AOS DESIGN OPTIMIZATION: SURFACE WATER MICROBE CELL COUNTS

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 BACKGROUND AND GOALS

1.1 Description of sample design and available data

Surface water microbe cell count (DP1.20138.001) is a count of fluoresced microbial cells by volume. In the field, grab samples are collected during water chemistry sampling at stream S2 locations and lake and river buoy locations. Stream samples are collected monthly (12x per year), while lake and river samples are collected every other month (6x per year). Twenty-five mL of sample water is added to a sterilized 50 mL conical tube with 2.8 mL of 10% buffered, 0.2 um filtered, formaldehyde. Samples are refrigerated after collection.

Within 60 days of collection, samples are shipped from the NEON Domain Support Facilities (DSFs) to the Battelle cell count lab for analysis. Samples are shipped cool on ice packs overnight. In 2024, NEON began collecting blank samples annually. Blank samples are 0.2 um filtered DI water, carried to the field, preserved, and analyzed in the same way as field samples.

At the lab, microbial cells are enumerated using propidium iodide (PI) staining, black filter filtration, and fluorescence microscopy.

Data are provided on the NEON portal as rawMicrobialAbundance, which is converted to microbial abundance per mL:

$$microbialAbundancePerMl_i = amc\_cellCounts.rawMicrobialAbundance_i \times \frac{(amc\_fieldCellCounts.cellCountSampleVolume_i + amc\_fieldCellCounts.cellCountPreservantVolume_i)}{amc\_fieldCellCounts.cellCountSampleVolume_i}$$

1.2 Analytical Goals

The purpose of this analysis is to determine whether the surface water microbial cell count data product is useful or necessary to data users. Blanks data collected in 2024 shows high cell counts in blank samples at some sites, which calls into question the reliability of sample data. NEON Surface water microbe group abundances (DP1.20278.001) provides quantitative PCR (qPCR) data which also provide an estimate of microbial abundance. The propidium iodine staining and count method may be an older method that is not widely used anymore by he scientific community, and group abundance data also provide estimates of microbial abundance.

In 2019, flow cytometry was explored as an alternative method for NEON data analysis. However, with the relatively low number of NEON samples analyzed annually, the time to maintain instrumentation and cost of changing the data product to a new method was not deemed worthwhile.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Reference Documents

RD[01]	NEON.DOC.001152	NEON Aquatic Sampling Strategy
RD[02]	NEON.DOC.003044	AOS Protocol and Procedure AMC – Aquatic Microbial Sampling
RD[03]	Battelle Epifluor Protocol v7.1	NEON Microbial Productivity from Aquatic Samples

2.2 Acronyms

Acronym	Definition
NEON	National Ecological Observatory Network
AOS	Aquatic Observation System
TWG	Technical Working Group
PI	Propidium iodide
qPCR	Quantitative polymerase chain reaction

3 METHODS AND RESULTS

3.1 Methods

The analysis was performed using data from RELEASE-2025. Surface water microbe cell count (DP1.20138.001) and Surface water microbe group abundances (DP1.20278.001) were downloaded into the R environment using the neonUtilities R package.

Surface water cell count abundance per mL was calculated using the equation above. Cell count abundance per mL and group abundance mean copy number data were log transformed to meet assumptions of normality, and regressed. Sample data and blank data were plotted in R using ggplot2.

Literature reviews searching for recent (since 2020) uses of count-based data were performed by Jennifer Edmonds and Stephanie Parker, and the NEON data portal was queried for recent downloads.

