PROJECT SUMMARY

OVERVIEW. Ecosystem management and conservation practices are often focused on the maintenance of diversity, with less attention paid to ecological connectivity. However, how species interact with other species in their environment can have profound impacts on the structure of the ecosystem as a whole. The vertebrate gut microbiome provides a unique opportunity to understand the role of ecological connectivity in the production of a functional ecosystem, as these microbial communities are essential for host physical functioning but change rapidly in response to biotic and abiotic environmental conditions. As these changes take place across multiple scales, metacommunity theory can provide a useful framework to understand how the ecological conditions and host evolutionary processes interact to produce a community's structure. By treating each host or species as a unique "patch" whose composition is determined by ecological and evolutionary processes, we can test the relative importance of these processes within a patch vs. between patches. Thus, to investigate how biotic and abiotic features interact to contribute to higher order host functioning, I will work with leading expert in gut microbial assembly, Dr. Kevin Kohl at the University of Pittsburgh (UPitt), to test hypotheses from metacommunity theory in a tractable multi-species aquatic system. Specifically, I will create an artificial metacommunity consisting of three different sympatric species of tadpoles. Through developing this system, I will also test the importance of dispersal on host performance and fitness, demonstrating whether a more diverse habitats results in beneficial host outcomes. Filling these gaps is foundational to understanding how microbiome structure and function scales across animal populations and their wider ecosystem as well as the implications of variation in structure for host physical functioning. More broadly, this system contributes to an understanding of how higher order functions are derived from interactions of organisms across scales.

INTELLECTUAL MERIT. The proposed research will help disentangle effects of host behavior and environment on gut microbial assembly at both the taxonomic and functional level, as well as understand the importance of ecological connectivity for the maintenance of ecosystem function. Furthermore, by identifying important drivers of gut microbial assembly while applying hypotheses derived from metacommunity theory, this work will also provide empirical evidence for metacommunity models. The proposed research will also test the Rules of Life by examining how functional and microbial elements are transferred between hosts, and the impact of this transfer on host physical functioning under changing environmental conditions.

BROADER IMPACTS. I have designed this project as a stepping stone to my ideal career as a researcher at an aquarium or museum. As my PhD work has leveraged observational data from a wild terrestrial species, a multi-species, experimental aquatic system will broaden my experience to include the impact of ecological connectivity and abiotic environments on gut microbial structure and host health. This project will also expand the conservation implications of my work, as understanding the transmission of beneficial microbes provides important foundational knowledge about amphibian physiology under changing environmental conditions relevant to management decisions. In addition to publishing open access journal articles and presenting at national and international conferences, I will work with UPitt's PittBio Outreach Office to develop and implement a free K-12 module for Pittsburgh public schools. The dissemination of this research to K-12 students may result in the recruitment of students from underrepresented groups to STEM, but I am committed to taking an active role in this as well: I will also work with the PittBio Outreach's "GeneTeam" Program, which pairs local public school students with researchers in an REU-like fashion over the summer. Specific to the implementation of my proposed research, I will recruit and mentor undergraduates from historically excluded groups to assist in my project aims and develop their own independent research projects related to host-associated microbial dynamics. I am well prepared to mentor these students based on the extensive mentorship I have done as a graduate student. As a women of color in STEM and a second generation immigrant, I am committed to recruiting more students from historically excluded backgrounds to STEM as well as creating a supportive and inclusive community in which they can thrive.

PROJECT DESCRIPTION: Using metacommunity theory to assess the impact of multi-species interactions on gut microbial assembly

BACKGROUND. Conservation efforts are often focused on the maintenance of unique species or high number of species, but the loss of highly interactive species may also lead to large declines in ecosystem composition and diversity[1]. As declines in ecosystem diversity are often linked to changes in ecosystem services and functions, understanding the importance of these interspecies interactions could be a key to better management practices that preserve ecosystem connectivity[2,3]. Host-associated microbiomes provide a unique opportunity to understand how ecosystem structure and function, as shaped by fundamental ecological and evolutionary processes, respond to variation in species interactions and environment. These complex, dynamic microbial communities are essential for host physical functioning and development. In particular, the gut microbiome breaks down complex carbohydrates, produces vitamins, trains the immune system, and resists pathogens. The gut microbiome maintains this level of functioning while also exhibiting a high degree of plasticity, fluctuating over time in response to age, diet[4], season[5], interactions[6], and shared environments[7,8]. Most research conducted thus far in microbiome assembly has been limited to examining the effect of social interactions within a single species. However, nearly all animal hosts live, interact, and share microbes with their own species as well as among other sympatric species[9]. *Despite these hypotheses, little is known about the consequences of interspecies interactions and shared environment on gut microbial assembly. As microbial symbionts are thought to promote ecological adaptation, the transfer of microbes between hosts could buffer hosts against environmental stressors.*

