UNRAVELING THE ROLES OF HOST GENETICS, MICROBIAL INTERACTIONS, AND ENVIRONMENTAL FACTORS IN SHAPING AQUATIC ANIMAL MICROBIOMES

by

Madelaine Laura Brown

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Microbiology

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2026

DEDICATION

After six relentless years of studying, reading, writing, extracting DNA, analyzing sequences, growing bacteria, teaching, mentoring, crying, laughing, and—most importantly—learning, I dedicate this dissertation to myself. I dedicate this dissertation to all the past versions of me who never dreamed of accomplishing so much, but who chose to believe in herself anyways. To the little girl who didn't know how capable she was, I hope I have made you immensely proud. To the many versions of myself I hope to become—a senior scientist, a professor, a mentor, a colleague, a mother, and a friend—I dedicate this work as a reminder. A reminder of who you were in these years: resilient, curious, and determined. I hope you remember how far you have already come, and how far you can still go.

ACKNOWLEDGEMENTS

I am deeply grateful to everyone who supported me academically, professionally, and personally, as I worked toward this degree. I would like to first thank my parents, S. and R. Brown for their unconditional support and love, and for instilling in me at a young age that I can do anything I put my mind to. To my sister A. Brown, thank you for always being my biggest supporter, fiercest protector, and trusted confidant. To, S. Russo and J. Welnak, thank you for always believing in me, your friendship means the world to me. To D. Cummings, and R. Botts, thank you for fostering my passion for microbiology, your mentorship was invaluable.

To the newest members of my chosen family, M. Rodrigues, C. Casey, H. Welhaven, T. Wolfe, K. Lyon, P. Brewster, K. Blackwell, A. Maritan, J. Crandell, and A. Adams-Beyea, thank you for allowing me to share this experience with the kindest people. It gives me so much hope that you all are the next generation of scientists. To my advisor F. Stewart, thank you for your mentorship and for creating the best work environment. Z. Pratte, thank you for helping me navigate the highs and lows of being a woman in STEM. My deepest gratitude for all members of the Stewart lab, I have learned so much from you all. Thank you, M. Griffin, and B. Bosworth, none of this would have been possible without your expertise and collaboration.

Additional thanks to the National Science Foundation (grant no. 2235325 to Z.A.P; grant no. OCE 1947522 to Z.A.P. and F.J.S.), Simons Foundation Investigators in Aquatic Microbial Ecology Award (to F.J.S.), Central Caribbean Marine Institutes' Women in Ocean Science Award (to Z.A.P.), USDA ARS (Project 6066-31000-016-000D to M.J.G) and USDA-NIFA Southern Regional Aquaculture Center (Project 2020-38500-32562 to M.J.G) for their financial support.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF FIGURES

Figure Page

LIST OF FIGURES CONTINUED

Figure Page

LIST OF FIGURES CONTINUED

Figure Page

LIST OF FIGURES CONTINUED

Figure Page

LIST OF FIGURES CONTINUED

Figure Page

LIST OF FIGURES CONTINUED

Figure Page

ABSTRACT

Aquatic animals experience a unique and increased pressure from their surrounding environment as they are in constant contact with billions of microorganisms, many of which may cause adverse health outcomes. Microorganisms that live within and/or on aquatic animals (microbiomes) can protect their host from potential pathogens along with aiding in nutrient metabolism and host immunity. These relationships are well documented in human and terrestrial systems, and in some model aquatic organisms; however, the majority of aquatic microbiomes are under characterized. 16S rRNA gene and metagenomic sequencing was used to study these microbial communities. 16S rRNA gene sequencing targets a conserved bacterial gene with variable regions that allow for the taxonomic identification of bacterial taxa, the quantification of their relative abundance, and the comparison of microbial diversity between samples. Metagenomic sequencing includes the whole genetic content within a sample, allowing for further taxonomic classification of bacteria within a complex community, while also identifying potential functions of those microorganisms. With these approaches, the impact of host hybridization on catfish digesta microbiome community structure and predictive function were analyzed. This study found that hybrid catfish harbor a unique microbiome from both parental microbiomes, with increased taxa and metabolic pathways associated with host immunity and growth. Additionally, catfish embryo microbiomes in aquaculture were characterized and how the potential impact those communities have on embryo protection during development was investigated. Catfish embryo microbiomes were distinct from water microbiomes, and many of the enriched taxa were associated with nutrient cycling and antioxidant production. Functional groups associated with antimicrobial production and resistance were identified and may contribute to host defense. Lastly, 16S rRNA gene sequencing was used to study the dominant and hypothesized coral symbiont, *Endozoicomonas*, and how its relative abundance varies by coral type, ocean depth, and marine protection status. *Endozoicomonas* was more abundant in soft coral microbiomes compared to hard corals, with higher abundances in unprotected marine areas for soft corals and in shallower depths in hard corals. Together, these studies demonstrate how host genetics, microbial interactions, environmental factors, and the complexity of these relationships, contribute to microbiome community structure and potential metabolic function.

CHAPTER ONE

INTRODUCTION

Host-Associated Microbiomes

Microorganisms (bacteria, fungi, viruses, and archaea) that live within or on the surface of a host (animals and plants) are collectively known as the hosts' microbiome. Host-associated microbiomes provide their host with several benefits including metabolism, protection against pathogens, and mucosa maintenance (Louis & Flint, 2009; Neish, 2014; King et al., 2016; Zhu et al., 2021; Qi et al., 2023). While much of what is known about host-associated microbiomes comes from organisms that live in terrestrial systems—such as humans and model non-human animals and plants—aquatic hosts represent a vast and comparatively understudied component of animal-microbe interactions.

Aquatic animals are immersed in microbe-rich environments, resulting in constant exposure to diverse microbial communities. Immediate microbial succession of aquatic hosts has important implications for microbiome establishment, stability and transmission, particularly during early life stages (Wilkins et al., 2015; Fujimoto et al., 2018; Nyholm, 2020). Unlike terrestrial organisms, where host-associated microbiomes may be shaped by discrete exposure events, aquatic microbiomes are dynamically influenced by the surrounding water, sediment, and organisms that live in these systems.

Despite these complexities, aquatic systems encompass some of the best-characterized and least-understood examples of animal-microbe symbioses. The symbiotic relationship between the bobtail-squid and *Vibrio fischeri*, in which *V. fischeri* provides bioluminescent

camouflage and in return the host provides essential nutrients, has provided fundamental insights into microbial colonization, host specificity, and immune recognition (Nyholm & McFall-Ngai, 2021). Additionally, zebrafish have emerged as important vertebrate models for studying microbiome effects on host development and immunological function under controlled conditions due to their similarities to humans in regard to intestinal structure and physiology, and their external fertilization nature (Xia et al., 2022). In contrast, the extent of the symbiosis and over all microbiome function in more complex systems like coral holobionts (the coral and its microbiome comprised of bacteria, fungi, viruses, archaea and dinoflagellate algae) remains poorly understood due to the complexity of multi-microbe interactions (Thompson et al., 2014). Indeed, research has been able to identify the key symbiotic microbial players in aquatic host-associated microbiome, but these systems also underscore substantial research gaps—such as the role of host associated microbiome in the early developmental stages of various fish species.

Understanding host-associated microbiomes in aquatic organisms is critical given their ecological importance, economic value, and relevance to food production through aquaculture. Aquaculture systems offer a unique and advantageous framework to study host-associated microbiomes due to their controlled environmental conditions, known genetic lineages, and capacity for experimental replication. These characteristics enable the disentanglement of host, environmental, and microbial contributions to microbiome assembly in ways that are not feasible in wild livestock populations (terrestrial and aquatic). Specifically, early life stages, and subsequent microbial succession and colonization can be readily sampled and manipulated, thereby providing a controlled aquatic system to examine microbiome establishment during critical developmental periods. Together, these aquaculture factors provide the ideal context for investigating the factors that shape microbiome structure and function.

Factors That Shape Microbiome Structure

Microbiome Structure

Microbiome structure refers to the composition of microbial communities, including the identity of community members, overall richness, and the relative abundance of taxa within the community(Nemergut et al., 2013). The factors shaping host-associated microbiome structure are commonly grouped into host and environmental factors, and microbe-microbe interactions. Host-associated factors include, host type (e.g., animals and plants), diet, age, physiological stress and genetics. Environmental factors encompass variables such as geographic location, habitat type (terrestrial vs aquatic), oxygen availability, temperature, environmental protection status, and depth (aquatic systems). Microbial interactions include processes such as metabolite production (e.g., bacteriocins), direct physical interactions (e.g., biofilm formation) and antagonisms or mutualistic relationships among community members. Importantly, these factors do not act independently, rather they interact in complex and often non-linear ways to shape microbiome composition and function (Benson et al., 2010; Bernardo-Cravo et al., 2020). As a result, disentangling the relative contribution of individual factors remains a major challenge in microbiome research. Utilizing controlled aquatic environments such as aquaculture, may help us begin to control for environmental influence, while asking more targeted questions on specific host-microbial interactions, the mechanisms that contribute to host health, and host resilience in aquatic systems.

Host factors

Host genetics are increasingly recognized as important determinants of microbiome structure, particularly in systems where environmental variation can be controlled (Benson et al.,

2010; Org et al., 2015). Host hybridization provides a robust framework to examine genetic influences on microbiomes by combining distinct parental genomes with diverse functional traits in a shared environment. Interspecific hybridization, in which two species mate to produce hybrid offspring, has been shown to alter host-associated microbiomes across a range of organisms (Miller et al., 2021); however, the direction (e.g. increase or decrease in microbial diversity, shifts towards one parent microbiome, or emergence of novel communities) and functional consequences of these changes remain inconsistent.

There is inconsistent knowledge on how hybridization impacts host microbiomes. In some systems, hybridization has been associated with negative outcomes, such as the increased abundance of parasitic microorganisms and reduced host fitness like sterility (Good et al., 2008; Miller et al., 2010; Brucker & Bordenstein, 2013), whereas in others it has been linked to shifts in microbiome function that may enhance host performance (Li et al., 2016; Zhu et al., 2021). Furthermore, several studies report minimal or no effects of hybridization on microbiome structure, underscoring the context dependence of host genetic influences (W. Li et al., 2018; Sevellec et al., 2019). These studies highlight significant gaps in our knowledge on understanding how host genetics and hybridization shape microbiome composition and function. Controlled aquaculture systems provide a unique opportunity to examine these relationships by reducing environmental variation and enabling direct comparisons among related host lineages.

Microbial Interactions

In addition to host factors, interactions among microbes themselves play critical roles in shaping microbiome structure and function. These interactions span from competitive to commensal processes, including antagonistic mechanisms such as bacteriocin and antibiotic production, polymicrobial behaviors such as biofilm formation, and mutualistic metabolic

relationships such as between methanotrophic and methylotrophic bacteria (Sanz-Lazaro et al., 2011; Amin et al., 2012; Penesyan et al., 2021; Deng et al., 2024). Together, these interactions influence community assembly by prompting or inhibiting the establishment of specific taxa, particularly during early life stages when microbial communities are newly forming and host immune defenses are not yet fully developed (Fujimoto et al., 2013; Fujimoto et al., 2018; Lokesh et al., 2019). Early colonizing microbes can therefore act as foundational species, shaping downstream community composition and function.

Through these processes, microbe–microbe interactions contribute to microbiome stability, colonization resistance against opportunistic pathogens, and the functional maturation of host-associated microbiomes as they transition from early, transient assemblages to more stable, functionally integrated ecosystems. These interactions are particularly relevant in aquatic systems such as those associated with fish, where microbial communities colonizing eggs, skin, and mucosal surfaces can form biofilms that mediate both commensal and competitive interactions, ultimately influencing pathogen resistance and early microbiome assembly (McMurtrie et al., 2025; Paralika & Makridis, 2025). However, inferring these interactions from community composition alone remains challenging, limiting our ability to fully resolve their role in shaping. Microbiome dynamics and representing a key ongoing challenge in microbiome research.