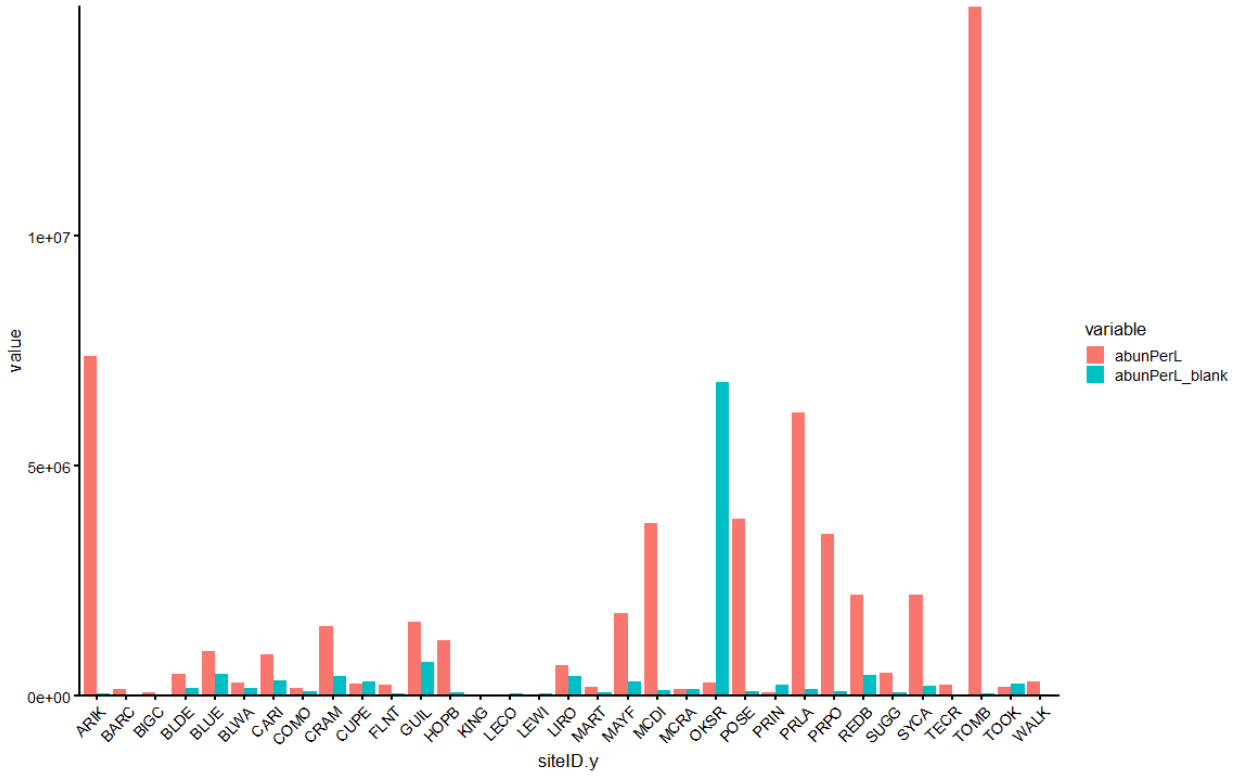
3.2 Results

Cell count blanks varied among sites in 2024 (Figure 1), some were greater than or similar to cell count samples numbers (e.g. OKSR, LIRO, MCRA, TOOK).

The regression analysis of cell counts vs. group abundance data showed a relationship (Figure 2). Because microbial data are inherently variable, the lack of significance is expected (adjusted R-squared = 0.1008, $p < 0.05$).

Surface water microbe cell count data have rarely been downloaded from the NEON portal. An apparently spike in April-May 2024 is seen across multiple NEON data products and is likely not reflective of real data downloads.

Figure 1: Blank samples cell count results by site in 2024.



4 DISCUSSION

Surface water microbial cell count data were widely used in the 1990s and earlier (Hobbie et al. 1977, Lisle and Priscu 2004). In the 2000s, sequencing methods became less expensive and more commonly used. Recent publications using total cell count data appear to use flow cytometry for drinking water distribution system applications. We were not able to find recently published papers using the method that NEON uses. Additionally, the Battelle lab was queried and does not provide this service for any other contracts.

