Anne Arundel Countywide Biological Stream Survey

Benthic Macroinvertebrate Laboratory Sorting/Subsampling

Tray Comparison Study Summary

Background

Anne Arundel County Department of Public Works, Bureau of Watershed Protection and Restoration has collected stream ecological data in streams and watersheds across the County since 2004. The County's Countywide Biological Stream Survey (CBSS) program collects data from randomly selected stream sites in each of the County's 24 subwatersheds, also referred to as the program's Primary Sampling Units. The CBSS is currently in Round 4, the fourth complete coverage of the 24 PSUs. CBSS data are used to assess ecological conditions and stream resources in each of the watersheds within Anne Arundel County to support data-informed management decisions. The CBSS is used to meet the County's current Municipal Separate Storm Sewer System (MS4) permit conditions for Assessment of Controls/ Watershed Assessment Monitoring.

Field data collection methods follow exactly those used by Maryland Department of Natural Resources' Maryland Biological Stream Survey (MBSS; Harbold et al, 2024). Laboratory benthic macroinvertebrate sorting/subsampling has evolved throughout the life of the CBSS program. During Rounds 1 (2004-2008) and 2 (2009-2013) a Caton tray (Caton 1991) was used for laboratory subsampling of samples. During Round 3 (2017-2012) subsampling was conducted using a Marchant box (Reynoldson et al, 2007; 2017 and 2018) and a modified Caton tray (2019-2021). The modified Caton tray was also used for the first two years of Round 4 (2023 and 2024). Starting in 2025 the CBSS will employ an exact copy of the MBSS sorting tray (Dziepak et al, 2024) to maintain consistency with MBSS methods as required by the County's MS4 permit.

To understand how differences in sorting/subsampling trays may affect the benthic macroinvertebrate subsamples, Anne Arundel County will conduct a side-by-side comparison of sorting trays. Understanding potential differences is important to the County for correct interpretation of the program dataset over time and important to Maryland Department of the Environment (MDE) for potential incorporation of CBSS data into MDE analyses and Maryland's Integrated Report of Surface Water Quality.

Anne Arundel County is partnering with the benthic macroinvertebrate lab that processed the vast majority of samples throughout the life of the program, EcoAnalysts of Moscow, ID. The following outlines the side-by-side comparison study design.

Side-by-Side Comparison Study Design

- 30 CBSS samples from 2025 (n of 45 total samples for 2025) will be used in the comparison study. A power analysis was completed which shows that this number of samples will

discriminate at least a 0.5 IBI unit change at P=0.05 and power = 0.8. The 30 samples will be selected at random.

- Prior to selecting the 30 samples for the side-by-side comparison, the 10% (5 samples) QC re-ID samples will be selected at random and removed from the pool of sites for this study.

- CBSS benthic macroinvertebrate samples will be first sorted using the MBSS sorting tray to the 120 +/- organism target and subsampled organisms will be identified following Dziepak et al, 2024 and the CBSS QAPP (KCI, 2023).

- For each sample, all sortate removed during the subsampling process and all identified organisms will be returned to the appropriate sample container along with all unused/unsorted sample material. The sample will then be sorted using the modified Caton tray (same as employed 2019-2021 and 2023-2024) to the target 120+/- organism subsample. The 120 +/- organism target is described in the MBSS laboratory methods manual (Dziepak et al, 2024). The resulting subsample will be identified following the MBSS laboratory methods manual (Dziepak et al, 2024).

- Novel taxa encountered during the comparison study will be removed from the sample and added to the project reference collection started in 2024. If novel taxa are encountered during either sorting phase (MBSS tray or modified Caton tray) the novel taxon will be added to the reference collection. If a novel taxon is encountered in both the MBSS tray and the modified Caton tray phase, only the first encounter from the MBSS tray phase needs to be retained in the reference collection.

- Paired subsamples from the same site will be compared to measure differences in samples potentially attributed to the difference in subsampling trays. Statistical analyses will be performed on site pairs, site pairs grouped by PSU, and the total population of grouped pairs to identify differences in Maryland's Coastal Plain Benthic Macroinvertebrate Index of Biotic Integrity (BIBI) or any of the component metrics. Anne Arundel County has support from University of Maryland Center for Environmental Science for the analysis of the data produced by the side-by-side study.

- Results will be shared with the County, EcoAnalysts, and MDE. It is likely that the results of this study are of interest to other jurisdictions in Maryland, and the scientific community at large. A peer-reviewed publication is a possible outcome of this study.

- **<u>OPTIONAL</u>** – The benthic macroinvertebrate sample picking/sorting process has an unknown/unquantified variability. Benthic organisms are likely not distributed across the sample evenly. To reduce this variability and limit the possibility that differences among sample pairs is due to purely to chance, it was suggested that 3 to 5 sample repeat sorts are completed on the same sample using both trays. These repeat sorts/IDs would be completed for 3 to 5 sites in total.

Generally, for each site the sample would be sorted and IDed using the MBSS tray, then all sortate and IDed organisms returned to the sample, then re-sorted and re-IDed using the MBSS tray, and repeated for a total of 3 to 5 times. This would be completed for the same sample 3 to 5 times for each tray. Example:

Site R4-04-11-25 is selected at random and is sorted and IDed 5 times using MBSS tray, and sorted and IDed 5 times using the modified Caton tray. This results in 5 replicates for site R4-04-11-25 sorted using the MBSS tray, and 5 replicates sorted using the modified Caton tray.

This repeated for the next randomly selected site.

Resulting dataset is 5 sites, each sorted 5 times with the MBSS tray and 5 times with the modified Caton tray.

References

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