Environmental Factors

Environmental conditions are also major determinants of host-associated microbiome structure, particularly in aquatic systems where hosts are in constant contact with surrounding microbial communities. In these systems, the surrounding water microbiomes represent a dynamic and functionally important reservoir of microbial diversity, maintaining

essential metabolic processes and acting as a primary source for host colonization (Sehnal et al., 2021). Factors such as temperature, oxygen availability, and nutrient concentrations influence which microorganism can persist and in what abundances in a given environment (Rubin & Leff, 2007; Ji et al., 2024; Zong et al., 2024). Notably, environmental microbiomes can vary substantially across geographical, seasonal, spatial, and temporal scales while still maintaining a conserved functional core, suggesting that ecosystem-level processes may be preserved even as community composition shifts (Sehnal et al., 2021).

For example, temperature has been identified as one of the strongest environmental drivers shaping aquatic microbiomes, with increases in temperature promoting proliferation of opportunistic pathogens, which can in turn alter host-associated microbiome structure and disease susceptibility (Sunagawa et al., 2015). Disruption of environmental microbiomes through anthropogenic factors such as antibiotic exposure can shift host-associated microbiomes and negatively impact aquatic animal growth and survival by disrupting host-microbiome interactions (Bentzon-Tilia et al., 2016; Bielen et al., 2017). In systems where disease outbreaks are particularly common, like aquaculture, these disruptions may be particularly detrimental by compromising beneficial and protective microbiomes.

Importantly while the surrounding environment provides the initial pool of microbial colonizers, host-associated microbiomes are not simply reflections of environmental communities. Instead, hosts can selectively filter and maintain specific microbial taxa, resulting in distinct microbiomes even within relatively homogenous environmental conditions (Sehnal et al., 2021). Additionally, within a single host, variation in environmental conditions across body sites further shape microbiome composition; for examples, fish gut microbiomes are often enriched with facultative anaerobes compared to skin or gill microbiomes due to differences in

oxygen levels (Pratte et al., 2018). These spatially distinct microbiomes are associated with different functional roles for the host, with gut-associated communities contributing beneficial metabolites like short-chain fatty acids (Fontinha et al., 2024), while gill and mucosal microbiomes are more closely linked to toxin and waste filtration, and protection against pathogens (Maina, 2002; Pratte et al., 2018). Together, these patterns demonstrate that environmental factors shape microbiome structure both by filtering external microbial communities and by interacting with host physiology to influence microbiome assembly and stability.

Studying Microbiomes

Given the complexity of host, microbial, and environmental factors that shape microbiome structure, studying aquatic microbiomes often requires both culture dependent and culture independent approaches, each with distinct strengths and limitations. Culture-dependent methods include bacterial enrichment and isolation, which can enable experimental manipulation of hosts and bacterial species. In contrast, culture-independent approaches rely largely on next-generation sequencing (NGS), such as 16S rRNA gene and metagenomic sequencing to characterize microbial communities and their potential functions without need for cultivation.

Culture-Dependent Methods

Culture-based methods rely on solid or liquid media to stimulate microbial growth either broadly or through selective enrichment of specific taxa. Common media include nutrient-rich agar or broths like tryptic soy broth/agar, which support the growth of the majority of culturable microorganisms (McIlwaine et al., 2024). More selective media, such as MacConkey agar (selective for gram-negative and differential for lactose fermenting bacteria), can target narrower

populations of bacteria in a given sample (McIlwaine et al., 2024). These media-based approaches are commonly used to isolate organisms for downstream analyses, including antibiotic resistance testing, pathogenicity assays, biochemical characterization, genetic manipulation, and whole genomes sequencing (Balloux et al., 2018). Culture-based methods remain important for diagnostic purposes like water quality testing and pathogen identification, (Balloux et al., 2018; McIlwaine et al., 2024); however, they are not comprehensive for complex microbial communities such as host-associated microbiomes.

Historically, culture-dependent methods were the standard for studying microbial communities, but their use is decreasing due to the inability to culture most microorganisms. Only a small fraction, often estimated at 1%, is recoverable using traditional culturing techniques, a limitation known as the "Great Plate Count Anomaly" (Staley & Konopka, 1985). Additionally, culture-dependent methods do not provide information of the original abundances of organisms within a sample, and growth biases favor species that thrive under the chosen conditions. This limitation complicates comparisons between samples or treatment groups and makes it difficult to identify taxa associated with specific host or environmental factors. Because culture-dependent approaches recover only a limited portion of microbial diversity, culture-independent, sequencing-based methods are now more commonly used to characterize entire microbial communities.

Culture-Independent Methods

Culture-independent methods, particularly NGS, enable comprehensive characterization of microbiome composition and function without the need for cultivation. One of the most widely used approaches is 16S rRNA gene sequencing, which targets a conserved bacterial gene that contains both highly conserved and variable regions (Johnson et al., 2019). There are nine

variable regions that provide taxonomic information that can be used to identify bacteria at varying levels of resolution. Typically, the V3–V4 region is targeted using universal primers that anneal to conserved regions flanking variable regions, allowing for polymerase chain reaction (PCR) amplification of the region across diverse taxa (López-Aladid et al., 2023). The amplified products (short-reads) are then sequenced, generating paired-end reads that can be quality filtered, merged, and classified against references databases. As a result, 16S rRNA gene sequencing is ideal for identifying differences in community structure, including diversity metrics, taxonomic composition, and relative abundances across samples or treatments. This approach is a relatively rapid and cost-effective way to compare microbial community composition across many samples; however, it is limited to taxonomic profiling. This method does not directly measure metabolic activity or functional capacity and therefore, inferences must be made cautiously (Janda & Abbott, 2007).

Other ‘omic approaches including metagenomics, transcriptomics and metabolomics, can provide information on the metabolic capability of the microbial community. Specifically, metagenomics identifies taxa and genes present in a community, which may be used to infer functional potential, transcriptomics indicates which genes are actively transcribed at the time of sampling, and metabolomics measures the metabolic products produced (Aguiar-Pulido et al., 2016). For understanding community functional potential and identifying which taxa may contribute to specific metabolic pathways, metagenomics provides the most robust culture-independent approach.

Metagenomics involves the sequencing of all genetic material present in a sample—including host and microbial DNA (Aguiar-Pulido et al., 2016). After DNA extraction, the entire genomic content is sequenced rather than targeting a specific region of a gene like in 16S rRNA

gene sequencing. Reads are quality filtered and then either assembled into contigs or scaffolds for community-wide analyses and further binned into metagenome-assembled genomes (MAGs) to reconstruct genomes of individual taxa. MAG-based analyses often provide higher taxonomic resolution than 16S rRNA gene sequencing and allow for more detailed functional inferences. Additionally, unlike amplicon-based approaches, metagenomics enables direct assessment of the functional potential encoded by the microbial community allowing correlations between taxonomic structure and microbial capabilities; however, the presence of a gene does not confirm its expression, and functional interpretations represent potential rather than actual microbial functions (Langille et al., 2013). Other limitations to metagenomic sequencing include its expense, high host DNA yields diluting microbial reads, and its relatively high demand for computational resources and complex bioinformatic pipelines.

Collectively, 16S rRNA gene sequencing and metagenomic analyses enable robust characterization of microbiome diversity, taxonomic structure, and relative abundance, forming the basis of the analytical approaches used throughout this thesis. The integration of multiple ‘omic techniques enhances researchers ability to unravel the complex taxonomic and functional systems within diverse microbiomes (Aguiar-Pulido et al., 2016).

Experimental Manipulation of Microbiomes

Like many microbial systems, experimental manipulation of microbiomes is considered the gold standard for establishing casual relationships between microbes and host phenotypes (Amato et al., 2019). These approaches typically involve the addition, removal, or alteration of specific microbial taxa or communities, followed by observation of host responses such as changes in growth or disease susceptibility. Manipulative experiments have been instrumental in demonstrating microbiome mediated effects in germ-free and gnotobiotic mouse and zebrafish

models where microbial colonization has been shown to influence host development, metabolism and immune function (Hill et al., 2016; Sonnenburg et al., 2016; Pindling et al., 2018). However, these are often difficult to implement in complex host-microbiome systems.

In aquatic animals, experimental manipulation of microbiomes presents additional challenges due to constant exposure to environmental microbes and the difficulties of maintaining controlled or gnotobiotic conditions. Early life stages, in particular, are characterized by rapid microbiome succession from surrounding water, making it particularly difficult to isolate host-driven effects from environmental influences (Wilkins et al., 2015; Fujimoto et al., 2018). As a result, many studies of aquatic host-associated microbiomes rely on comparative and observational approaches to infer these host-microbiome relationships. While these approaches do not directly establish causality, they provide critical insights into patterns of microbiome structure, and potential function within ecological and economically relevant systems.

Hypotheses

Together, these limitations highlight the need for integrative approaches to better resolve the relative contributions of host, microbial, and environmental factors in shaping aquatic microbiomes. This thesis aims to disentangle these factors to better understand their roles in structuring freshwater and marine animal microbiomes. Specifically, it is hypothesized that host genetics contributes to microbiome divergence, such that hybrid catfish harbor microbiomes distinct from both parental species and are enriched in growth-associated microbial metabolic pathways. It is further hypothesized that early-life microbiome assembly is influenced by environmental exposures, with channel catfish microbiomes sharing taxa with surrounding water while selectively enriching host-associated bacteria. Finally, it is hypothesized that

environmental gradients structure microbiome composition across ecological contexts, such that *Endozoicomonas* remains a dominant symbiont across coral types and depths, with increased relative abundance in corals from marine protected areas. Using culture-independent approaches, including 16S rRNA gene and metagenomic sequencing, these hypotheses are tested across the studies presented in this thesis

CHAPTER TWO

HYBRIDIZATION SHAPES THE TAXONOMIC COMPOSITION AND METABOLIC POTENTIAL OF DIGESTA MICROBIOMES IN CATFISH

Contribution of Authors and Co-Authors

Manuscript in Chapter 2
Author: Madelaine L. Brown
Contributions: formulated guiding hypothesis, performed sample extraction and preparation, devised and performed all bioinformatic analyses, wrote manuscript

Co-Author: Zoe A. Pratte
Contributions: formulated guiding hypothesis, advised on bioinformatic analyses, wrote manuscript

Co-Author: Cynthia C. Ware
Contributions: performed sampled collections

Co-Author: Noor-ul-Huda
Contributions: performed sampled collections

Co-Author: Fernando Y. Yamamoto
Contributions: performed sampled collections

Co-Author: Brian G. Bosworth
Contributions: performed catfish rearing, performed sampled collections

Co-Author: Matt J. Griffin
Contributions: formulated guiding hypothesis, performed sampled collections,

Co-Author: Frank J. Stewart
Contributions: formulated guiding hypothesis, advised on bioinformatic analyses, wrote manuscript

Manuscript Information

Madelaine L. Brown, Zoe A. Pratte, Cynthia C. Ware, Noor-ul-Huda, Fernando Y. Yamamoto, Brian G. Bosworth, Matt J. Griffin, Frank J. Stewart

Animal Microbiome
Status of Manuscript:
☒ Prepared for submission to a peer-reviewed journal
☐ Officially submitted to a peer-reviewed journal
☐ Accepted by a peer-reviewed journal
☐ Published in a peer-reviewed journal

Abstract

Interspecific hybridization can produce offspring with beneficial traits, potentially mediated by changes in the host-associated microbiome. In the U.S. aquaculture industry, channel (*Ictalurus punctatus*) and blue (*Ictalurus furcatus*) catfish are hybridized to produce offspring with enhanced growth rate and disease resistance. The mechanisms underlying hybrid vigor in these catfish are unclear, but in addition to host genetic effects (heterosis), they may also involve modifications in the digesta microbiomes, which can influence host nutrient and energy acquisition as well as immune system regulation. To explore microbial contributions to hybrid performance, we used 16S rRNA gene amplicon sequencing and shotgun metagenomics to compare the digesta microbiome of hybrid catfish to that of the parental blue and channel catfish. Taxonomic analysis revealed the hybrid microbiome is a blend of parental microbiomes with enrichment of potentially beneficial taxa. Hybrid microbiomes are enriched in lactic acid-producing bacteria compared to blue catfish and vitamin B_{12}-producing bacteria compared to channel catfish, while lacking potential pathogens observed in both channel and blue catfish. Hybrid microbiomes are also enriched in microbial genes for antibiotic and vitamin B synthesis, and energy and carbohydrate metabolism. Relative to parental fish microbiomes, hybrid microbiomes contain features of potential host benefit and may therefore contribute to increased host health, growth, or fitness in aquaculture. These findings warrant future studies to test if alteration of the digesta microbiome contributes significantly to hybrid vigor.