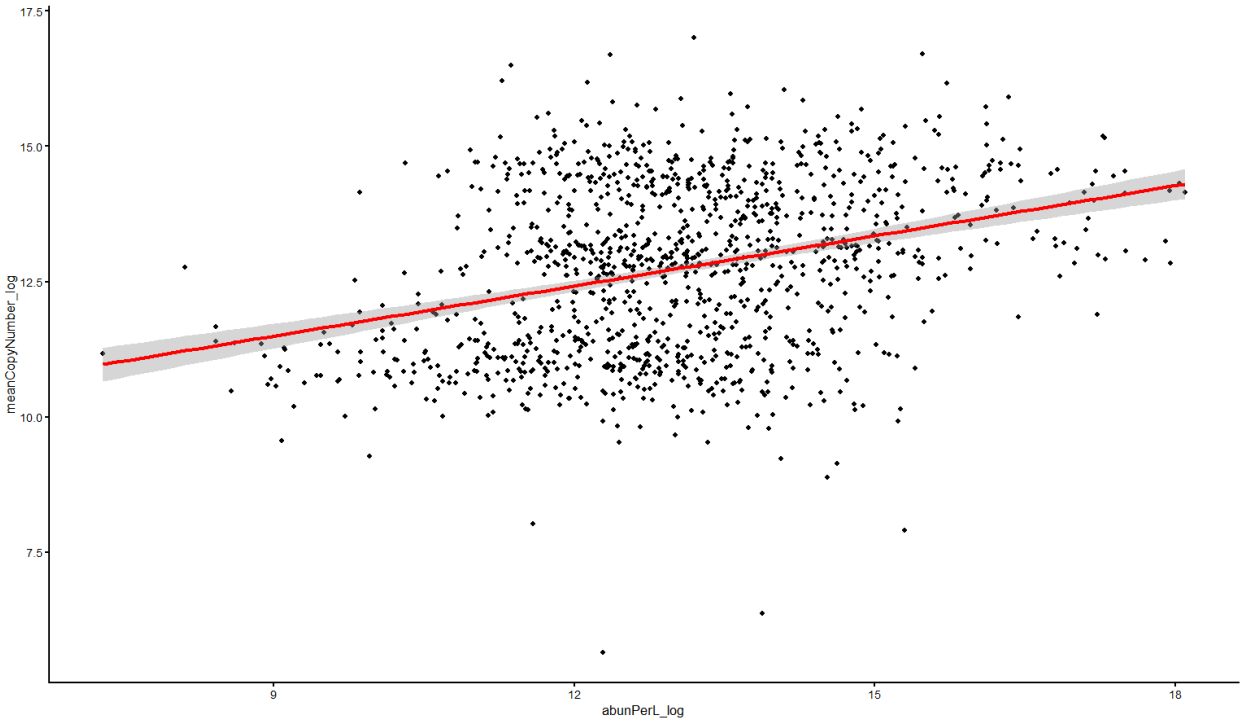
Variability in cell count blank data show no trends, but do show concerning levels of contamination at some sites. Sibling blank samples were also collected and sent to the Battelle sequencing lab. None of these samples had enough microbes to pass dnaExtraction QC.

Regression analysis of cell count data and group abundance (qPCR) data showed a relationship, suggesting that data users interested in microbial abundance could use the group abundance data provided from the sequencing lab.

Responses from the community include the following:

- Jennifer Edmonds, Aquatic Biology TWG and STEAC: “I spoke with Dr. Sujung Lim, a postdoc at UNLV in August. She thought the use of a stain to quantify bacteria as just part of a descriptive ef-

