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 a study that will focus on characterizing the gut microbiomes of estuarine fish in the lower Quinnipiac River and New Haven Harbor, as well as assessing the relationships between environmental conditions, diet, microplastic prevalence, and these microbial communities.

Total Grant Amount

19,000.00

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).

This grant application had four specific objectives; to 1) monitor the prevalence of microplastics in Fundulus heteroclitus fish in the lower Quinnipiac River and New Haven Harbor and link this to differences in their microbiomes based on location along the river, 2) monitor abiotic factors along the river and assess how these may influence the microbiome of F. heteroclitus, independent of microplastic contamination, 3) assess how the microbial composition of the F. heteroclitus gut relates to the overall health of the fish in terms of physiology and growth, and 4) using temporal monitoring, determine any seasonal effects on the gut microbiome of F. heteroclitus that may affect fish health.

The project was sectioned into several stages, the first of which was to collect and begin to process the F. heteroclitus fish that were sampled over a several month period in the summer for 2021. Five sites were monitored for fish collection (listed in order from the most upriver to most downriver site): North Haven boat ramp (41.36481944, -72.87945278), Tidal Marsh (41.348725, -72.8732722), Dover beach (41.3196945, 72.8882722), Long Warf (41.28536944, -72.923989), and Sandy Point (41.267367, -72.92939722). All sites were seined for fish approximately every 4-5 weeks (May 25th/27th, June 28th, July 28th) with the final sampling pushed back due to weather conditions not conducive to effective or safe seining (September 15th). Certain sites and dates were more successful than others, producing some variability in the dates/locations that are present in our analysis. When sampling, a maximum of 10 F. heteroclitus at each site per date were collected for health monitoring, internal microplastics analysis, and fecal extraction for microbiome analysis (Attached ‘picture 1’). Briefly, a total of 108 F. heteroclitus were obtained for analysis. Fish were consistently obtained at the Tidal Marsh site throughout all sampling periods. Dover beach was also consistent, however no fish were obtained during the September sampling. The other sites were less consistent in fish catch rates, but during the June sampling fish were obtained from all sites. Abiotic factors such as water temperature and salinity were also recorded.

The second stage of the project involved processing the collected F. heteroclitus. Several parameters were measured as part of the fish processing, including length, total weight, gutted weight, and fecal weight. From this information we were able to calculate Fulton's body condition factor (K), a measure of overall physiological condition in fish corrected for body length (Ricker 1975). In general, this provides information about overall health of the fish; as it gets longer it should be relatively heavier if it is healthy and well fed. The fecal material of the fish was extracted and processed to extract DNA from the sample. These samples were processed to preserve high molecular weight DNA and the DNA was frozen until the next stage of the project. Digestive tracts and livers were removed and stored at –20C for further later processing.

The third stage of the project involved quantifying and characterizing microplastics present in the digestive tract and liver and sequencing the microbial contents of the fish gut. The microbial sequencing of the DNA extracted from the fecal samples gives us a glimpse of the gut microbiome of F. heteroclitus; temporally and at the separate locations along the waterway. DNA libraries were created by amplifying and tagging the 16S rRNA gene, a major bacterial species-defining metric. Utilizing Oxford Nanopore Technologies' sequencing allowed us to sequence and analyze nearly the entire 16S rRNA gene sequence of the fish gut microbiota, allowing for more specific bacterial clade identifications. Microplastics were isolated from previously digestive tracts and liver tissue using chemical digestion. Tissue samples (digestive tracts and livers, separately) were thawed, combined with a 10% KOH solution, and held in a lab oven at 60°C for 48 hours. Following digestion, samples were vacuum filtered on cellulose 11 µm cellulose filter paper, stored in aluminum foil, and dried for 24 hours in a lab oven at 60°C.

Together, these stages culminated in our ability to study the microbial communities that existed within the intestines of the fish we sampled. Linking these communities to the biological factors we cataloged about the fish, and the abiotic factors from where they were collected, have linked overall fish health to the gut microbiome. it was found that the location where the fish live does influence their gut microbes. Looking at the Shannon diversity index, linking richness and evenness of species in a community, the bacterial diversity is statistically lower at both Long Warf and Sandy Point than compared to the other three sites (Attached 'picture 2'). This decrease in diversity seems to correlate with the increased salinity detected in the water at both of those sites. Interestingly, we also see a correlation between these two sites and an increase in the Fulton's condition factor, indicating that the fish at these sites are, overall, healthier than those at the more freshwater sites. Delving further into the microbiome data, there appears to be an inverse correlation between the amount of Proteobacteria and Firmicute bacteria that are present in the fish gut (Attached 'picture 3'). Specifically, an increase in the number of Firmicutes present in the gut microbiome predicts a lower number of Proteobacteria. Slightly more concerning is that, looking at the Firmicute species present, Clostridium perfringens, a common cause of food poisoning, is quite abundant in these samples (ranging from <1% all the way up to >64% in one case). Microplastics isolated from tissues are currently undergoing analysis under magnification. Microplastics are being characterized (color, shape, and size) and quantified. Results to date suggest greater microplastic loading in digestive tract samples, but also the present of some microplastics in all liver samples analyzed.