Introduction

Interspecific hybridization blends genetic lineages through the mating of different species and is common in both natural ecosystems and controlled breeding environments (Miller et al., 2021). Under controlled breeding, hybridization is often done to produce offspring with advantageous traits relative to the parent species (hybrid vigor; (Reichley et al., 2018; Hegde et al., 2022)). The mechanisms underlying these advantages may be mediated, in part, by the host's intestinal microbiome. Gut microbiomes benefit host health and physiology in diverse ways, for example by aiding nutrient metabolism (Krajmalnik-Brown et al., 2012; Scully et al., 2014; Zhu et al., 2021) and immune function (Futo et al., 2015; Zheng, 2020), fighting pathogens (Louis & Flint, 2009; Miljkovic et al., 2015; King et al., 2016; Qi et al., 2023), and maintaining mucosal integrity (McDermott & Huffnagle, 2014; Neish, 2014; Morshed & Lee, 2023). The host similarly influences its microbiome through a combination of factors that determine the timing and pathways by which microbes are acquired and the physiochemical conditions surrounding the microbiome (Sevellec et al., 2019). For example, intestinal microbiome composition can vary based on whether microbes are acquired vertically from a parent versus horizontally from the environment or a non-parent individual, with the extent of vertical or horizontal transmission shaped by factors such as birth mode, social structure, and transovarial transmission of microbes (Nyholm, 2020). Hybridization between species might therefore result in a hybrid microbiome distinct from those of parental species due to a reshaping of microbiome acquisition pathways or to changes in host physiology or certain behaviors known to have strong effects on the microbiome (e.g., feeding, social interaction).

The impact of hybridization on microbiome composition and function has been explored for a small subset of animals. In certain insect hosts (e.g., mites, wasps, flies) and mice,

hybridization reshapes the microbiome but leads to unfavorable offspring outcomes like sterility and lethality (Good et al., 2008; Miller et al., 2010; Brucker & Bordenstein, 2013; Miller et al., 2021). In other animals, including carp and cervids, hybridization reshapes the microbiome but confers host benefits, for example by enhancing nutrient absorption (Li et al., 2016; Zhu et al., 2021). Hybridization may also reshape the microbiome with no obvious impact on host physiology or health, as reported in some freshwater fish species (W. Li et al., 2018; Sevellec et al., 2019). These studies together suggest hybridization can influence host microbiomes, potentially due to variation in the genetic and ecological relatedness of the hybridizing species. What seems clear, however, is that hybridization is likely to reshape microbiome composition. In instances in which hybrids exhibit beneficial traits relative to parental species, analysis of a compositionally reshaped microbiome might uncover key bacterial groups and metabolic pathways that drive hybrid fitness.

Aquaculture provides a controlled setting for studying how hybridization influences aquatic animal microbiomes. The Thad Cochran National Warmwater Aquaculture Center (NWAC) in Stoneville, MS, USA conducts research on the husbandry of commonly reared freshwater animals, including both channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus furcatus*). Channel catfish are common in aquaculture due to their fast maturation, high feed conversions, and spawning success (Wolters et al., 1996; Li et al., 2014; Reichley et al., 2018). In comparison, blue catfish are less widely farmed because of slower growth rates and lower spawning success but have increased resistance to certain diseases (Reichley et al., 2018). Hybrids of channel and blue catfish (♀*I. punctatus* ♂ *I. furcatus*) have beneficial traits of both parents, notably quick growth, tolerance to crowding, and enhanced pathogen resistance (Wolters

et al., 1996; Reichley et al., 2018). These hybrids offer a substantial (up to 30–40%) increase in aquaculture biomass production compared to parent species (Li et al., 2014).

The biological basis for success in hybrid catfish is not fully characterized but could involve changes in the intestinal microbiome that influence energy yield or other host properties. Indeed, fish microbiomes—primarily composed of Proteobacteria, Bacteroidetes and Firmicutes—aid in digestion, nutrient absorption, immune function, and pathogen defense, with beneficial members such as lactic acid and vitamin B_{12} producers, nitrogen fixers, and fiber degraders contributing to these roles in aquaculture (Etyemez & Balcazar, 2015; Giatsis et al., 2016; Akhila Dharnappa Sannejal & Deekshit Vijaya Kumar, 2023; Qi et al., 2023). We hypothesize that, compared to parent species microbiomes, hybrid catfish microbiomes are enriched in microbial taxa and genes that contribute to disease resistance and fast growth rates, contributing to their overall fitness in aquaculture.

To begin testing this hypothesis, we compared digesta (intestinal content) microbiome diversity and metabolic potential of hybrid catfish (♀*I. punctatus* x ♂ *I. furcatus*) to that of parental species in aquaculture at NWAC. We quantified diversity and metabolic potential via 16S rRNA gene amplicon sequencing and shotgun metagenomics, respectively, using DNA extracts of digesta from blue, channel and hybrid catfish housed in discrete tanks and sampled over two years. These studies indicated catfish hybridization results in a digesta microbiome that blends features of both parental species, with enrichment of potentially beneficial taxa. Hybrid microbiomes are also enriched in functions related to antibiotic and vitamin biosynthesis, as well as carbohydrate and energy metabolism.

Methods

Fish and Sample Collection

2022 Sampling. Channel catfish (~6 months old; 7–13 cm) were obtained as fry from a local producer and reared indoors at the Mississippi Agricultural and Forestry Experiment Station (MAFES) rearing facility on the NWAC campus in Stoneville, MS, USA. Blue and hybrid fingerlings were reared indoors at the Warmwater Aquaculture Research Unit (WARU) hatchery on the same campus, then transported to the MAFES facility for use in the study. Fish were held in discrete, 2000 L circular holding tanks (~4000 fish/tank; 1 tank per group (channel, blue, hybrid)) sourced with groundwater at a flow rate of 3.8 L/min (~25°C) with supplemental aeration provided by a regenerative blower. Photoperiod was maintained at 12:12 light:dark by fluorescent lights controlled by timers. All fish were fed a commercial catfish diet (32% protein, 6% fat, <5% fiber, <9% ash; Rangen Inc., Buhl, ID, USA) at approximately 2% body weight every other day for 27 days. On the day before sampling, fish (~7 months old) from each group were fed in 30-minute increments to ensure that food was at approximately the same stage of digestion in all fish. Fish were euthanized by an overdose of buffered tricaine methanesulfonate (MS-222; 1:1 $NaHCO_3$), and individual fish (n=25/group) were randomly selected and aseptically dissected with sterile scissors and tweezers to remove the gastrointestinal tract. Digesta was squeezed out of the intestine using sterile forceps and placed into sterile 1.5 ml microcentrifuge tubes containing 1 ml of RNA preservation buffer (25 mM sodium citrate, 10 mM EDTA, and 70 g ammonium sulfate per 100 ml solution, pH 5.2). Tubes were stored at -20°C until DNA extraction. Water (50 ml) from each holding tank (n=5), as well as water (50 ml) from the source spigot for each tank (n=3), was passed through 0.2 µm Isopore™ polycarbonate filter membranes, which were then stored in 1 ml of RNA preservation buffer at -

20°C. All samples were shipped on ice packs to Montana State University, Bozeman, MT, USA, for further processing.

2023 Sampling. Channel, blue, and hybrid catfish fingerlings (~3 months old; 4–7 cm) were obtained from the USDA WARU hatchery and held at the MAFES rearing facility as previously described. When fish were ~6 months old (7–13 cm), they were transferred to the MAFES Aquaria Facility at NWAC and distributed into 80-liter aquaria (3 aquaria per group; ~30 fish per aquarium), each containing 22 L of groundwater supplied at a flow rate of 3.8 L/min at approximately 25°C. Supplemental aeration was provided using a regenerative blower. Photoperiod was maintained at 12:12 light: dark by LED lights controlled by timers. All fish were injected with 0.1 ml of sterile PBS to serve as procedural controls for an ancillary study investigating bacterial pathogenesis. Fish were fed to satiation daily with a commercial catfish diet (32% protein, 6% fat, <5% fiber, <9% ash; Rangen Inc., Buhl, ID, USA) for 57 days. On the day before sampling, fish from each group were fed in 5-min intervals to ensure that food was at approximately the same stage of digestion in all fish. Fish (~8 months old) were euthanized by an overdose of buffered MS-222 (1:1 $NaHCO_3$), and fish (~ n=10/aquaria) were randomly selected and aseptically dissected with sterile scissors and tweezers to remove the gastrointestinal tract. Digesta was squeezed out of the intestine using sterile forceps and placed into sterile 1.5 ml microcentrifuge tubes containing 1 ml of RNA preservation buffer. Tubes were stored at -20°C until DNA extraction. Water (50 ml) from each tank, as well as water (50 ml) from the source spigot for each tank, was passed through 0.2 µl Isopore™ polycarbonate filter membranes, which were then stored in 1 ml of RNA preservation buffer at -20°C. All samples were shipped on ice to Montana State University, Bozeman, MT, USA, for further processing.

DNA Extractions and Amplicon and Metagenomic Sequencing

DNA was extracted from catfish digesta and water samples using the DNeasy® 96 PowerSoil® Pro Kit (QIAGEN). Digesta (150 µl) was placed directly into PowerBead tubes, and DNA was extracted following the manufacturer's protocol. For water samples, half of each filter membrane was aseptically cut into smaller pieces, which were then placed into PowerBead tubes for DNA extraction following the manufacturer's protocol. For each extraction kit, we created an extraction blank consisting of a tube containing RNA preservation buffer but no digesta. These blanks (n = 6) were processed through the extraction protocol alongside the samples. DNA extracts were used for PCR amplification of the V3–V4 region of the 16S rRNA gene using primers F515 and R806, modified as previously described (Amy Apprill, 2015; Parada et al., 2016). A two-step protocol was employed to first amplify the 16S rRNA gene and then append Illumina specific adapters, following Illumina's suggested 16S rRNA sequencing protocol. PCR reactions included 2-5 µl template DNA, 0.25 µl of each forward and reverse primer, 0.5 µL bovine serum albumin (BSA, 20 mg/ml; New England BioLabs Inc), 2.5 µl Hot Start Taq PCR MasterMix (VWR, Radnor, PA, USA), and sterile nuclease free water to a total volume of 25 µl. Additionally, we created negative controls (n = 15) for PCR amplification by replacing template DNA with sterile, nuclease-free water. PCR amplification included an initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 2 min, and extension at 72°C for 90 sec. This was followed by a final extension step at 72°C for 10 min. Amplicon libraries were purified with Diffinity RapidTip PCR purification tips (Diffinity genomics, NY, USA), quantified on a Qubit Fluorometer (Thermo Fisher Scientific), and pooled at equimolar concentrations. Amplicons from 2022 were sequenced at the University of Georgia's Georgia Genomics and Bioinformatics Core (GGBC) on a MiSeq (Illumina Inc.,

San Diego, CA, USA) using a V2 500-cycle kit (250 X 250 bp) with 5% PhiX to increase read diversity (Padilla et al., 2015). Amplicons from 2023 were sequenced at Michigan State University's Research Technology Support Facility on a MiSeq using a V2 500-cycle kit (250 X 250 bp) with 5% PhiX to increase read diversity.

For shotgun metagenomics, we selected three 2022 extracts with high DNA yield for each fish group (n = 9 total). Aliquots of these samples were sequenced on a NovaSeq (Illumina Inc., San Diego, CA, USA) at GGBC.

Microbiome Data Processing and Analysis

16S rRNA gene sequences from 2022 and 2023 sample collections were analyzed in parallel following the DADA2 (v1.22.0; (Callahan et al., 2016)) and QIIME2 (v2-2022.11; (Bolyen et al., 2019)) pipelines. Using DADA2, raw sequences were demultiplexed, quality filtered, trimmed by 40 bp at only the 5' ends, truncated to 200 bp, and denoised. To analyze both datasets together, all sequences and feature tables were merged in QIIME2. Sequences were clustered at 99% identity using q2-vsearch (v2024.10.0) resulting in operational taxonomic units (OTUs) for downstream analyses. Next, through q2-alignment, OTUs were aligned using MAFFT (Katoh et al., 2002), and phylogeny was estimated using FastTree (Price et al., 2010). All OTUs were assigned a taxonomic designation using the SILVA-132 database (Quast et al., 2013). Sequences identified as chloroplast or mitochondria were removed from all datasets. Next, all samples were rarefied to 27,000 sequences, a threshold chosen based on the plateau of Shannon diversity rarefaction curves and denoised feature table summaries. Samples falling below this threshold, including extraction blanks, PCR negative controls, and three poorly sequenced water samples, were removed for downstream analyses. Additionally, any OTUs that

were identified in blanks or negative controls at a higher average relative abundance than in the digesta samples were removed (Altman-Kurosaki et al., 2025).

