TCF - Quinnipiac River Fund Final Report

Instructions

QUINNIPIAC RIVER FUND GRANT AWARD - FINAL REPORT QUESTIONS

This form is to be completed by all nonprofit organizations that received a grant through the Quinnipiac River Fund.

Grant Details

Grant Details

Organization Name
University of New Haven

Grant Description
to support the study of biofilm composition and growth on polypropylene microplastic versus natural stone in unimpaired and impaired sections of the Quinnipiac River.
Total Grant Amount
15500.00

Report Questions

1. List the specific objectives/outcomes of the project and tell how they were met during the grant period. Also, provide an update on any special conditions of the grant (if applicable).

140 samples were successfully harvested from two sites within the Quinnipiac River Watershed. We evaluated the microbial diversity of the river-cultured microplastic and stone substrates from the impacted Quinnipiac River (Quinnipiac River and Canoe Launch, Cheshire, CT) and nonimpacted Honeypot Brook (Cheshire Park, Cheshire, CT) to determine the influence on biofilm assemblage. We also evaluated possible preferential coliform colony accumulation in the microplastic substrate over that of stone in conjunction with site location. Generalized linear modeling demonstrated that the influence of both site location and substrate explained the presence of total coliform attachment. Total coliform colony counts were greater in the impaired Quinnipiac River site than in the unimpaired Honeypot Brook tributary ($W=583$, $p=0.037$) and on the microplastic substrate than the stone substrate ($W=1038$, $p=0.022$). (Figure 1).

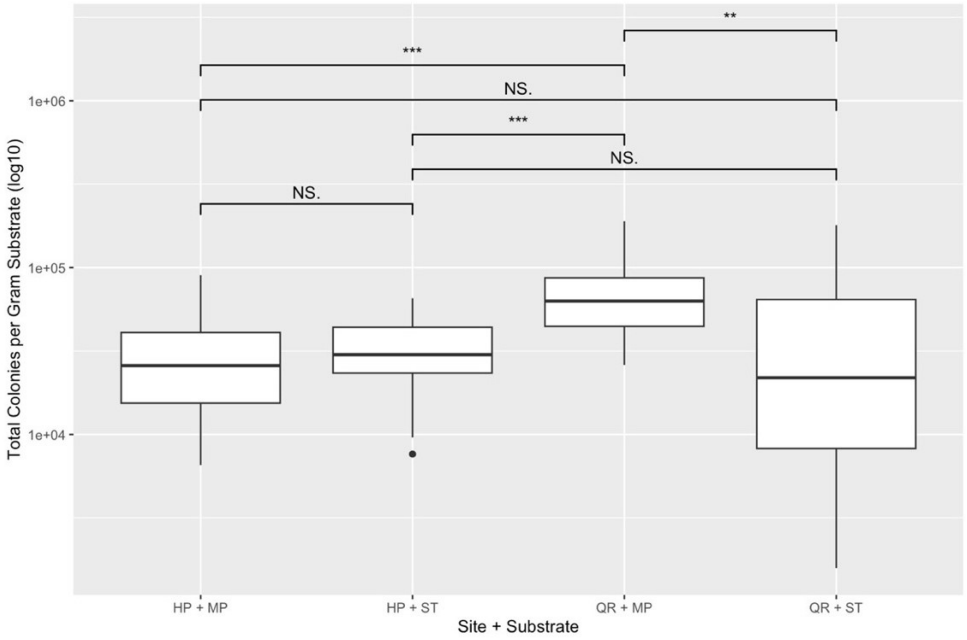

Figure 1: Total coliform colonies taken from Day 90 samples and diluted to 1:1000. QR=impaired, HP=unimpaired, MP=microplastic, ST=stone. Median (middle horizontal bar in box), interquartile range, minimum, maximum, and outliers are represented. n=80. *** = <0.01, ** = <0.05, NS = not significant

Sequenced features to the class level were dominated by *Alphaproteobacteria, Betaproteobacteria,* and *Gammaproteobacteria,* comprising 75% of the community biome. Simpson's Diversity indices indicated that within the two substrates, there was little variation between the communities. (Figure 2). It was noted that microplastic alpha diversity trended slightly lower than the stone.