Taken together, we have found several correlations with both biotic and abiotic factors that link directly to the gut microbiome diversity of F. heteroclitus. As our data analysis continues to evolve and we fully integrate our microbiome, microplastics, and environmental data we expect to find novel interactions that will warrant further study.

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?

Our initial goals aimed at studying F. heteroclitus to monitor and study the influence of microplastics on aquatic health. The funding through the Quinnipiac River Fund grant has allowed us to take the first important step in defining factors that can be used in the future to monitor fish health in the Quinnipiac River.

Our DNA library preparation and amplification went very well and we got a large number of reads from the actual sequencing run. This study has helped inform how long we need to run our sequencing machine in order to get enough data for analysis. This will save us time in the future and allow us to have a more efficient sequencing protocol.

While planned fish sampling frequency was monthly (i.e., every 4-5 weeks), weather conditions and the timing of favorable tides during the month of August precluded seining. This resulted in a 1.5 month gap in sample collections. Given more favorable conditions, sampling in both August and September might have been possible. Additionally, the number of F. heteroclitus collected at each site varied considerably. While variance is to be expected in field ecology, greater sample frequency or the inclusion of additional sites could have eliminated some of this variance.

Also, F. heteroclitus included in this study were subsampled from those captured during seining. During the sample collection, a representative subsample of F. heteroclitus captured during seining were chosen for inclusion in this project. During early sampling, this subsample included individuals selected from the full range of sizes available and included a number of smaller individuals. Following initial lab processing of collected fish, it became clear that the digestive tracts of smaller individuals may not hold enough material for effective gene sequencing. This led to a change in subsample selection and an acknowledged bias for larger individuals, albeit while still including some smaller individuals.

Overall, our project went smoothly, especially the sampling and initial processing. We did have to ask for an extension of this grant, which was mainly due to the full sequencing and analysis steps. With such a large number of samples to sequence we were not initially prepared for the time it would take to process the large amount of data that we would obtain. In the future we'll be able to budget our time more accordingly, considering analysis time and the resources needed to perform these analyses.

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?

Based on preliminary findings from the work funded through this grant, a number of interesting trends and potential relationships have been identified. While the study of both fish gut microbiomes and the impacts of ingested microplastics are in their infancy, these initial results are promising and deserve additional attention.

Immediate research needs include resources to move beyond visual assessment of collected microplastics to polymer identification using spectroscopy. Already collected microplastics may be analyzed to determine their composition, which would provide new insights on already completed research projects. Additionally, resources to facilitate a network of labs and researchers to consistently conduct polymer identification would provide great insight on the status of plastic pollution in the Quinnipiac River.

Medium term research needs include resources for further studies with greater scope. Such studies could focus on increasing sampling frequency and density, which could provide clearer and more resolved characterizations of microplastic prevalence and distributions in the Quinnipiac River and how they change over time. Additionally, greater spatial resolution may lead the identification of microplastic pollution point sources.

Long term research needs include resources for both reducing the rate of microplastic pollution and mitigating microplastics already in riverine environments. This will require interdisciplinary projects focused on detection and collection or in situ elimination of microplastics.

In addition to microplastic-focused research, the study of the fish gut microbiome, the ecological influences on gut microbial communities, and how these impact fish condition, survival, fecundity, and possibly population stability and resilience are currently growing areas of study.

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.

Detailed Accounting

accounting document info.docx

Pictures (optional): Please attach 1 to 3 pictures of activities that have occurred throughout the grant period (with a description for each) as a result of grant funding. All pictures should be submitted in JPEG format and may be uploaded to www.thequinnipiacriver.com and used in Foundation publications.

Picture 1

Picture1.jpg

Description

Picture 1 is a graph representing the number of fecal extracts that were obtained from each site during each of the sampling visits.

Picture 2

Picture2.jpg

Description

Picture 2 is a box plot graph representing the Shannon diversity index of the gut microbiomes that were obtained from each of the sites samples. There is statistical support to conclude that the diversity of bacteria at the Long Warf and Sandy Point sites was decreased compared to those at the other three sites.

Picture 3

Picture3.jpg

Description

Picture 3 is a scatter plot looking at the correlation between the number of Proteobacteria and Firmicutes that were sequenced in each sample. Each point on the graph represents one fish. There is an indication that as the number of Firmicutes increases in the gut the range in the number of Proteobacteria decreases.