Statistical Analyses

Statistical analyses were conducted using RStudio (v2023.03.0+386; (Team, 2023)) to test for differences in alpha diversity and community composition among channel, blue, and hybrid catfish. Alpha diversity was measured as the number of observed features (99% identity OTU clusters) and via the Shannon diversity index and compared pairwise between catfish types using Wilcoxon rank-sum tests calculated from rarefied data and implemented with ggpubr (v0.6.0; (Kassambara, 2025)) and microbiomeutilities (v1.00.17; (Shetty, 2012-2019)) R packages, with comparisons visualized via boxplots. Weighted UniFrac distances were calculated from rarefied data and used to compare community structure via Principal Coordinates Analysis (PCoA) using the phyloseq (v1.42.0; (McMurdie & Holmes, 2013)) and ggplot2 (v3.5.2; (Wickham, 2016)) R packages. Differences in community composition between sample types were tested using permutational multivariate analysis of variance (PERMANOVA, adonis2 from the vegan package (v2.6-4; (Oksanen, 2022)) with 999 permutations, and pairwise comparisons were conducted using the pairewiseAdonis package (v0.4.1; (Arbizu, 2017)) with Bonferroni-adjusted p-values. Homogeneity of group dispersions was evaluated using permutational multivariate analysis of dispersion (PERMDISP, betadisper and permutest from vegan (Oksanen, 2022)) followed by pairwise comparisons with Tukey's HSD test. DESeq2 was used to identify OTUs with significantly varying relative abundance among the three catfish types, with p-values adjusted to control for the false discovery rate using the Benjamini-Hochberg method. Heat maps were generated in ggplot2 and only included the top 5 most

abundant taxa that varied significantly (as determined in DESeq2) among blue, channel and hybrid catfish microbiomes.

Metagenomic Data Processing and Analysis

Metagenomic datasets were quality filtered to retain reads greater than 100 bp with a Phred score greater than 25 (Trim Galore! v0.6.10; (Martin, 2011)). Host sequences were identified by using Bowtie (v2-2.5.1; (Langmead & Salzberg, 2012)) with very sensitive local parameters to align reads against blue (*I. furcatus*) and channel (*I. punctatus*) catfish genomes downloaded from NCBI (Accession numbers CM041687 JAMCCP010000000, and NC_030416, respectively). Host reads were removed from further analysis.

Relatedness of metagenome datasets was assessed based on k-mer frequencies. Simka (v1.5.1; (Benoit, 2015)) was used to calculate Bray-Curtis dissimilarity values based on 21-mer frequencies, with sample clustering patterns visualized in ggplot2. PERMANOVA and PERMDISP were implemented to test for significant variation in k-mer composition as described above, but using 21-mer-based rather than OTU-based distances.

A contig-based approach was used to compare functional potential among metagenomes. Reads were normalized with BBnorm (https://sourceforge.net/projects/bbmap/) and assembled into contigs with metaWRAP (v1.3.2; (Uritskiy et al., 2018)), both with default parameters. Contigs were quantified by read alignment using CoverM contig (v0.7.0; (Samuel T N Aroney 2025)) for each metagenomic sample and Prokka (v1.13; (Seemann, 2014)) was used to identify and annotate open reading frames based on metabolic function, both with default parameters. Additionally, gene count data and Prokka annotations were merged based on contig IDs for statistical comparisons. Genes were then categorized (e.g., genes *ackA*1–20 were grouped into the gene category "*ackA*"), to assess differential enrichment of gene categories across all catfish

samples using DESeq2. Only gene categories that were significant (adjusted $P < 0.05$) and had a baseMean greater than 50 (to exclude minimally expressed genes) were kept for further analysis. To account for differences in sequencing depth, counts for each gene category (obtained from CoverM) within each sample were individually normalized by dividing by that sample's *rpoB* gene category count. The gene *rpoB* was chosen due to its conserved expression across all bacteria (Adekambi et al., 2009). Relative abundance was calculated using the normalized gene counts for each sample to represent how much of each sample's metagenome was dedicated to each potential function. Gene categories with a minimum relative abundance of 0.5% in at least one sample were included. Using gene annotations and KEGG enzyme numbers from Prokka, metabolic pathways were assigned via KEGG enzyme (Kanehisa & Goto, 2000; Kanehisa, 2019; Kanehisa et al., 2025). Genes lacking enzyme numbers or enzyme numbers without associated pathways were excluded. Super-pathways of interest (e.g. biosynthesis of secondary metabolites, carbohydrate metabolism, energy metabolism, and metabolism of cofactors and vitamins) as well as relevant sub-pathways within each, were kept for their association with fish growth and disease resistance. Metabolic potential was visualized with a heatmap using ggplot2 in R.

Gene sequences identified as partial and full-length 16S rRNA genes from metagenomic data were individually queried using BLAST Nucleotide (BLASTn; (Chen et al., 2015)) and taxonomy was assigned based on the best hit. These metagenome-derived 16S sequences were longer than typical amplicon reads, providing higher-resolution taxonomic classification. To determine whether metagenomics 16S rRNA gene sequences corresponded to OTUs detected in the amplicon dataset, sequences matching taxonomically annotated OTUs from differential abundance analyses (previously described in *Statistical analyses*) were aligned using megaBLAST to identify highly similar sequences. This approach enabled cross-validation

between metagenomics and amplicon datasets and improved confidence in taxonomic assignment of the most abundant and differential OTUs between catfish types.

All computational efforts were performed on the Tempest High Performance Computing System, operated and supported by University Information Technology Research Cyberinfrastructure (RRID:SCR_026229) at Montana State University, Bozeman, MT, USA (https://www.montana.edu/uit/rci/tempest/). All raw sequences are publicly available through the NCBI's SRA database under PRJNA1449012.

Results

Across 2022 and 2023, we collected 314 samples spanning catfish digesta and tank and source water (Figure 1, Table A1). Unless otherwise indicated, all results presented here combine samples from both years. After quality control, the final 16S rRNA gene amplicon dataset included 285 samples and 1,756 OTUs (sequences sharing >99% identity), with an average of 86,485 sequences per sample and average read length of 212 base pairs. For metagenomic analysis, nine digesta samples representing three biological replicates per fish group (channel, blue, and hybrid catfish) were shotgun sequenced and assembled, yielding an average contig length of 553 base pairs (range: 200-466,821 bp) and an average of 38.3x coverage (range: 4.3-95.7x) per sample, with most samples having >20x coverage.

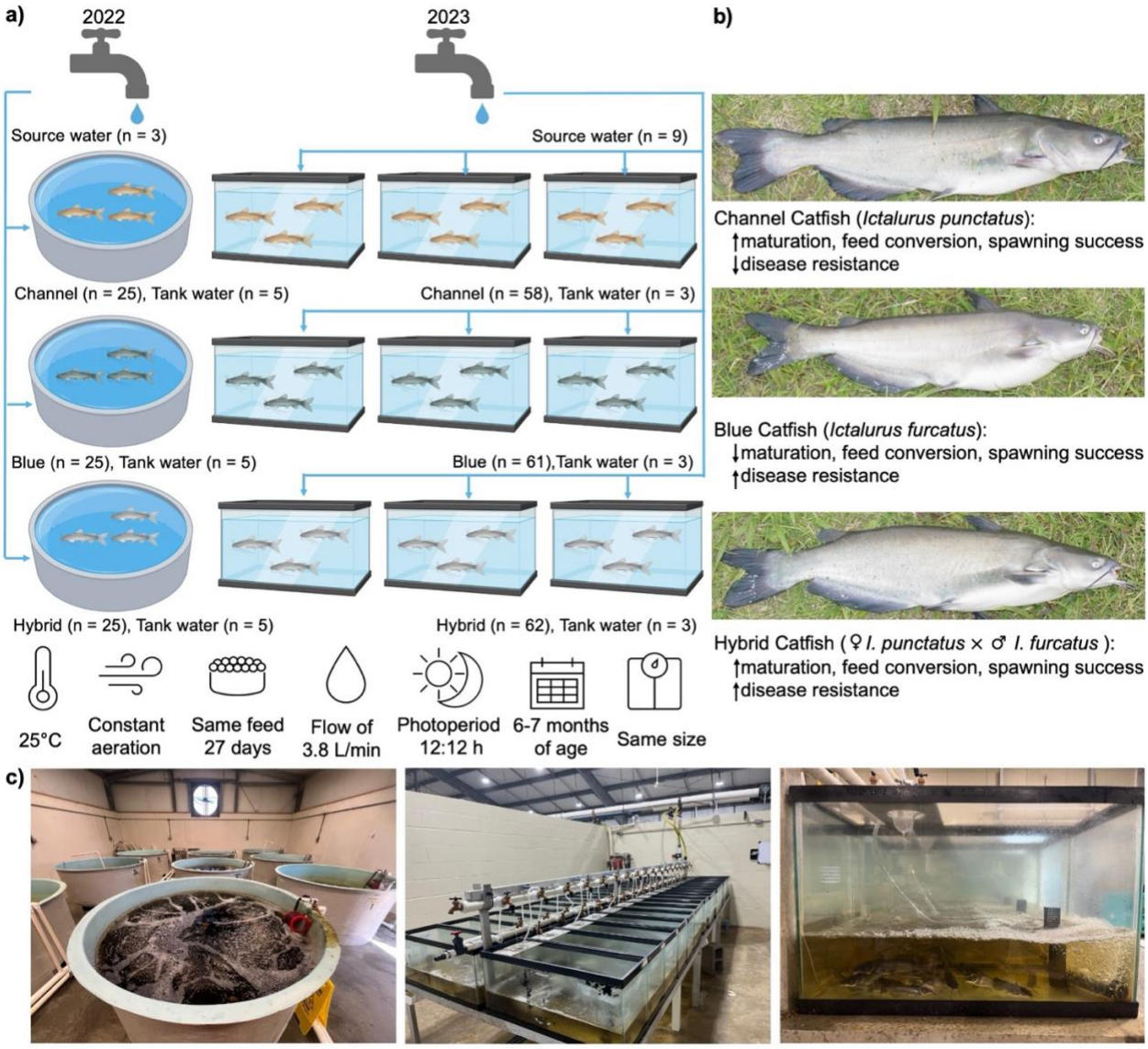

Figure 1: Experimental Design. a) Catfish tank set up, with blue, channel and hybrid catfish residing in their separate tanks. Catfish digesta and water samples were collected, with the sample numbers indicating the total number of samples for each type per sampling year. b) Channel catfish are popular in aquaculture due to their increased maturation, feed conversion and spawning success, while blue catfish are beneficial due to their increased disease resistance. Hybrid catfish have beneficial effects from both parental species. Photo credits: Brian G. Bosworth. c) Catfish rearing and tank set up at the Mississippi Agricultural and Forestry Experiment Station (MAFES) in 2023 and 2024. Photo credits: Matt J. Griffin.

Hybrid Catfish Microbiome Composition is a Unique Blend of Both Parental Microbiomes

Digesta microbiomes differed among channel, blue, and hybrid catfish based on alpha and beta diversity comparisons of 16S rRNA gene amplicons (Figure 2). Observed features (unique 99% OTUs) were significantly higher in channel compared to both blue and hybrid catfish, which did not differ (Figure 2a; Table A2). Channel catfish had significantly lower Shannon Diversity than both blue and hybrid catfish, and blue catfish were significantly higher than hybrids (Figure 2a; Table A3). Beta diversity comparisons of 16S rRNA gene sequencing data using a NMDS with Bray-Curtis distances revealed separation among digesta microbiomes by catfish types and water samples, which were statistically confirmed with one-way (pseudo-F = 47.5 , df =3, $P = 0.001$) and pairwise PERMANOVAs (all $P = 0.001$; Table A4) with no significant impact from dispersion except between channel and blue catfish microbiomes (PERMDISP $P = 0.03$; Figure 2b). Although based on a smaller sample number, beta diversity analysis of metagenomes showed a similar separation of catfish types based on PCoA of Bray-Curtis distances (Figure 2c). One-way PERMANOVA showed significant differences among metagenomes based on catfish type ($P = 0.02$), although pairwise PERMANOVA comparisons did not show significant variation ($P > 0.05$).