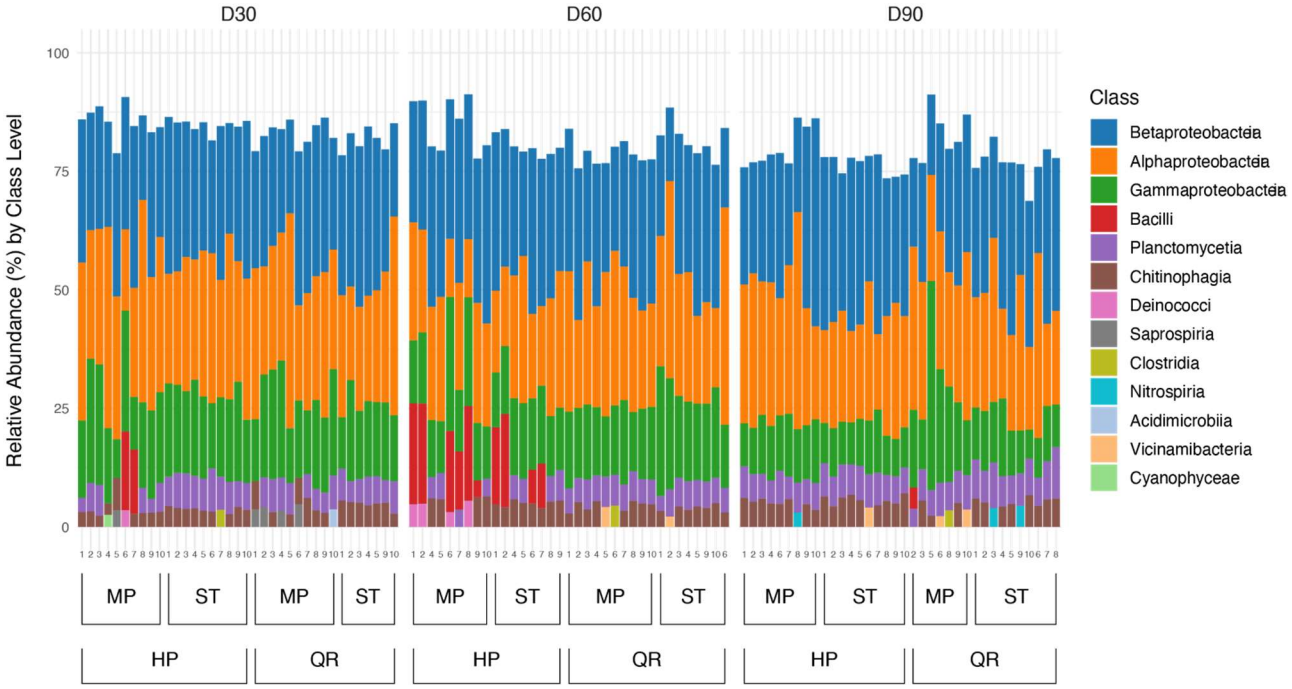

Figure 2: Total relative abundance (percent) of top classes on microplastic (MP) and stone (ST) substrate at the impaired (QR) and impaired (HP) sites over the sampling period. Each bar represents one replicate sample. n=108

Further analysis of common aquatic enteropathogens showed that the genus *Citrobacter* was significantly more abundant on the microplastics at both locations. (Figure 3).

