**Impact of Habitat, Dissolved Oxygen, and Disease on Benthic Fish Species in the St. Lawrence River**

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**Background**

Although the gut microbiome in humans has been the subject of extensive study, research pertaining to the importance and shaping of the microbiome in fish is still emerging. A layer of this which has yet to be explored within many freshwater habitats is how the gut microbiomes of different fish species respond to abiotic and biotic stressors. The impacts of human disruption such as climate change, pollution, and invasive species introduction have long been studied in a variety of aquatic environments. However, how the impacts of these disruptions within an ecosystem elicit a response in the microbiomes of different aquatic species is still a burgeoning topic of research, especially in freshwater systems and fish. Preliminary work regarding the gut microbiome of fish has shown that there are many biotic and abiotic factors which are impacted by and impact its gut microbiome, such as species, metabolism, disease susceptibility, growth and development[MT1]  (Gallo, Farrell, & Leydet 2020; Ghanbari, Kneifel, & Domig 2015). Conversely, there are many outside factors which shape and impact the composition of the gut microbiome, a phenomenon observed throughout microbiome analysis. These factors include many different aspects of the host’s diet, environment, and characteristics. As the habitat evolves, it is important to understand how the niche these species occupy within their food web may also change[MT2] , sparking interesting in understanding the relationship between trophic position and gut microbe acquisition.  Diseases such as Viral Hemorrhagic Septicemia Virus and hypoxia resulting from climate change are both issues which plague the upper St Lawrence River system.  Understanding the impacts these changes have on the microbiomes of fish can provide useful insight regarding overall fish health, as well as a knowledge base in aquaculture settings where maintaining the health of fish is key. [MT3]

**Methods and Preliminary Results**

The main objective of this research is to obtain a greater breadth of information regarding the evolving relationship between gut microbiomes development, fish condition, and changes to the ecosystem. Specifically, we want to characterize changes in the gut microbiota of fish due to differences in species, habitat (reference vs. manipulated), dissolved oxygen concentrations, and affliction with viral hemorrhagic septicemia virus (VHSV). We hypothesize that there will be significant differences in the gut microbiota at the level of difference of habitat, and a decrease in microbiota diversity in low dissolved oxygen levels and presence of VHSV. While currently understudied in VHSV, the correlation between many diseases and gut microbiota modulation has been well documented in aquatic hosts, including zebrafish, a popular model organism in gut microbiome studies (Ganz 2017). Differences in hypoxia response across many species has also been well documented, and a comparison across species with previously studied hypoxia resistance could prove insightful on how the microbiome differs in fish of varying resistance to such conditions.

         In the summer of 2021, I worked in collaboration with the Thousand Islands Biological Stations (TIBS) to collect samples including fish (hindgut, stomach when applicable), zooplankton, and water. Data collection was also performed in conjunction with the Lake Ontario Division of Fish and Wildlife of the New York Department of Environmental Conservation to determine areas of interest concurrent with the trophic interaction data collection. [MT4] This included additional sample collection of key species from reference and restored wetlands in both Flynn Bay and Eel Bay that were at the time experiencing unprecedented hypoxia conditions in July. All fish collected were euthanized and stored on ice, with dissection and gut preservation occurring within the same day as capture. During the July sampling, I collected 22 Brown bullhead (*Ameiurus nebulosus*), 19 Bluegill (*Lepomis macrochirus*), 15 Tadpole madtom (*Noturus gyrinus*), and 23 Pumpkinseed (*Lepomis gibbsus*). During the August sampling, I collected 13 Brown bullhead, 10 Bluegill, 18 Goldenshiners (*Notemigonus crysoleucas*), 19 Largemouth bass [MT5] (*Micropterus salmoides*), 21 Pumpkinseed, 16 Round goby (*Neogobius melanostomus*), 10 Tadpole madtom, and 19 Yellow perch (*Perca flavescens*) from Flynn Bay Eel Bay, Rose Bay, Millens Bay, and Cobb Bay. All gut samples were retrieved using sterile methods and stored in NAP buffer until ready for DNA extraction.

**Current and Future Work**

This work is ongoing. Currently [MT6] samples from the summer of 2021 are undergoing DNA extraction and PCR amplification to prepare for Illumina® MiSeq sequencing, which will occur in the winter session through the spring of 2022, and spring of 2022 through the summer session respectively. [MT7] The opportunities provided by the Sussman internship allowed me to explore and expand my original fish gut microbiome focus with the help of my mentors and other researchers working at TIBS. I have the opportunity to incorporate YOY muskellunge samples (n=30) for a comparison between wild vs stocked rearing in conjunction with otolith analysis, additional YOY and year 1 fish collected from the reference and restored wetlands from May-July 2021, and Round goby preserved gut samples from the summer of 2019 for a comparison between a multitude of factors including infection with VHSV, time permitting. Once it has been determined what I will be able to include, these samples will also be used for DNA extractions, PCR, and sequencing within the same timeline as highlighted above. Results of these studies will be submitted for publication, and the Sussman fund, TIBS, and my mentor from the DEC Jessica Goretzke will be acknowledged in any subsequent publication.[MT8]

**Works cited**

Gallo, B.D., Farrell, J.M., Leydet, B. 2020. Use of next generation sequencing to compare

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Ghanbari, M., Kneifel, W., & Domig, K. J. 2015. A new view of the fish gut microbiome:

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