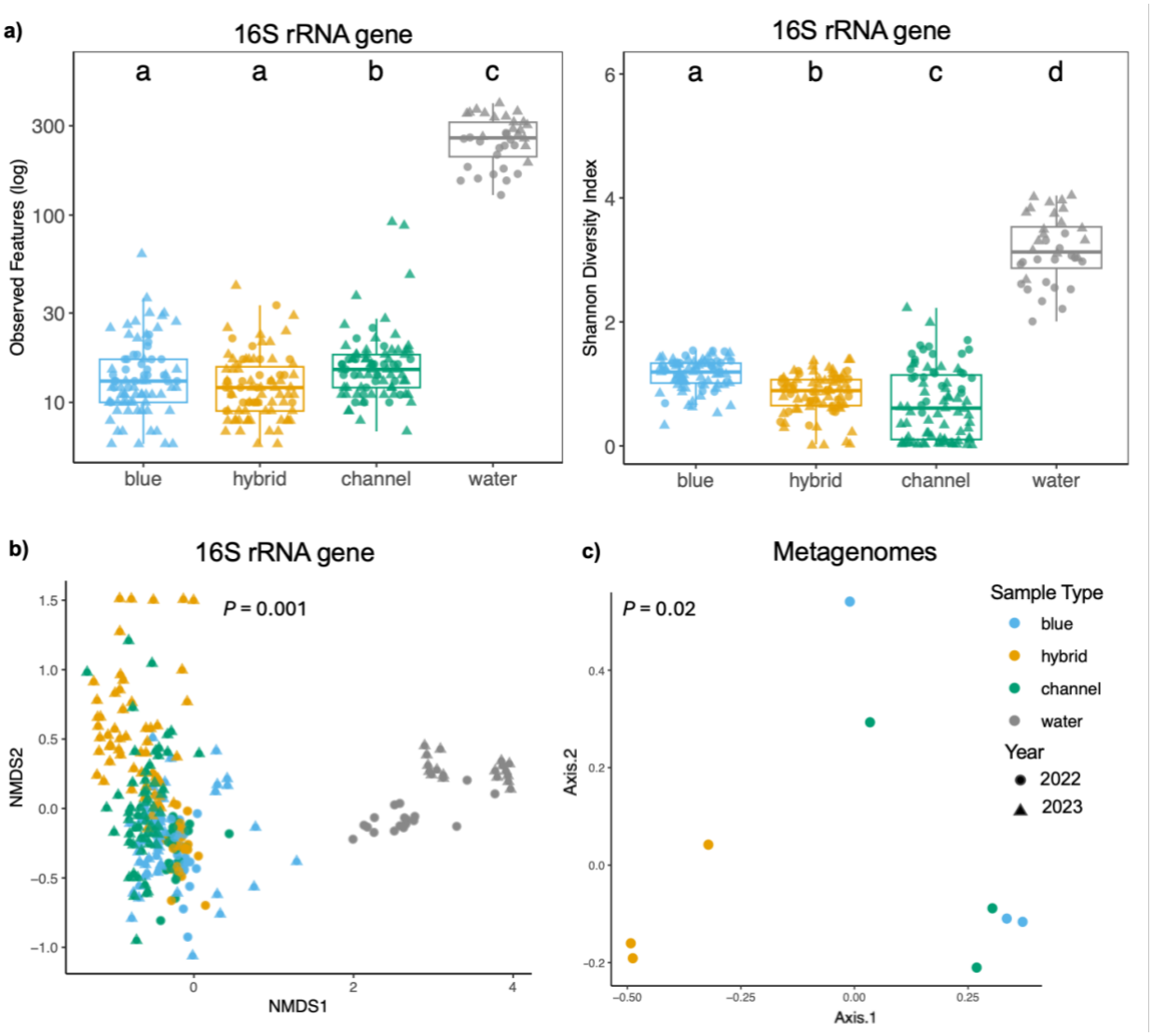

Figure 2: Hybrid catfish microbiomes differ significantly from blue and channel catfish. a) Alpha diversity measures (observed features and Shannon diversity index) of digesta and water microbiomes based on rarefied data. Hybrid catfish microbiomes are significantly different from channel catfish for observed features and Shannon diversity, and blue catfish for Shannon diversity. Water microbiomes show higher alpha diversity than all digesta samples. Channel catfish Shannon diversity is lower in 2023 than in 2022, no other digesta samples vary by year. Box plots show median (center line), 25–75% quartiles (area within the box), minimum and maximum values (whiskers), outliers (large circular data points) and jittered data points. b) NMDS (Bray-Curtis dissimilarity index) of microbiome composition shows that digesta microbiomes differ significantly among catfish types and from water microbiomes (PERMANOVA). Pairwise results are given in Table A4. c) PCoA (Bray-Curtis dissimilarity index) of digesta metagenomes reveals significant differences among catfish types (PERMANOVA).

Microbiome taxonomic composition was similar across catfish types (Figure 3), although some bacterial groups differed significantly in representation among channel, blue, and hybrid catfish (Figure 4, Table 1). Across catfish types, digesta microbiomes were mainly comprised of Streptococcaceae (0–99.9%), Fusobacteriaceae (0–94.8%), Peptostreptococcaceae (0–63.4%), and Aeromonadaceae (0–27.6%), with Hafniaceae also prominent in channel catfish in 2023 samples only (0.05–84.1%; Figure 3). In some samples, a single family comprised nearly 100% of all sequences, with such instances most often involving dominance by Streptococcaceae, notably in hybrid and channel microbiomes (Figure 3), despite no clinical signs of disease. Several abundant OTUs (99% identity clusters) varied significantly across catfish types (Figure 4). The most abundant OTU across all samples for both sampling years was classified as *Lactococcus* (Streptococcaceae) and was significantly enriched in hybrid and channel compared to blue catfish (adjusted $P \leq 0.05$), with its average abundance in hybrids (45% of sequences) intermediate between that in channel (60%) and blue (20%) parental species (Figure 4). However, in 2022 samples, the *Lactococcus* OTU was most abundant in hybrid and blue catfish, but at lower relative abundances in channel catfish. An OTU in the genus *Edwardsiella* (Hafniaceae family) was significantly enriched in channel catfish (adjusted $P < 0.0001$) but largely absent from blue and hybrid catfish; however, this pattern was primarily observed in 2022, as *Edwardsiella* was not prominent in 2023 microbiomes (Figure 4). Conversely, the second most abundant OTU in the data, classified to the genus *Cetobacterium* (Fusobacteriaceae family), was ~2.5–4.5X more abundant in blue compared to channel (adjusted $P < 0.0001$) and hybrid catfish (adjusted $P = 0.014$), with its abundance in hybrids intermediate between its abundance in the parental species. Similarly, an OTU classified to *Aeromonas* (Aeromonadaceae family) was on average 20X more abundant in blue compared to channel (adjusted $P < 0.0001$)

and hybrid (adjusted $P = 0.003$) catfish. Lastly, an OTU in the bacterial family Peptostreptococcaceae was on average 5–6X enriched in blue and hybrid compared to channel catfish (adjusted $P < 0.0001$) (Figure 4).

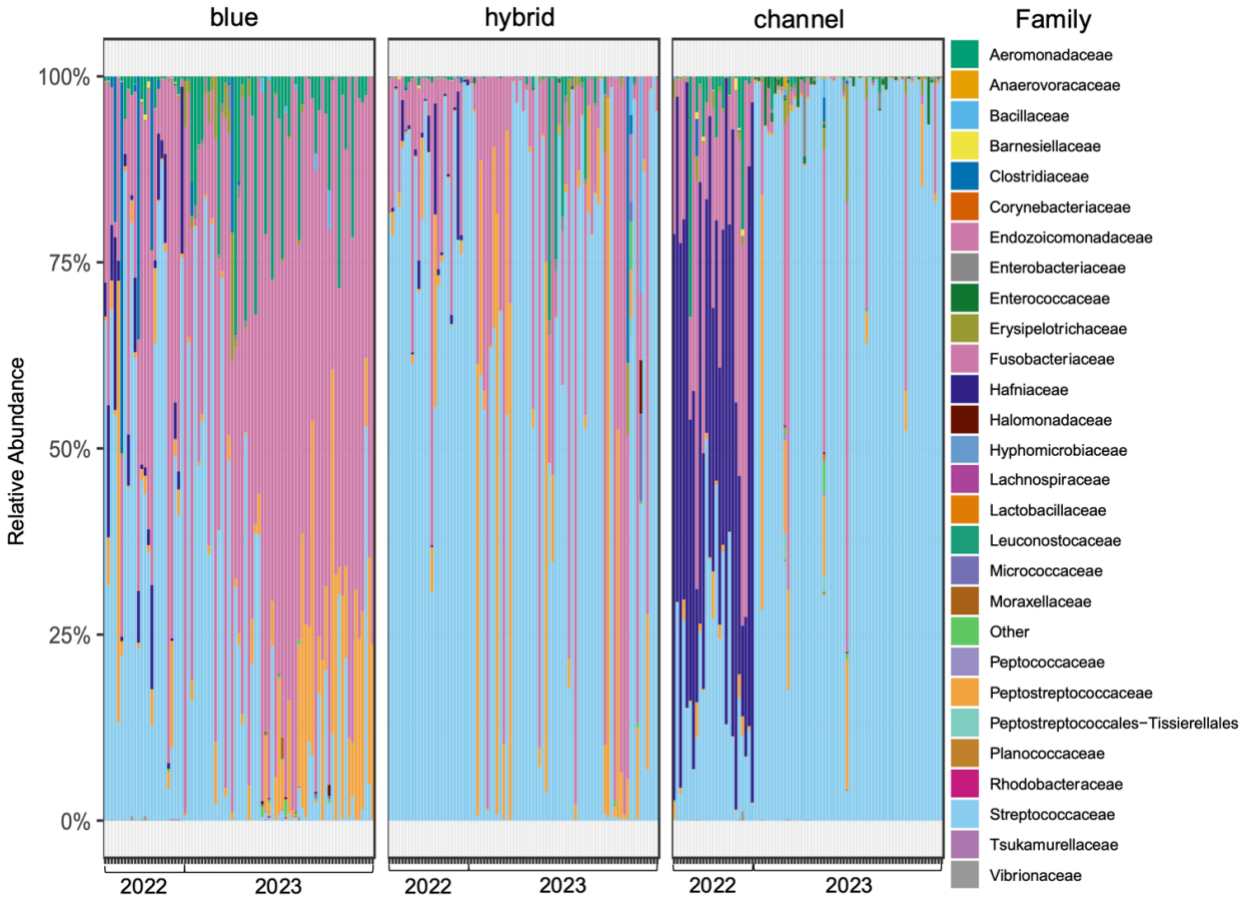

Figure 3: Blue, channel and hybrid microbiomes are dominated by Streptococcaceae, Fusobacteriaceae, Peptostreptococcaceae and Aeromonadaceae. Microbiome community composition at the level of bacterial family, Families included in this plot contained OTUs present at a minimum relative abundance of 1% in 5% of all samples. "Other" represents all OTUs that did not reach these criteria for abundance and prevalence.