The transfer of gut microbes, especially across host individuals and species, could be primarily driven by metacommunity processes. Specifically, ecological processes such as dispersal between hosts or environments have been shown to be important drivers of microbiome assembly, and limiting dispersal can significantly alter community composition[9–12]. In a multi-species context, we can extend the traditional use of community ecological theory in microbiome research to a metacommunity approach, treating individual hosts and host species as "patches" and tracking ecological and evolutionary processes (dispersal, selection, priority effects) within or between patches (Figure 1). By creating a tractable aquatic system in which to compare the relative importance of these eco-evolutionary processes, I will apply hypotheses proposed by metacommunity models to the microbiome and thus identify causal drivers of gut microbial assembly. Further, I will also test the importance of dispersal on host performance and fitness, demonstrating whether a more diverse habitats (here increased number of host species) results in beneficial host outcomes. Filling these gaps is foundational to understanding how microbiome structure and function scales across animal populations and their wider ecosystem and their implications for host functioning. This system also contributes to a broader understanding of how higher order functions are derived from interactions of organisms across scales.

My long-term research goal is to understand the complex evolutionary and ecological relationships between the host and their gut microbiome on host health and fitness from a metacommunity perspective. My objective in this proposal is to test how host social dynamics and abiotic environment impact microbial dynamics at the taxonomic level and the genic level and characterize the consequences of these dynamics on host health. The central hypothesis is that rates of host microbial uptake differ based on both the type of interactions between hosts and the abiotic environment hosts occupy. Specifically, I predict that dispersal between hosts will increase when

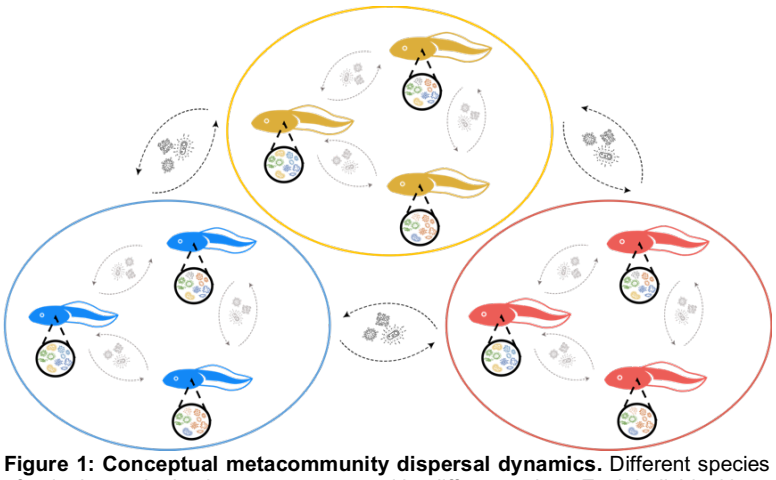


Figure 1: Conceptual metacommunity dispersal dynamics. Different species of tadpoles and microbes are represented by different colors. Each individual host could be considered a patch, as well as each group of hosts, as represented by the ovals. Interspecies dispersal (black) is shown between species groups, and intraspecies dispersal (grey) are within species groups.

hosts have access to diverse sets of interactions or host species are more stressed, and that such dispersal will improve host performance under stressful environments.

I plan to test my central hypothesis by developing a tractable aquatic study system using three species of wild-derived frog tadpoles: the bullfrog (*Lithobates catesbeianus*), the leopard frog (*Lithobates pipiens*) and the green frog (*Lithobates clamitans*). *Well-mixed, artificial aquatic environments provide an even environmental pressure of host-host transmission, allowing me to test the ability of the host to regulate their gut microbiome in the context of a diverse microbial environment by treating environmental dispersal as the null model.* Amphibians exhibit plastic development, allowing me to test the effects of dispersal regimes or abiotic environmental factors on host development and fitness as adults. Lastly, these *Lithobates* species co-occur in natural settings in western Pennsylvania, replicating a system resembling wild assembly processes and ecological contexts. This system thus balances the need for experimental manipulation and preserving natural processes. *Using the Lithobates system, I will pursue the following two aims*:

Aim 1: Disentangle the effects of intra- and interspecies interaction on gut microbial assembly.

Aim 2: Understanding the role of a multispecies environment on microbial dispersal and host health under stressful environmental conditions.