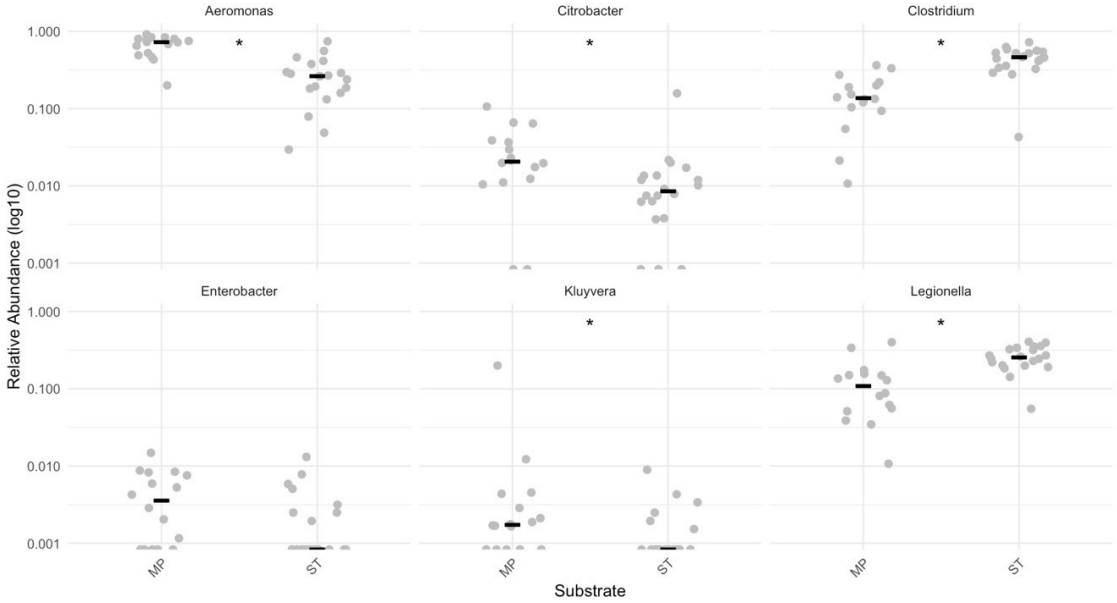

Figure 3: Representative abundances (log10) of select genera on microplastic (MP) and stone (ST). n=38, * = $p<0.05$

2. Please share your successes, challenges and any lessons learned through the implementation of your project. Were there any unintended consequences or lessons learned that may affect how you operate your program moving forward?

At the Day 60 harvest date, it was noted that the sample cage at the HP location was removed from the water and placed on a rock. A previous visit approximately a week prior confirmed the position, and it is unclear how long the cage had been out of the water. Before the D60 sample harvest, the cage was replaced in the HP and allowed to soak for several minutes to rehydrate desiccated biomass**.** We do not know the extent of biofilm disruption due to remaining out of the Honeypot Brook site around Day 60. While we did have signage and a QR code to visit a project landing page, it was clear that the apparatus could be better identified as a scientific project. It is suspected that by Day 90, the accumulated detritus on the exterior of the biome tea infuser may have played some role in reducing flow-through to the substrate itself. Alternatives for future studies could include a less fine mesh that would contain the 3 mm substrate size while allowing for uninhibited water flow.

3. What are the opportunities and needs of your organization as it continues to move forward with its work to positively impact the Quinnipiac River?

We acknowledge that this study was not a full replication as only one site was used for the impaired and unimpaired locations; however, these results indicate that further within-condition replication is warranted in a future study. Future directions for this study include additional exploration of community richness and evenness over time, which could be an interesting focus. Though outside of the scope of our analyses, a similar observation using Pielou's evenness suggested significant change within groups between D30 and D60 ($p=6.13e07$). Indeed, this was underscored in our beta diversity analysis, wherein the collection date influenced clustering between site and substrate pairing.

Examination of successional growth over an extended study period may provide more information into early and later biofilm development and the influence of seasonal variability. Comparing environmental communities from water samples with that of the adhered community to the microplastic substrate would be another approach to analyze the incident of preferential selection.

The limitation of selective media use for coliform monitoring is that the methodology indicates the presence or absence of *E. coli* and non-*E. coli* organisms. While we did not see a significant abundance of *E. coli* in the substrate microbiomes, it was evident that the latter was more problematic and too general to determine what fraction of these organisms may be pathogens. Although more costly, using 16S rRNA gene sequencing elucidated the specific genera included in this category. The microbiomes of the microplastic and stone substrate were not overwhelmingly distinct at the class level as first hypothesized; however, there appears to be support that an impaired waterbody with microplastics may suffer from an additive impact of their presence. That is, the discharge of these particles in riverine systems alone does not address the issue's totality; the system's condition must also be considered.