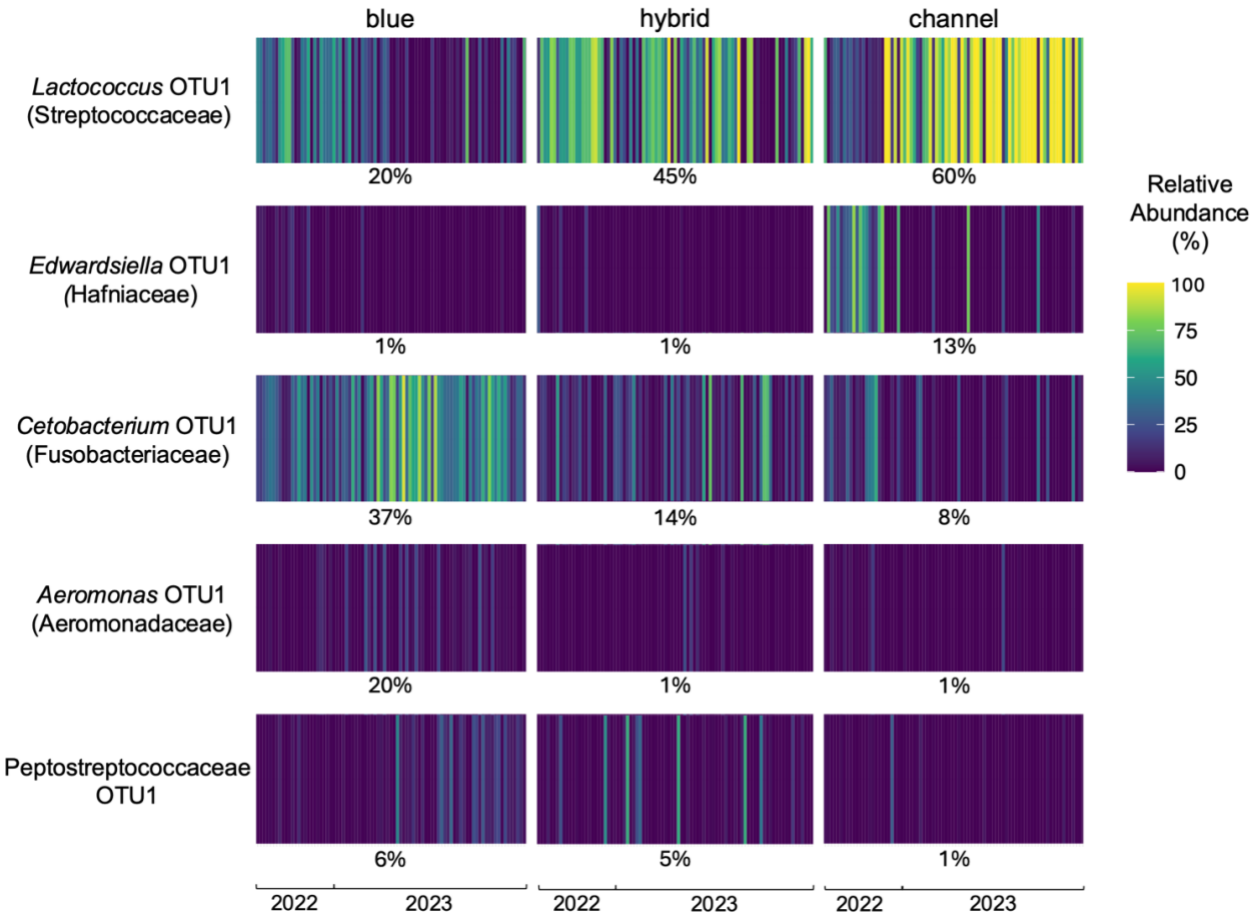

Figure 4: Hybrid microbiomes have intermediate relative abundances of dominant taxa in blue and channel catfish digesta microbiomes. Heat map showing the most abundant OTUs that contribute to significant compositional variation among blue, channel and hybrid catfish digesta microbiomes (adjusted $P > 0.05$, DESeq2 package, RStudio). Average relative abundances are listed below each OTU.

To further resolve taxonomy, we aligned the top five most abundant OTUs recovered from amplicon sequencing (Figure 4) to 16S rRNA gene sequences identified from metagenomes (metagenome screening identified six full length (1,495–1,546 bp) and 24 partial (401–1,224 bp) 16S rRNA gene sequences). The *Lactococcus* OTU was 97.2% similar to a metagenome-derived 16S rRNA gene identified as *Lactococcus cremoris subsp. tructae* (*L. cremoris subsp. tructae*). Similarly, the *Cetobacterium*, *Aeromonas, Edwardsiella*, and Peptostreptococcaceae OTUs

shared 100% similarity with metagenome-derived 16S rRNA genes identified as *Cetobacterium somerae* (*C. somerae*), *Aeromonas veronii* (*A. veronii*), and *Edwardsiella anguillarum* (*E. anguillarum*), and *Paraclostridium* spp., respectively.

Hybrid Catfish Microbial Metabolic Potential May Promote Growth and Disease Resistance

All catfish microbial metagenomes contained genes associated with KEGG super pathways, including, amino acid, carbohydrate, energy, lipid, nucleotide, and glycan metabolism; biosynthesis of secondary metabolites; metabolism of cofactors, vitamins, terpenoids, and polyketides; translation; and xenobiotic degradation (Figure A1). Figure 5 highlights KEGG pathways within super pathways that contribute significantly to differences among blue, channel, and hybrid catfish digesta metagenomes and may have relevance to substrate turnover or synthesis in the host intestine. All reported differences were statistically significant (adjusted $P < 0.05$, DESeq2). Within the biosynthesis of secondary metabolites super pathway, the pathway for synthesis of the antibiotic streptomycin was more abundant in hybrid and blue compared to channel catfish. A similar trend was observed for pathways of biosynthesis of various antibiotics. In the carbohydrate metabolism super pathway, pathways of starch and sucrose, galactose, and fructose and mannose metabolism were enriched in hybrid and blue compared to channel catfish. In contrast, central metabolic pathways of pyruvate metabolism and the TCA cycle were more abundant in channel catfish. Glycolysis/gluconeogenesis was the most abundant pathway overall, with higher relative abundances in blue and hybrid compared to channel catfish. Within the energy metabolism super pathway, methane metabolism was the most abundant pathway across all catfish types, being enriched in channel catfish. Sulfur metabolism and carbon fixation by the Calvin cycle pathways were similar across groups but significantly higher in hybrid and blue

catfish. Nitrogen metabolism was enriched in channel catfish and with similar abundances in one blue catfish metagenome, while oxidative phosphorylation was the least abundant energy metabolism pathway across all catfish types. Within the metabolism of cofactors and vitamins super pathway, the pathway for vitamin B_7 (biotin) synthesis was most abundant, being enriched in blue and hybrid compared to channel metagenomes. Vitamin B_{12} (cobalamin) synthesis genes were also enriched in blue and hybrid catfish. In contrast, vitamin B_6 (pyridoxine) synthesis genes were more abundant in channel catfish.

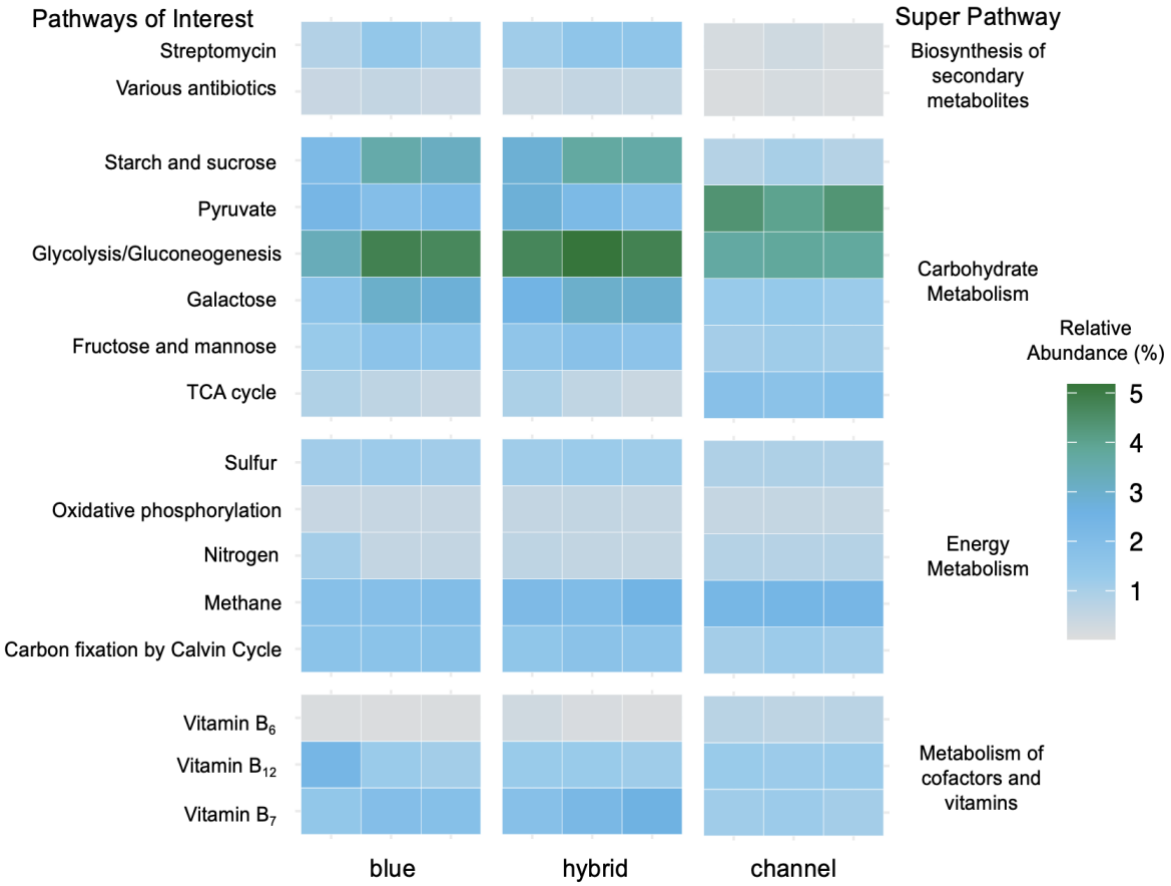

Figure 5: Hybrid metagenomes have higher relative abundances of genes associated with carbohydrate and vitamin metabolism, and antibiotic biosynthesis. Heat map of metabolic potential with pathways of interest that contribute to the significant differences between blue, channel and hybrid catfish digesta metagenomes (adjusted $P < 0.05$, DESeq2). Relative abundance of normalized gene counts associated with metabolic pathways of interest (y-axis) and KEGG metabolic super-pathway (secondary y-axis) for all catfish digesta metagenome samples (x-axis). Hybrid catfish and Blue catfish microbial metagenomes have higher relative abundances of genes associated with antibiotic biosynthesis, vitamin B_{12} and B_7 metabolism, and sugar metabolism. Channel catfish microbial metagenomes have higher relative abundances of genes associated with vitamin B6 metabolism, pyruvate metabolism, and the TCA cycle.

Discussion

Digesta microbiomes of hybrid catfish are similar to, but exhibit differences from microbiomes of parental catfish species, raising the question of whether a unique hybrid

microbiome may influence the physiology or fitness of the hybrid host. Beta diversity analyses revealed clear separation of hybrid microbiomes from both parental groups, independent of tank effects (Figure 2c–d). Further, hybrid catfish microbiomes differed in richness and evenness from those of blue and channel catfish, although diversity patterns were not consistent between comparisons and varied slightly from 2022 to 2023, highlighting the dynamic nature of microbiome structuring (Figure 2a–b). Taxonomic and functional gene profiling (Figures 3–5) further showed that hybrid microbiomes are enriched in certain microbial groups and pathways of potential benefit to holobiont metabolism or disease resistance. The potential contributions are discussed below.

Pathways for B vitamin production were enriched in hybrid relative to channel catfish metagenomes, raising the possibility that the microbiome may supply important compounds for host immunity, growth, and development. Notably, vitamin B_{12} (cobalamin) contributes to fatty acid metabolism, strengthens the intestinal barrier, primes innate immune responses, and is essential for generation of blood cells (Green et al., 2017). The bacterium *Cetobacterium somerae* is a dominant B_{12}-producing taxon in freshwater fish microbiomes (Etyemez & Balcazar, 2015; Qi et al., 2023). In a study of zebrafish, B_{12} production by *C. somerae* was linked to protection against pathogens, while B_{12} supplementation increased microbial diversity and upregulated tight junction proteins, suggesting enhanced intestinal barrier function (Qi et al., 2023). That study concluded that since the majority of gut microbes need B_{12} but only a minority can synthesize the vitamin, the presence of B_{12}-synthesizing *C. somerae* may have outsized probiotic effects in helping support other important members of the fish gut microbiome (Qi et al., 2023). Notably in our study, one of the most abundant OTUs in catfish digesta was classified as *C. somerae* (Figure 4). Notably in our study, one of the most abundant OTUs in catfish digesta

was classified as *C. somerae* (Figure 4). This OTU constituted up to 92% of sequences in certain samples, was most abundant (average of 37% of sequences) in blue catfish, and was enriched in hybrids (14% of sequences) compared to channel catfish. Further, pathways for vitamin B_7 synthesis were also enriched in hybrid and blue catfish compared to channel catfish. Vitamin B_7 (biotin) serves as a coenzyme for carboxylases involved in important steps in the metabolism of carbohydrates, proteins, and lipids in animals (A.-C. Hansen, 2015; Angela Liu, 2022). These patterns raise the hypothesis that B vitamin provisioning by the gut microbiome—notably B_{12} provisioning by *C. somerae*—may provide a functional benefit to hybrid catfish, at least compared to channel catfish, and may help explain the differential susceptibility to some enteric catfish pathogens reported for blue catfish (Wolters et al., 1996; Reichley et al., 2018; Armwood et al., 2022).