STUDY SYSTEM. In order to control for priority effects in the microbiome stemming from the egg environment, wild captured frogs will be bred in captivity and eggs will be reared for these experiments[13]. The sponsoring scientist's lab, Dr. Kevin Kohl's lab at the University of Pittsburgh (UPitt), has previously undertaken similar breeding efforts and is well equipped to support my training in this system[14]. Tadpoles are quite social and have been shown to prefer environments with similar sized conspecifics available[15,16]. Interactions range from schooling to competitive behaviors[17–19]. Importantly, the use of tadpoles to test the influence of the microbiome on host physiology and performance is well-documented: numerous studies have shown that tadpoles with disrupted microbiomes exhibited increased susceptibility to disease and parasites[20–22]. While amphibians are a tractable system in which to study gut microbial assembly, understanding amphibian ecological connectivity has important implications for conservation. Amphibians are indicator species of overall ecosystem health, but due to shifting ranges from climate change, ecological connectivity within these communities is deteriorating[23].

AIM 1: Disentangle the effects of intra- and interspecies interaction on gut microbial assembly. Social interactions are known to be an important contributor to the host microbiome[6,24,25]. Most studies investigating these interactions have done so in populations or groups that also cohabitate, thus it is difficult to fully disentangle social interactions from shared environments[9,26–30]. While social interactions have been causally linked to changes in host physiology and thereby host performance and fitness, the mechanism mediating this relationship is less well understood[31–34]. I will use a series of experiments to test whether dispersal rates and the resulting microbiome structure differ based on the type of interaction through two specific hypotheses:

> *H1: Gut microbial structure is impacted by cohabitation and interaction between hosts.*
>
> *H2: Gut microbial communities follow patterns of metacommunity theory and thus host microbiome diversity will differ between mixed and single host species environments.*

Experimental design: To accomplish this aim, I will assign tadpoles to tanks with and without plastic barriers in a factorial design (Figure 2). Tanks will be designated either same species or mixed species hosts, and with various designs of barriers that differentially allow visual interactions using one-way glass, direct contact through co-housing, and/or chemical and microbial interactions using a flow-through water system. These barriers will serve as a proxy for interaction vs cohabitation: hosts in tanks with no barrier are assumed to interact, those in tanks with permeable or clear barriers are assumed to cohabitate, and those with a solid barrier are neither interacting nor cohabitating. The clear barriers are testing for physiological response related to the perception of access. This experimental design will identify if interactions between species are necessary for gut microbiome community similarities and thus the causal relationships between host behavior and microbiome assembly. In addition to microbial community structure, I will measure the impact of socialization on physiology by measuring tadpole growth (length, weight, Gosner stage, scaled mass index) and corticosterone to assess if animals are exhibiting signs of physiological stress[35–37]. After at least 7 weeks of treatment (exact timing based on pilot study), I will sacrifice tadpoles to collect gut contents and extract microbial DNA. For each gut sample, extracted DNA will be prepared for 16S

