Methods

- Ultra-micro size-fraction (UMSF; pictured right): bacteria that can pass through the 0.2 µm pore membranes employed in environmental surveys.
- Despite being ubiquitous and having high metabolic activity, UMSF remain elusive and largely uncultured.
- Investigations of UMSF are skewed by difficulties in culturing and a lack of techniques for measuring UMSF biogeochemical signatures.
- According to a 2019 study, one out of seven global rivers surveyed had antibiotic levels above safe water standards.¹

Rationale:
To study the impact of man-made carbon sources, introduced through human movement and migration, on the metabolic response of a local stream water UMSF community.

Driving Question:
What effect does the introduction of man-made carbon sources have on the UMSF community?

Hypotheses:
H1: In the presence of cyclodextrin, the UMSF community will undergo significant metabolic response activity.
H2: In the presence of cyclodextrin, the UMSF community will experience significant growth in terms of cell counts and DNA concentration.
H3: During this experiment, we will identify effective methods & procedures for filtration and culturing of the UMSF community.

Microcosm Preparations
1. Water sample collection at Beaver Creek
2. Initial (1F) 0.2 µm filtration
3. Re-filtering with second 0.2 µm filter (2F)
4. 1F inoculation (+ Cyclodextrin in ½)
5. 2F inoculation (+ Cyclodextrin in ½)
6. Biofilm DNA extracted from bottle walls
7. DNA extraction & analysis – 0.2 µm filters
8. DNA extraction & analysis – 0.1 µm filters
9. Geochem: anions, organic acids, pH
10. 16S rRNA amplicon sequencing

(+) Community Metabolic Analysis

“Novel” Rapid Biofilm Extraction

Conclusions
H1: In the presence of cyclodextrin, the UMSF community underwent significant metabolic response activity as indicated by increased sulfate and nitrate reduction post-injection as well as the increase in organic acid production from day 10 to day 24.
H2: In the presence of cyclodextrin, the UMSF community experienced significant growth as indicated by higher DNA concentration counts observed post-injection.
H3: We developed methods & procedures for effective filtration and culturing of the UMSF community.

Future work
- Isolate characterization and 16S rRNA amplicon community analysis.
- The lab techniques employed here will guide future research into the UMSF and understanding their relationship with man-made carbon sources.
- Future research directions include looking at the potential utilization of UMSF to biodegrade antibiotics and pharmaceutical by-products in rural community water systems.

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