Catfish health may also be influenced by antibiotic production or resistance in the intestinal microbiome. Microbiomes containing naturally produced antibiotics or other bactericidal compounds have been shown to provide protection against host pathogens (Silo-Suh et al., 1994; Amin et al., 2012; Donia et al., 2014; Alam et al., 2022). Our metagenomic analysis shows that hybrid and blue catfish microbiomes are enriched in genes associated with streptomycin biosynthesis and overall biosynthesis of various antibiotics, compared to channel catfish (Figure 5). This pattern may also help explain known disease resistance in blue catfish, a potential microbially mediated advantage that may extend to hybrid catfish (Figure 5).

Taxonomic analysis of the hybrid catfish microbiome identified other bacteria of potential significance for host health, specifically lactic acid-producing bacteria. By combining 16S rRNA gene sequences extracted from both the amplicon and metagenomic data, we identified a strain of *Lactococcus* sharing 97.2% 16S rRNA gene similarity to *L. cremoris subsp.*

tructae. This variant was by far the most abundant taxon in our datasets, representing an average of 20%, 45%, and 60% of 16S rRNA gene amplicons in blue, hybrid, and channel catfish, respectively (Figure 4). *L. cremoris* subsp. *tructae*, a lactic acid-producing bacterium used in dairy fermentation (Li et al., 2019), has been isolated from the digesta microbiomes of rainbow (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*; (Perez et al., 2011)). In aquaculture, pre-exposure to *L. cremoris* WA2-67 reduced rainbow trout mortality by 20% following challenge by the pathogenic *L. garvieae,* with antipathogen activity linked to the production of an antibacterial peptide by *L. cremoris* (Araujo et al., 2015). Given the pathogenicity of *L. garvieae* in catfish aquaculture (Shahin, Mukkatira, et al., 2022), the presence of *L. cremoris* subsp. *tructae* in catfish suggests a potential probiotic function that may reduce host mortality and enhance resilience (Giatsis et al., 2016). Its high relative abundance in hybrid catfish, particularly in 2022 samples, suggests a potential role in enhancing host health and highlights a possible mechanism underlying hybrid vigor in aquaculture.

In contrast, OTUs representing potential pathogens were detected in blue and channel catfish but at much lower abundance in hybrid catfish. Specifically, OTUs identified as *Aeromonas* and *Edwardsiella* were 100% identical to known pathogens *A. veronii* and *E. anguillarum*, respectively. *A. veronii*, which causes disease in freshwater fish (Dong et al., 2017; Li et al., 2020) and antibiotic-resistant infections in channel catfish (Truong Dinh Hoai, 2019), was most abundant (an average of 20% of amplicons) in blue catfish but nearly absent in hybrids. *E. anguillarum* is extensively studied in this aquaculture system (Reichley et al., 2017; Reichley et al., 2018; Armwood et al., 2022) and can cause mortality at high concentrations, with blue and hybrid catfish exhibiting greater mortality than channel catfish (Reichley et al., 2018; Armwood et al., 2022). In our study, the *E. anguillarum* OTU appeared only in 2022 samples

and primarily in channel catfish, being effectively absent from blue and hybrid species. The near absence of *Aeromonas* and *Edwardsiella* in hybrid microbiomes suggests that although these putative pathogens are more prevalent in the parental species, any potentially harmful effects may not extend to hybrids.

Certain broad categories of microbiome functions were at relatively even representation across catfish species but nonetheless contributed to variation in metabolic potential among host types. For example, blue and hybrid catfish metagenomes were relatively enriched in genes of sulfur metabolism (Figure 5). These included genes such as *cysDNCHIJKM* involved in sulfur assimilation and biosynthesis of sulfur-containing amino acids like cysteine and methionine, which are essential for protein production, cellular growth, and immune function (He et al., 2018; Zhang et al., 2020; de Bont et al., 2022). Specifically, within the NF-κB pathway, cysteine and methionine increases homeostasis and intestinal barriers and decreases oxidative stress, TNF-α, and IL-1β in the host (Kim et al., 2009; Chen et al., 2014; Song et al., 2016; Liu et al., 2017). Conversely, nitrogen metabolism genes were primarily detected in channel catfish metagenomes and in one blue catfish metagenome, with minimal representation in hybrids (Figure 5). Most identified genes were involved in nitrate reduction (e.g., *napA*, *narGHIYVZB*), dissimilatory nitrate reduction to ammonium (DNRA; *nirD*, *nrfA*), and nitrogen assimilation (*glnA*, *gdhA/B*, *gltB/D*). The latter two processes may contribute to the bioavailable nitrogen pool in the microbiome, although effects of these microbial functions on host growth remain to be determined (She et al., 2023).

Within the carbohydrate metabolism super pathway, hybrids exhibited a profile of pathway representation intermediate between profiles from both parental species, being enriched in sugar metabolism (e.g., starch and sucrose, galactose, fructose, and mannose pathways)

pathways similar to what was observed in blue catfish, but also enriched in central carbon metabolism pathways (pyruvate metabolism and the TCA cycle) similar to what was observed in channel catfish (Figure 5). In contrast, representation of glycolysis/gluconeogenesis genes was highest in hybrids compared to both blue and channel catfish. Carbohydrate processing by the gut microbiome may be valuable in aquaculture where high-carbohydrate diets are common (Biju Sam Kamalam, 2017; Zhang et al., 2023) and enhanced microbial carbohydrate digestion, via fermentation and short-chain fatty acid production (e.g., acetate and butyrate; (Louis & Flint, 2009; Ponnusamy et al., 2011; Tian et al., 2017)), has been linked to improved growth, feed efficiency, and resilience (Chenglong Wu, 2016; Zhang et al., 2023). Indeed, an OTU classified to the butyrate-producing genus *Paraclostridium* was detected at relatively high abundance (~5%) in both hybrid and blue catfish. Butyrate production by members of this genus has been associated with improved growth and immunity in fish (Louis & Flint, 2009; Estensoro et al., 2016; Tian et al., 2017; Bakky et al., 2025), raising the possibility that microbiome organic acid production during carbohydrate metabolism may benefit growth in hybrid catfish.

A limitation of this study is the inconsistency in early rearing conditions among blue, channel, and hybrid catfish across sampling years. Channel catfish (2022 cohort) were initially obtained as fry from a commercial hatchery and held the entire time in the MAFES/NWAC rearing facility. Comparably, blue and hybrid catfish were hatched at the USDA WARU and reared indoors at the WARU facility prior to transfer to the MAFES/NWAC facility as fingerlings. These differences in hatchery origin and early-life rearing environments may have introduced a hatchery or origin effect that could influence observed microbiome differences. Although the inclusion of 2023 samples helped mitigate this concern, slight differences in taxonomic trends were observed between years in the amplicon sequencing data. Additionally,

because metagenomic data were only generated from 2022 samples, the inferred metabolic potential may differ had 2023 samples been included. Another important limitation is that host behavioral and physiological differences, particularly feeding behavior, were not directly measured in this study. Hybrid catfish exhibit more aggressive feeding relative to parental species, which may independently influence gut microbiome structure through increased food intake, altered nutrient flux, changes in gut transient time, or differences in digesta composition. These host-driven factors could contribute to microbial community composition and functional potential, raising the possibility that observed microbiome differences are, at least in part, a consequence rather than a cause of hybrid vigor.

Conclusion

Hybrid catfish harbor microbiomes with taxonomic and functional traits distinct from those of parental blue and channel catfish. An increased abundance of *C. somerae* and vitamin B_{12} biosynthesis pathways in hybrids highlights a potential role of microbial-derived B vitamins in supporting host immunity and nutrient metabolism. Additional enrichment of antibiotic biosynthesis genes and lactic acid-producing bacteria like *L. cremoris*, coupled to the relative absence of potential pathogens such as *Aeromonas and Edwardsiella* species, further suggest a disease-protective microbiome in hybrids, although this hypothesis awaits testing. The metagenome data also highlight variation in broad functional category representation among catfish microbiomes, suggesting that microbiome metabolic contributions may indeed differ among closely related catfish types. It remains to be tested whether these variations underlie differential contributions to nutrient use and immune function in hybrids relative to parental

species. Together, these microbial features reflect a unique microbiome in hybrid catfish that may underpin hybrid vigor and growth success in aquaculture.

CHAPTER THREE

MICROBIOME-MEDIATED DEFENSE POTENTIAL ASSOCIATED WITH CHANNEL CATFISH EMBRYOS

Contribution of Authors and Co-Authors

Manuscript in Chapter 3
Author: Madelaine L. Brown
Contributions: formulated guiding hypothesis, performed sample extraction and preparation, devised and performed all bioinformatic analyses, wrote manuscript

Co-Author: Zoe A. Pratte
Contributions: formulated guiding hypothesis, advised on bioinformatic analyses

Co-Author: Cynthia C. Ware
Contributions: performed sampled collections

Co-Author: Dyvia Rose
Contributions: performed sampled collections

Co-Author: Brian G. Bosworth
Contributions: performed catfish rearing, performed sampled collections

Co-Author: Matt J. Griffin
Contributions: formulated guiding hypothesis, performed sampled collections

Co-Author: Frank J. Stewart
Contributions: formulated guiding hypothesis, advised on bioinformatic analyses, wrote manuscript

Manuscript Information

Madelaine L. Brown, Zoe A. Pratte, Cynthia C. Ware, Dyvia Rose, Brian G. Bosworth, Matt J. Griffin, Frank J. Stewart

ASM Animal Microbiology
Status of Manuscript:
☐ Prepared for submission to a peer-reviewed journal
☒ Officially submitted to a peer-reviewed journal
☐ Accepted by a peer-reviewed journal
☐ Published in a peer-reviewed journal

04-18-2026

Abstract

Externally hatched fish embryos are susceptible to microbial pathogens upon exposure to the aquatic environment. Host-associated microbiomes are hypothesized to protect embryos from pathogens and environmental stressors through antagonistic mechanisms such as antimicrobial production. In this study, we used 16S rRNA gene profiling to taxonomically characterize microbiomes associated with embryos from 23 spawns of channel catfish sampled before and after embryo surface disinfection. Shotgun metagenomics was then used to explore potential defense-associated functional traits in embryo microbiomes. Embryo microbiomes were dominated by Comamonadaceae, Flavobacteriaceae, Rhizobiaceae, Methylomonadaceae, and Methylophilaceae, with Methylomonadaceae and Methylophilaceae significantly enriched following disinfectant treatment. Rhizobiaceae, Xanthobacteraceae and Sphingomonadaceae were enriched relative to microbiomes in tank water, suggesting potential roles in nutrient cycling and protection against environmental pollutants. Metagenomic analysis revealed genes associated with antimicrobial production (NRPS, RiPPs, terpenes, acyl amino acids) and resistance (vanW, qacJG), suggesting that embryo microbiomes may contribute to host defense. Together, these results indicate that catfish embryos harbor diverse microbiomes taxonomically distinct from the surrounding environment and equipped with functional properties potentially important for host defense and resilience against environmental stressors.

Introduction

Externally fertilized embryos of fishes are vulnerable to a wide range of environmental stressors, including attack by pathogens and exposure to pollutants or excess nutrients (Wilkins et al., 2015; Bentzon-Tilia et al., 2016; Fujimoto et al., 2018; Kerwin & Nyholm, 2018).