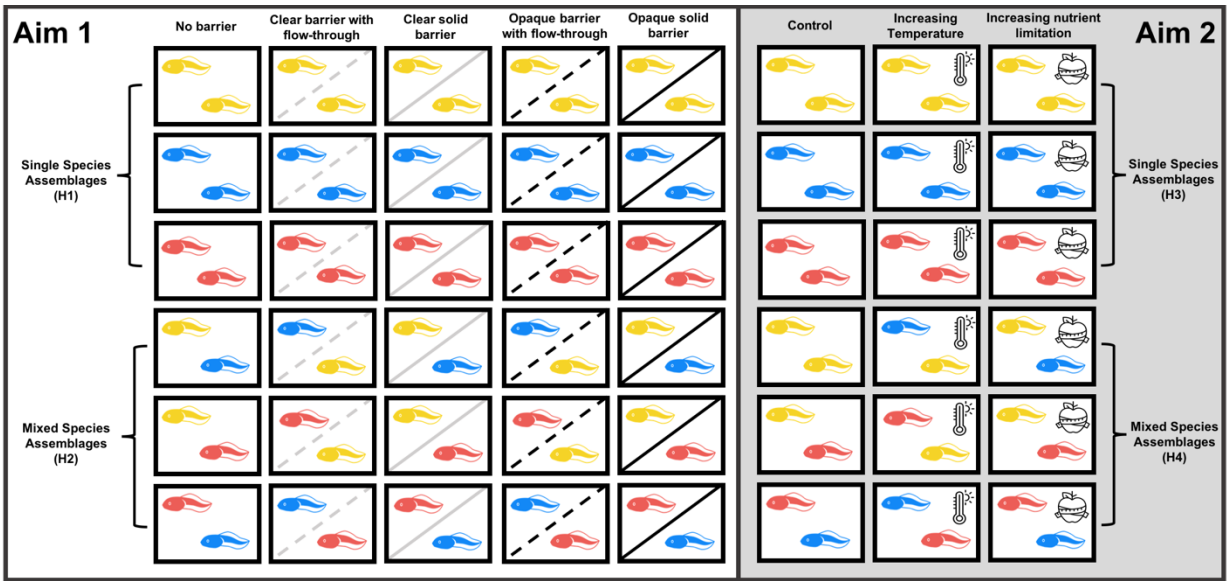


Fig 2: Experimental set up. Experimental set up of tanks for Aim 1 is on the left and Aim 2 is on the right with a grey background. The hypothesis each set of tanks corresponds to is indicated on the left and right of the Aim 1 and 2 tanks, respectively. Each tank will be set up in replicates of 3. Multiple tadpoles of each species will be assigned to each tank as appropriate, allowing for the appropriate level of interaction assigned as well as sampling without replacement over time. Tadpoles will be kept in these treatments for at least 7 weeks (based on preliminary data in the Kohl lab) prior to sampling.

sequencing on an Illumina NovaSeq[38,39]. Trimming, quality filtering, and generation of the amplicon sequence variant table will be completed using DADA2[40]. Beta diversity will be calculated using multiple metrics of community composition such as Bray-Curtis dissimilarity and weighted Unifrac distances. I will quantify distinguishability between microbiomes using PERMANOVAS and visualize this with non-metric multidimensional scaling (NMDS). Multivariate statistical models will control for experimental treatments and be used to determine whether microbial community dispersal between host species is a function of host habitat characteristics. As these molecular, bioinformatic, and statistical techniques are similar to techniques I used during my PhD research, I am well-qualified to undertake these analyses. The support provided by UPitt's Center for Research Computing will also help bridge any gaps between my current bioinformatic or statistical workflows and the newest pipelines and analyses.

Expected Results: **H1:** The type of socialization, as determined by the type of barrier in the tank, will result in physiological changes governing microbiome community structure. For example, if tadpoles have access or perceived access to one another their microbiomes will be compositionally more similar to one another, regardless of barrier flow-through, than animals housed in tanks where the barrier was opaque. Alternatively, perception of sociality could be less important than physical interactions or cohabitation. In this case, I predict that pairs in tanks without a barrier would be the most similar to one another and pairs in tanks with a solid barrier would be the least similar to one another. Pairs in tanks with barriers preventing direct interaction but allowing water through will be of intermediate similarity. **H2:** Species differences between hosts, or heterogeneity between patches, and dispersal limitation (barrier) will result in differences in alpha diversity (richness) and beta diversity (evenness or similarity) predicted by metacommunity models[41]. Hosts housed in single species assemblages will exhibit lower alpha and beta diversity than in mixed species assemblages. If the barrier is more permeable or removed (higher dispersal), alpha diversity will be higher than if the barrier was present. Beta diversity will decrease (become more similar) under higher dispersal. Alternatively, if patch differences are not an important determinant of overall community composition, tadpoles from mixed species assemblages or in tanks with lower dispersal limitations will harbor gut microbial assemblages with similar beta diversity compared to single species groups or tanks with higher dispersal limitations.

AIM 2: Understanding the role of a multispecies environment on microbial dispersal and host health under stressful environmental conditions. Intraspecies sociality and cohabitation have profound effects on the composition of the gut microbiome, and are associated with increased microbial taxa diversity and

increased similarity between hosts[11,24]. These metrics are also often proposed to be important markers of healthy microbiomes and even overall host health and fitness[42–46]. However, under duress, metrics of the gut microbiome, such as microbial community stability and resilience, have been shown to change across a few host taxa[47–50]. Outside of allergen research, little research has been done examining interspecies interactions and cohabitation's impact on the gut microbiome and consequences of this variation on host health[51,52]. The beneficial impacts of interspecies sociality and cohabitation is fundamental to understanding the role of interspecies interactions in ecosystem functioning. Here, I will fill this gap by testing whether stress alters metacommunity dynamics and interspecies sociality buffers it:

H3: Microbial dispersal between hosts will increase in stressful environments.

H4: Increased taxonomic and functional diversity in host species will be associated with a buffering effect against stressful environments on host fitness.