Additional developments of this project present the opportunity to engage the public in 'citizen science' to understand the fate of microplastics in the environment. Much of the field activity is hands-on and would be a wonderful learning experience in aquatic ecology, fieldwork, and scientific methods. Similar small-group field experiences could be developed and tailored to the level of the attendee. This project has components that can be featured at clean-up events to highlight the research and importance behind it. The value of seeing deployed materials created from a sketchpad will inspire others to become interested in field studies.

Link to open-access publication: https://rdcu.be/edXQs

Attachments

Financial information (required): Please provide a detailed accounting of how the specific grant dollars were spent based on the budget submitted in the grant application.

DATE	ITEM	CATEGORY	COST
3/20/2023	Location Scouting	Mileage	$48.73
4/7/2023	YSI Phosphate Reagent (50)	Field	$44.65
4/7/2023	YSI Nitrate Reagent (50)	Field	$123.51
4/7/2023	WhirlPak 13oz (500)	Field	$144.13
4/7/2023	Brilliance (TM) E.coli/fecal coliform dehydrated media, 500g	Lab	$868.38
4/7/2023	Fisherbrand Disposable Petri dishes (500ct) FB087571	Lab	$44.55
4/7/2023	DNeasy PowerSoil Pro Kit (250)	Lab	$1,830.00
4/7/2023	3 in stainless steel tea infuser	Field	$157.39
4/25/2023	Amphotericin B 1g	Lab	$106.76
4/27/2023	Lowes-PVC Piping/Sand	Field	$71.53
4/27/2023	SupplyHouse.com-Fittings	Field	$163.26
4/29/2023	Home Depot-PVC piping/Cement	Field	$11.63
5/5/2023	3 in stainless steel tea infuser	Field	$41.17
5/15/2023	Saurav Shretha	Personnel	$1,200.00
5/15/2023	Anne Gilewski	Personnel	$2,400.00
5/7/2023	Fishing bobs (8)	Field	$16.08
5/11/2023	3mm polypropylene beads	Field	$15.73
5/11/2023	150lb monofilament	Field	$11.69
5/7/2023	Substrate Collection	Mileage	$48.73
5/19/2023	VistaPrint-Field signage	Field	$42.06
5/24/2023	Wal-Mart-Cage tethers/rope	Field	$40.34

5/25/2023 Cage Deployment	Mileage	$48.73
5/26/2023 Site Visit Post Deploy	Mileage	$48.73
5/30/2023 Nanopore MinION R9 flow cell	Lab	$4,500.00
5/30/2023 Nanopore 16S rRNA library kit	Lab	$1,800.00
6/8/2023 Site Visit Check	Mileage	$48.73
	Publication	$976.97
3/14/2025 APC-Environmental Microbiome		
	Total	$14,853.48

Pictures (optional): Please attach one to three pictures in JPEG format, in the highest resolution possible, of activities that have occurred throughout the grant period as a result of grant funding. By providing pictures, your organization is consenting to unlimited use of the pictures by The Community Foundation for Greater New Haven and/or the Valley Community Foundation in publications in print and online (including www.thequinnipiacriver.com). Please include a description of each photo and, when known, the photographer to be credited.

Picture 1

University of New Haven graduate students Saurav Shrestha and Osasenaga Otuomagie removing samples from the Honeypot Brook location, Cheshire CT, July 21, 2023. Photo: Anne Gilewski

Picture 2
University of New Haven graduate students Anne Gilewski and Saurav Shrestha positioning sample cage in the Quinnipiac River, Cheshire CT, May 25, 2023. Photo: Dr. Jean-Paul Simjouw

Picture 3
University of New Haven graduate student Saurav Shrestha prepares sample cage for deployment, Quinnipiac River, Cheshire CT, May 25, 2023. Photo: Anne Gilewski