | F | Hydropsychidae | 30.00 |
|  |  | C | Caenis | 19.68 |  | F | Pseudocloeon | 20.00 |
|  |  | C | Tanytarsus | 8.33 |  | F | Polypedilum | 20.00 |
| 1281 | littoral | A | Pseudocloeon | 27.66 | snag | D | Rheotanytarsus | 50.00 |
|  |  | A | Tanytarsus | 13.32 |  | D | Tanytarsus | 25.00 |
|  |  | A | Maccaffertium | 11.27 |  | D | Pseudocloeon | 8.33 |
|  |  | B | Tanytarsus | 16.43 |  | E | Rheotanytarsus | 100.00 |
|  |  | B | Pseudocloeon | 15.61 |  | -- | - | -- |
|  |  | B | Rheotanytarsus | 9.86 |  | -- | -- | -- |
|  |  | C | Tanytarsus | 20.40 |  | F | Polypedilum | 66.67 |
|  |  | C | Hemerodromia | 13.33 |  | F | Rheotanytarsus | 33.33 |
|  |  | C | Pseudocloeon | 13.13 |  | -- | -- | -- |

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| site | habitat | sample | taxa | $\begin{gathered} \text { abundance } \\ \% \end{gathered}$ | habitat | sample | taxa | abundance \% |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1285 | littoral | A | Caenis | 16.07 | snag | D | Pseudocloeon | 53.85 |
|  |  | A | Thienemannimyia group | 12.50 |  | D | Atrichopogon | 23.08 |
|  |  | A | Pseudocloeon | 10.71 |  | D | Rheotanytarsus | 7.69 |
|  |  | B | Caenis | 16.25 |  | E | Pseudocloeon | 22.22 |
|  |  | B | Pseudocloeon <br> Thienemannimyia | 13.07 |  | E | Atrichopogon | 16.67 |
|  |  | B | group | 10.60 |  | E | Tanytarsus | 16.67 |
|  |  | C | Pseudocloeon | 19.87 |  | F | Pseudocloeon | 40.00 |
|  |  | C | Caenis | 14.33 |  | F | Rheotanytarsus | 40.00 |
|  |  | C | Tanytarsus | 10.75 |  | F | Atrichopogon | 20.00 |
| 1297 | littoral | A | Pseudocloeon | 29.44 |  |  |  |  |
|  |  | A | Tanytarsus | 15.15 |  |  |  |  |
|  |  | A | Oligochaeta | 10.39 |  |  |  |  |
|  |  | B | Pseudocloeon | 34.44 |  |  |  |  |
|  |  | B | Oligochaeta | 22.08 |  |  |  |  |
|  |  | B | Trichoptera | 8.39 |  |  |  |  |
|  |  | C | Oligochaeta | 49.90 |  |  |  |  |
|  |  | C | Trichocorixa | 8.77 |  |  |  |  |
|  |  | C | Polypedilum | 6.26 |  |  |  |  |

Appendix 2.
To compare within and between site variability among samples, we examined the standard deviations by creating error bar charts in which standard deviations are shown as lines extending from zero. To center the standard deviations on zero, the average of the 3 samples of a given method collected at each site was subtracted from the metric value of each sample. $\mathrm{E}=$ Ephemeroptera, $\mathrm{P}=$ Plecoptera, $\mathrm{T}=$ Trichoptera, Chiron. $=$ Chironomidae.

## Littoral samples




## Snag samples





