Experimental design: Tanks will be set up in 3 replicate mixed or single species assemblages as in Aim 1 (Figure 2). Instead of barriers, I will introduce stressful environmental variables: temperature and nutrient limitation (Figure 2). Environmental temperature has been shown to have profound effects on the structure of the ectothermic gut microbiome[53–55]. Nutrient limitation has similarly been shown to have a strong causal relationship with structural changes in the gut microbiome[4,56–59]. Non-control assemblages will include the stressful variable gradually increasing from 20°C and a high quality (protein) diet to a maximum stress level or temperature of 33°C or a low protein diet[60]. I will also measure corticosterone to assess if animals in mixed species assemblages are exhibiting fewer signs of physiological stress. Members of the Kohl lab are well versed in assays for determining host tolerance of temperature, assessing growth from nutrient limited tests, and measuring survival. To assess performance related to increased temperature tolerance, I will use locomotor performance metrics including the righting response, or time it takes an individual to recover an upright state after being placed upside down[61,62]. To assess performance post nutrient limitation, I will assess scaled mass index and distance traveled[37]. Extracted DNA will be prepared for both 16S rRNA and shotgun metagenomic sequencing on an Illumina NovaSeq[38,39]. Following trimming and quality filtering, species-level taxonomic abundances will be inferred using MetaPhlAn2. HUMAnN 2.0 will be used to functionally profile the metabolic potential of each sample's microbial community. Diversity metrics will be calculated as detailed in Aim 1.

Expected Results: **H3:** Sociality may increase the rate of dispersal between hosts (tested in Aim 1), and thus provide a mechanism for the transfer of beneficial microbes that buffer the host against environmental change. Specifically, across all tanks, host alpha diversity will increase in stressful environments, and host beta diversity will decrease in stressful environments. **H4:** Mixed species assemblages will exhibit higher tolerance to stressful environments than single species assemblages, leading to higher tolerance to increased temperature and diet stressors and increased survival compared to tadpoles housed in single species assemblages.

Preliminary Data: Previous work from the Kohl lab has shown that alterations in early life were associated with increased susceptibility to disease[20,21]. Work currently being done has also shown the gut microbiome contributes to survival under thermal stress in ectotherms.

INTELLECTUAL MERIT. The proposed work provides the first rigorous and causal test of interspecies interactions on gut microbial assembly using a novel sympatric tadpole system. My research will also advance our understanding of the role of ecological connectivity on ecosystem function by testing hypotheses derived from metacommunity theory and the impact of dispersal modification on transmission between hosts during shifting abiotic conditions. My proposal will greatly increase our understanding of the "Rules of Life" as these experiments examine the relationship between genetic elements, gut microbial community structure, and how variation in these components scales up to observable host performance and fitness phenotypes. We add additional complexity by varying the social environment and abiotic conditions to further test how a host's microbial communities interact with host physiology, and provide empirical evidence to test theoretical metacommunity models.

BROADER IMPACTS. Impacts in Conservation and Ecology: My experiments conducted are relevant to the conservation of amphibian populations. Amphibians are a bellwether of ecosystem health, but are declining globally due to habitat degradation and increased disease prevalence[63,64]. Understanding the mechanisms that govern climate change related temperature tolerance and disease risk as well as the

transmission of beneficial microbes provides important foundational knowledge about amphibian physiology relevant to management decisions of at-risk species.

Dissemination of Research: By testing hypotheses derived from metacommunity theory and host-associated microbial communities my work will be applicable to basic research in ecology and animal health. Experimental and metagenomic data as well as ASV tables, will be made available to the public via public archives (Dryad, Qiita). R scripts used for analyses will also be available on GitHub and published on Zenodo. I publish open access articles in broad readership journals such as *eLife*, *Ecology*, *Molecular Ecology*, and *Integrative and Comparative Biology*. I will present my discoveries at broad national and international conferences such as the Ecological Society of America, Animal Behavior, and the International Society of Microbial Ecology.