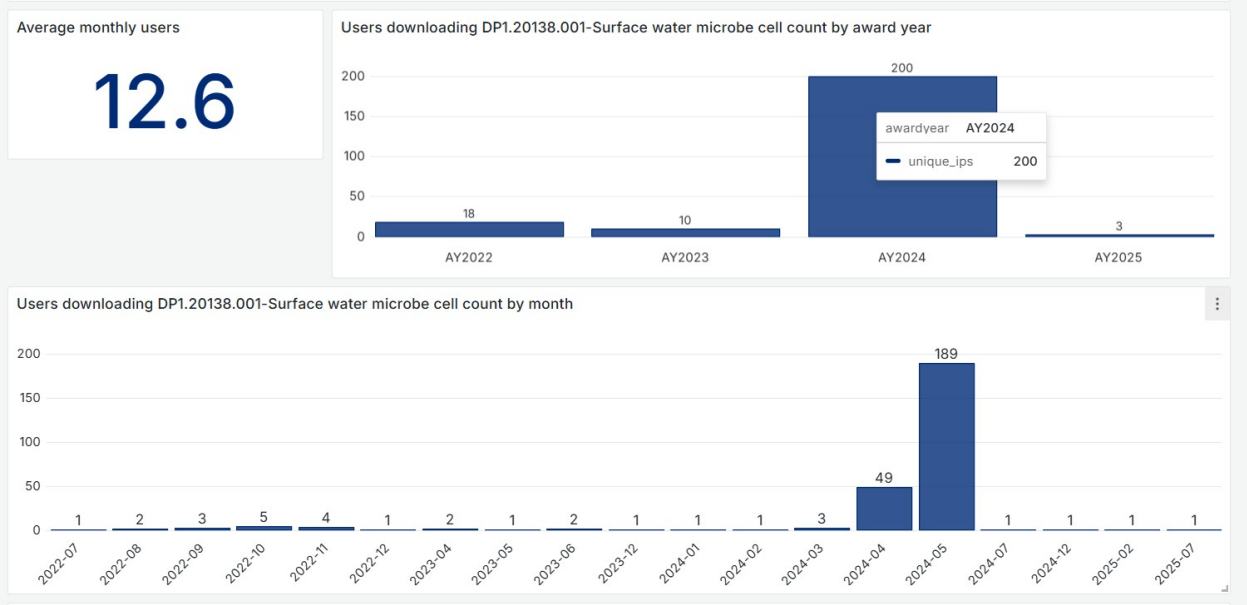
Figure 2: Regression analysis of surface water cell counts (x-axis) vs. surface water group abundances (y-axis).



fort, documenting total numbers was not useful and not something anyone does any more. She thought in an experiment where you want to confirm that your control is much different than a treatment, or that you are properly sterilizing something, that it is a useful measure, but doesn't tell you anything meaningful about the community when used more for correlational analysis. So we are both in agreement that it has no utility in the NEON sampling protocol."

- Andrew Rypel, Aquatic Biology TWG: "This makes sense to me Steph. I support the recommendation in your document."
- Nathan Ruhl, Aquatic Biology TWG: "I agree with your overall conclusion that this dataset might not be worth a continued investment, but it would be good to understand who is actually using/accessing the data before discontinuing it, if possible. Someone used it for something…" [Refers to spike in user numbers in spring 2024.]
- Jeff Blanchard, Microbe TWG: "I don't have any comments to offer for or against discontinuing the data product. I have recently started working the the metagenomic data and that is the limit of my experience with aquatic data to date."

Figure 3: Data downloads from the NEON portal for surface water cell counts



5 RECOMMENDATIONS

Cell count data are only collected in surface water samples, while group abundance data are collected for both surface water and benthic microbe samples. Due to the relationship between data products, the assumption that data users are more likely to use data produced by sequencing methods, and the apparent lack of data downloads from the NEON portal to date, it is recommended that the Surface water microbe cell count (DP1.20138.001) data product be discontinued.

6 ACKNOWLEDGEMENTS

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7 REFERENCES

Hobbie, J.E., J. Daley, and S. Jasper. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Applied Environmental Microbiology 33:1225-1228.

Lisle J.T. and J.C. Priscu. 2004. The occurrence of lysogenic bacteria and microbial aggregates in the lakes of the McMurdo Dry Valleys, Antarctica. Microbial Ecology 47(4):427-439.