Participation of underrepresented groups: As a woman of color in STEM and a daughter of immigrants, I am committed to creating inclusive environments that empower people from all backgrounds, but especially from historically excluded groups. *To do this, I will focus my efforts on both recruitment of K-12 students to STEM and retention of underrepresented undergraduate and graduate STEM students through mentoring and peer support* [65]. To increase recruitment efforts, during the proposed research I will work with UPitt's PittBio Outreach Office. This office has been part of the department for 20 years and partners with Pittsburgh Public Schools and UPitt researchers to create STEM outreach modules for K-12 students based on current research taking place in the department. These modules are then implemented in the classroom by training teachers and providing free resources in the form of "Pitt Kits". *Together, we will develop and implement a module for K-12 students based on understanding how beneficial bacteria present in the gut microbiome can help protect us from disease.* In the first year of my fellowship, we will develop the concepts and activities for the module in collaboration with local teachers and to Next Generation Science Standards. During the second year of the fellowship, we will then have a short pilot period in 1-2 classrooms to assess implementation procedures. After this pilot, we will broaden to 5 classrooms and assess learning outcomes. During this process, I will be an active collaborator, ready to modify the concepts and implementation to fit the needs of the students and teachers. *To retain and empower students interested in pursuing STEM, I will leverage my strong track record of mentoring students from diverse backgrounds and facilitating larger mentoring programs.* The PittBio Outreach Office also maintains a month-long summer mentoring program for public high school students (GeneTeam). I will volunteer as a mentor for this REU-like program, mentoring a student as they navigate implementing their experiments. At the outset of the mentoring relationship, we will collaboratively develop a mentoring roadmap, based primarily on the student's career goals, that will be revisited every two months[66]. Between mentoring assessment meetings, I will meet with my students biweekly to check in on both their research progress and general wellbeing. Specific to my proposed research, I will recruit and mentor undergraduates from historically excluded groups, both to assist in my project aims and to develop their own independent research projects related to the host-associated microbial dynamics. I will also contribute to larger mentorship programs aimed at creating inclusive environments for students from historically excluded groups[67]. For example, at my PhD institution I led a vertical mentoring program for female students, postdocs, and faculty for 2 years. The aim of our program was to support and elevate women in STEM rather than enforce a narrative of assimilation to an unsupportive system[68]. If similar programs do not currently exist at the UPitt, I will implement a program similar to the program I developed at my PhD institution. As the needs of the community are likely different between these institutions, I will implement surveys at the beginning, half-way point, and end of the pilot year to assess their effectiveness and fit for the community in Pittsburgh. Based on the feedback in these surveys, I will work with the administration at the UPitt to improve the program and ensure its longevity after I move on to my next career stage.

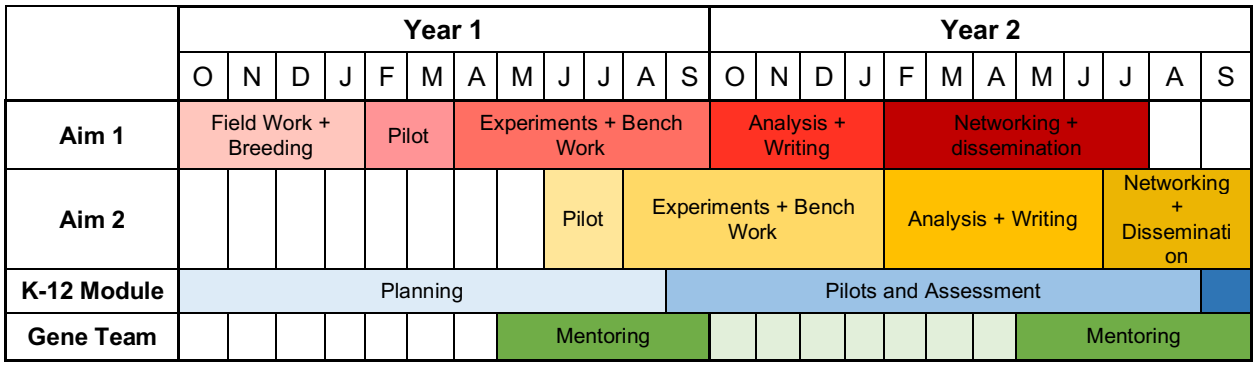
TRAINING PLAN. Justification of sponsoring scientist and institution: My sponsor is a leading expert animal physiologist, combining novel approaches from physiology to understand the consequences of variation in a host's microbiome across numerous vertebrate species. He and his lab have the expertise in the husbandry of ectothermic animals and assays to assess thermal tolerance and growth. Through that work, Dr. Kohl has collaborated with numerous scientists working at the intersection of behavior, evolution, and microbial ecology. The facilities at UPitt provide the ideal support as well. The Health Sciences Core Research Facility and Genomics Analysis Core will provide access to additional laboratory equipment, analysis software, and technical support I may need during the course of this research. In addition to computational power, the Center for Research Computing also provides direct consultation on

computational analysis and workshops on genomics and bioinformatics in R that will support the further development of my bioinformatic skill set.

Training Objectives: I will use this training period to develop three of the core competencies described by the National Postdoctoral Association: 1) gain discipline specific conceptual knowledge, 2) develop important research skills, and 3) improving my professionalism and communication skill. First, to gain specific conceptual knowledge, I will expand upon my observational statistical techniques by incorporating more experimental design. Increasing my familiarity with different experimental approaches will broaden my toolkit for addressing ecological and conservation-based questions. Additionally, once I have the empirical data from the proposed research, I will work with Dr. Mart Turcotte and Dr. Justin Kitzes at UPitt to create a new metacommunity model for the host-associated microbiome. Second, to develop important research skills, I will expand my technical skillset through animal husbandry, handling, and dissection. Animal handling and behavioral assays will complement my currently molecular toolkit and provide a foundation for my future career as an independent researcher at a zoo or aquarium. I will also sharpen my computational and analytical skills by gaining experience in metagenomic and bioinformatic workflows. The technical implementation of metagenomic workflows as well as the newest 16S rRNA pipelines will position me as a strong microbiome researcher and collaborator: I will be better equipped to collaborate with non-microbiome researchers and add new dimensions to research at the intersection of host health and conservation. Third, I will improve my professional network and communication skills by seeking out collaborations at the Pittsburgh Zoo and PPG Aquarium. This will provide an opportunity to create more community engaged research based on their needs and the needs of their animals. Through work on the proposed research and side projects I undertake as a member of the Kohl lab, I will gain invaluable experience collaborating with multiple renowned scientists and working in a broad variety of taxa, all of which will prepare me for my ideal research career.

Career Development and Future Directions: I have designed this project as a critical next step towards my ideal career as a researcher at an aquarium or museum. During my PhD, I worked to understand the role of the gut microbiome in the aging and development of a long-lived primate species. I found that the gut microbiome is a noninvasive metric of biological aging: the gut changes predictably with age in a clock-like fashion. Further, I showed that host behavior and early life experience modulate how quickly a host's microbiome ages, and demonstrated that hosts whose microbiomes age quickly may reproduce sooner. In my future career, I will continue to explore the consequences of variation in host-associated microbiomes in host health and fitness, while also contributing to community outreach and engaging in community-based research: I will also be able to collect data from all the components of the ecosystem and examine microbial community structure across multiple scales and in a variety of habitats. Many zoos and aquariums also partner with local and international wildlife rehabilitators and conservation researchers, allowing me to engage in the conservation applications of my research as well as research how the host-associated microbiome is impacted in captivity, the health consequences of that variation, and how we might "rewild" their microbiomes to improve host longevity.

TIMELINE.



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DATA MANAGEMENT PLAN

RESEARCH PRODUCTS. The proposed research will generate four types of research products: 1) behavioral, physiological, and genomic data (growth and stress data, samples, sequences), 2) field and lab protocols, 3) code, and 4) microbiome outreach teaching modules.

DATA TYPE AND FORMAT.

Data Type	Data Format
Physiological data	Mass (g), length (cm), and hormone data in Excel spreadsheets, photographs of Gosner stages, host tissue samples in 70% ethanol, extracted DNA
Behavioral data	Paper lab notebook to word documents
Genomic data: Sequences, amplicon sequence variant tables, taxonomies and proposed pathways	.fasta/.fastq sequencing files; .R bioinformatic files; .csv and .rds data files
Field protocols: Capture protocols and initial measurement data, breeding protocols and initial measurement data,	Paper lab notebook to word documents
Laboratory protocols: DNA extraction and amplification, library preparation, quality control and sequencing protocols	Paper lab notebook to word documents
Code: Bioinformatic pipelines and filtering information (Unix and R code), statistical analyses (R and Python code)	.sh, .job, .R, .Rmd, and .py scripts
Outreach: Microbiome teaching modules (including any videos) and mentoring plans	Google Docs (~.doc), PDF, Google sheets (!.xls), .mp4, .png or .jpg images

DATA STORAGE. All host tissue, DNA extracts, and genomic amplicon libraries will be stored in a -80 freezer in the Kohl lab. Physical lab notebooks and data will be kept in the Kohl lab, as well as scanned to be stored with protocols, and code in both the PI's personal Box and GitHub accounts as well as external hard drives and the UPitt's cloud storage. Big data will be stored using UPitt's secure big data storage as administered by the Center for Research Computing. All data and analyses will be backed up to via third party cloud storage systems daily.

DISSEMINATION, ACCESS, AND SHARING. Data generated through this fellowship will be published as open-access articles in peer-reviewed scientific journals. The data that contributed to those publications will be made freely available at the time of publication via databases like Dryad and Qiita. The PI's R and Unix code for the bioinformatic pipelines and statistical analyses will also be available via the PI's personal GitHub and Zenodo accounts. Prior to publishing, manuscripts will be uploaded as pre-prints to biology (biorxiv.org) or ecology and evolution (ecoevorxiv.org) servers. The PI retains rights to unpublished data but will be open to collaboration for organizations interested in using the data. Unpublished data will be stored on the PI's personal cloud storage for use in future open access publications and collaborations. Outreach modules developed with the PI will be disseminated by the PittBio Outreach Office, and, with their permission, available on the PI's personal website.

DISSERTATION ABSTRACT

The gut microbiome is the most plastic organ in a vertebrate host. It breaks down complex carbohydrates, produces vitamins, trains the immune system, and resists pathogens. It also changes substantially throughout life, helping the host adapt to its current environment and next developmental stage. These age-related changes are proposed to be important markers of individual development, senescence, and health. Yet to date, scientific understanding of these dynamics is hampered by the fact that most research on the microbiome is cross-sectional. Collecting longitudinal biological samples in the same individuals from birth to death is logistically prohibitive. Without this fine-grained data on microbiome composition across the life course, we do not know how gut microbiomes change in individuals over time, what factors drive variation in gut microbiome development and aging, or whether these changes serve as markers of maturational milestones or mortality risk.

The objective of my Ph.D. research is to characterize age-related changes in the gut microbiome across the life course, understand what host and environmental factors predict these changes, and determine whether microbial changes are linked to host maturation and survival. To achieve these goals, I collaborated with the Amboseli Baboon Research Project, which has been monitoring a wild population of baboons (*Papio cynocephalus*) for nearly 50 years. I helped generate an unprecedented gut microbiome data set for these animals, consisting of over 17,000 16S gut microbial profiles collected from 601 known individually-known, wild baboons over a 14-year period. For each baboon, we also have data on drivers of microbiome dynamics, including diet, environmental conditions, and social relationships.

To test for age-related changes in the gut microbiome, I used a machine learning approach to build a microbiome-based predictor of chronological age, called a "microbiome aging clock". I built a Gaussian process regression model of the gut microbiome that exhibits clock-like changes with baboons' chronological age, as indicated by a R^2 of 0.488 and a median age prediction error of only 2 years (the samples in the data set span birth to over 25 years of age, Figure 1). These changes are heteroskedastic such that variance in microbial age estimates are highest in the oldest animals. Such heteroskedasticity may indicate a breakdown of the host's processes that regulate the gut microbiome, as an increase in chronological age is often associated with the breakdown of physiological processes (e.g. aging). I also found that microbiome age and pace of aging are affected by common social and environmental conditions: female baboons exhibit microbiomes age acceleration (i.e. have old-for-age microbiota) during the dry season when resources are scarce. Further, low-ranking females exhibit faster rates of gut microbial aging than high-ranking females, indicating that resource limitation leads to a fast pace of microbial aging. Interestingly, microbiome aging is not correlated with the timing of sexual maturity, suggesting that these age-related changes in the microbiome capture unexplored dimensions of aging that are not correlated with traditional markers of biological age. However, this lays the groundwork for future studies of the functional consequences of an aging gut microbiome.

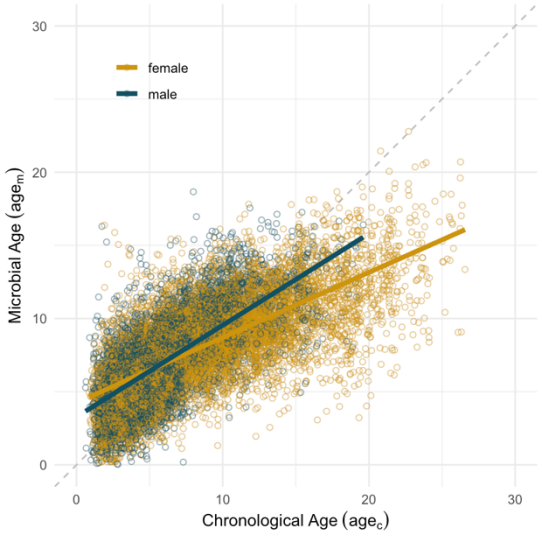

Figure 1: Microbiome aging clock based on a heteroskedastic gaussian process regression. Microbial age estimates (age_m) were highly correlated to chronological age (age_c) and exhibited low prediction error (Pearson's R = 0.698, median error = 1.962 years). Points are colored by sex with yellow indicating a female sample and blue indicating a male sample. Grey dashed line indicates a 1-to-1 relationship between age_c and age_m.