Microbial colonization of egg and embryo surfaces begins immediately after deposition into the environment, raising the possibility that an embryo-associated biofilm could exert effects, potentially positive or negative, on embryogenesis (Fujimoto et al., 2013; Fujimoto et al., 2018; Lokesh et al., 2019). In other animal-microbe partnerships, microbiomes play positive roles in defense against pathogens by outcompeting pathogens or producing compounds (e.g., antibiotics) that inhibit pathogen activity (Hanif et al., 2004; Boutin et al., 2012; Wilkins et al., 2016). As fish embryos lack a fully developed immune system, antipathogen effects of an embryo-associated microbiome may be particularly important for fish survival (Liu et al., 2014; Wilkins et al., 2016). However, despite growing evidence from other systems that microbes influence embryo survival and development (Krajmalnik-Brown et al., 2012; King et al., 2016; Zheng, 2020; Qi et al., 2023), the taxonomic composition and functional contributions of fish embryo microbiomes remain largely uncharacterized.

Biofilm formation may be an important mechanism by which microbiomes protect or otherwise benefit a host embryo. Biofilms provide a physical barrier that limits exposure to antibiotics and other environmental pollutants by slowing diffusion to the embryo surface and sequestering toxic compounds (Angoshtari et al., 2023). Thicker more mature biofilms contain internal oxygen and chemical gradients that support metabolically diverse microbes potentially capable of degrading a range of chemical stressors (Iijima et al., 2009; Sanz-Lazaro et al., 2011; Penesyan et al., 2021; Harbi, 2025). Beneficial microbes in biofilms may suppress pathogens by occupying attachment sites, competing for energy or nutrient substrates, or producing antagonistic compounds such as bacteriocins (Penesyan et al., 2021; Harbi, 2025). Many biofilm-associated microbes also produce secondary metabolites such as B vitamins and antioxidants that may enhance embryo immune function or inhibit pathogen growth (Beemelmanns et al., 2019;

Penesyan et al., 2021). Although biofilms are often associated with pathogenic infections, growing evidence shows that host-associated biofilms also provide beneficial protection (Fujimoto et al., 2018; Harbi, 2025). Whether embryos support a stable surface-attached biofilm with benefits to the host remains unexplored for the overwhelming majority of fish species.

Fish embryo-associated biofilms could serve a protective role for the host while also being susceptible to disruption by environmental or human-sourced antimicrobial compounds. Diverse antibiotics, including broad-spectrum compounds (e.g., streptomycin), are synthesized naturally by common environmental bacteria such as *Streptomyces* and *Bacillus* spp. (Amin et al., 2012). Studies in model systems (e.g., zebrafish) show that antibiotic exposure can disrupt embryo-associated microbiomes, reduce microbial diversity, and cause severe developmental abnormalities, underscoring the sensitivity of fish embryos to environmental pollutants (Zhang et al., 2015; Bielen et al., 2017; Pindling et al., 2018). Embryo biofilms likely also encounter antimicrobial compounds introduced intentionally or unintentionally by human activity. For example, in aquaculture systems, standard management practices may expose embryos to antimicrobial compounds, including iodine (betadine) treatment used commonly for fungal control. The extent to which such management strategies affect prokaryotic members of an embryo-associated biofilm is largely unknown.

The externally brooded embryos of channel catfish (*Ictalurus punctatus*) are a valuable model for exploring embryo-microbiome relationships in fish. Channel catfish are one of the most important aquaculture species in the United States, accounting for more than 60% of all U.S. food fish production (Liu et al., 2016; Reichley et al., 2018). Efforts to improve production have included hybridization with blue catfish (Li et al., 2014; Reichley et al., 2018) and the use of pre- and probiotics (Etyemez & Balcazar, 2015; Giatsis et al., 2016; Qi et al., 2023), but these

approaches are generally targeted at juveniles or adults rather than early life stages. Female channel catfish deposit their egg mass into secluded, semi-dark areas such as hollowed out logs, holes in the bank, or rock piles (natural), or into buckets/boxes (aquaculture) where the male catfish then fertilizes the eggs to produce embryos. In the wild, males will guard the mass during the embryonic stage (~5–10 days) while also aerating and removing waste by fanning the embryos with their fins (Wellborn, 1990; Dunham & Elaswad, 2018). Despite parental care or controlled spawning practices, catfish embryos experience immediate environmental exposure to pathogens and pollutants in natural and aquaculture environments (Bentzon-Tilia et al., 2016; Fujimoto et al., 2018; Najafpour et al., 2021; Shan et al., 2022). Specifically, fungal pathogens such as *Fusarium* and *Saprolegnia* spp. are a major cause of embryo mortality in aquaculture and can spread rapidly across embryo masses, leading to significant losses (Liu et al., 2014; Kerwin & Nyholm, 2018). Consequently, catfish aquaculture typically involves administering the iodinating antiseptic betadine to control fungal disease in embryos. However, whether betadine treatment disrupts embryo biofilms is uncertain, due partly to an overall lack of data describing the microorganisms associated with catfish embryos.

This study characterizes the microbiome associated with channel catfish embryos in aquaculture with the goal of testing how microbiomes may contribute to early host defense. Using 16S rRNA gene sequencing, we examined microbiome taxonomic composition of embryos from 23 controlled spawns, before and after betadine treatment, and across two channel catfish strains. Taxonomic characterizations were complemented with shotgun metagenomic analysis performed on a subset of samples to evaluate metabolic functions with potential defensive roles. Specifically, this study aimed to 1) determine whether embryo-associated microbiomes differ from those of the surrounding water and represent a distinct embryo surface

niche, 2) assess how common aquaculture practices, such as betadine treatment, alter these microbiomes, and 3) identify microbiome taxa and functional traits with the potential to protect embryos against pathogens or environmental stressors. These results show that community composition is consistent across spawns and strains but shifts significantly following betadine treatment, identifying embryo-associated bacteria possessing functional traits potentially valuable for host health and defense.

Methods

Catfish Embryo Rearing and Sample Collection

Spawns (N= 23) were obtained from mate pairings conducted in April of 2022 as part of the USDA-ARS Warmwater Aquaculture Research Unit (WARU) selective breeding program. Channel catfish in this study belonged to one of two selection strains: Delta Select (DS) and Delta Control (DC). The DS strain is selected for increased growth and carcass yield (Bosworth et al., 2020). The DC strain is a randomly bred population from the same base population used to produce the DS strain. For each of the two strains, one male and one female catfish broodstock were placed into each of 60 2,000-L holding tanks (30 tanks/strain) located along the level of a 0.8 ha brood pond at the Thad Cochran National Warmwater Aquaculture Center. Each tank was supplied with a submerged 40-L milk can that acted as the spawning cavity. Tanks were covered with mesh netting to prevent harassment and predation by birds. Water from the brood pond was pumped to each spawning tank (3.8 L min^{-1}), then flowed back to the brood pond. All spawning tanks received water from the same common pond source. Broodfish were fed a maintenance ration of a commercial catfish diet (32% protein) at approximately 1% body mass on Tuesdays and Thursdays. Milk cans were inspected for the presence of spawns on Mondays, Wednesdays,

and Fridays. When spawns were detected, embryo masses were removed from the milk cans using a plastic ice scraper, and a subsample of approximately 100 embryos was collected from each spawn and placed into sterile 50-mL tubes containing 25 mL of hatchery water. Samples were transported on ice to the laboratory, where embryo samples were placed in sterile Petri dishes. Five individual embryos were separated from each mass using sterile scalpel blades and forceps and transferred into 1.5-mL microcentrifuge tubes containing 1 mL of nucleic acid-stabilizing buffer (25 mM sodium citrate, 10 mM EDTA, 5.3 M ammonium sulfate, pH 5.2). Water samples (N=23, pre-betadine) were filtered through 0.2 µm polycarbonate membrane filters (Isopore™; MilliporeSigma), with filters stored in 1.5-mL microcentrifuge tubes containing 1 mL of the same nucleic acid-stabilizing buffer.

The remaining embryo mass was placed into a 7.6-L bucket containing groundwater from the WARU hatchery with betadine (100 ppm) to prevent introduction of potential outside contaminants to the hatchery. Spawns were transported by ATV ~0.5 km to the hatchery where each betadine-treated spawn was placed into an individual 80-L hatching trough supplied with groundwater and operated as a flow-through system (3.7 L min^{-1}, approximately 27 °C), with supplemental aeration provided by airstones connected to a regenerative blower. After 24 h in the hatchery, a second embryo subsample was collected from each spawn and processed as described above. In addition, 50-mL water samples were collected from both spawning tanks and hatching troughs for each spawn. Water samples (N= 25, post-betadine) were filtered through 0.2 pore size Isopore™ polycarbonate membrane filters (Isopore™; MilliporeSigma), with filters stored in 1.5-mL microcentrifuge tubes containing 1 mL of the same nucleic acid-stabilizing buffer. Embryo and filter samples were stored at -20 °C until cold shipment to Montana State University (MSU). Upon receipt at MSU, samples were stored at -80°C until DNA extraction.

DNA Extractions and Amplicon and Metagenomic Sequencing

Genomic DNA was extracted from embryo and water samples using the DNeasy® 96 PowerSoil® Pro Kit (QIAGEN). For each embryo sample (N = 23), five embryos from the same spawn were placed in a PowerBead tube, and DNA was extracted following the manufacturer's protocol. All embryos lysed during bead beating in the PowerBead tube. Therefore, we cannot determine whether microbial DNA originated from embryo surface-associated versus intracellular (within embryo) microbes. However, we have no evidence that microbes are found inside fish embryos and consider this scenario unlikely. For water samples, half of each filter membrane was aseptically cut into smaller pieces and placed into a PowerBead tube for DNA extraction following the manufacturer's protocol. For each extraction kit (N = 2), we processed an extraction blank consisting of a tube with nucleic acid-stabilizing buffer but without an embryo or water sample.

DNA from each sample and blank was used for PCR amplification of the V3–V4 region of the 16S rRNA gene using primers F515 and R806, modified as previously described (Amy Apprill, 2015; Parada et al., 2016) with the addition of barcodes and Illumina-specific adapters. PCR reactions contained 2–5 µl template DNA, 0.25 µl of each forward and reverse primer, 0.5 µL bovine serum albumin (BSA, 20 mg/ml; New England BioLabs Inc), 2.5 µl Hot Start Taq PCR MasterMix (VWR, Radnor, PA, USA), and sterile nuclease-free water to bring the total volume to 25 µl. Additionally, negative controls (N = 2) for PCR amplification were created by replacing template DNA with sterile nuclease-free water. PCR amplification included an initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 2 min, and extension at 72°C for 90 sec. This was followed by a final extension at 72°C for 10 min. All embryo samples were PCR-amplified twice, to generate

technical duplicates. PCR amplicons were purified with Diffinity RapidTip PCR purification tips (Diffinity Genomics, NY, USA), quantified on a Qubit (Life Technologies), pooled at equimolar concentrations, and sequenced at the University of Georgia Genomics and Bioinformatics Core, Athens, GA on a MiSeq (Illumina Inc., San Diego, CA, USA) using a V2 500-cycle kit (250 X 250 bp) with 5% PhiX to increase read diversity (Padilla et al., 2015). For shotgun metagenomics, the five catfish embryo samples with the highest DNA yields (two pre-betadine and three post-betadine) were chosen and sent to the Georgia Genomics and Bioinformatics Core for library preparation and sequencing on Illumina's NextSeq using a P2 150 x 150 flow cell (Illumina Inc., San Diego, CA, USA).

Microbiome Data Processing and Analysis

16S rRNA gene amplicons were analyzed following the DADA2 (v1.22.0; (Callahan et al., 2016)) and QIIME2 (v2-2022.11; (Bolyen et al., 2019)) pipelines. Using DADA2, raw sequences were demultiplexed, quality filtered, and trimmed at 40 bp from the 5' ends of both reads (--p-trim-left-f 40, --p-trim-left-r 40) and truncated at 170 bp (--p-trunc-len-f 170, --p-trunc-len-r 170), based on read quality profiles. Sequences were denoised, merged, and chimeras were removed to generate amplicon sequence variants (ASVs). Next, through q2-alignment, ASVs were aligned using MAFFT (Katoh et al., 2002), and phylogeny was estimated using FastTree (Price et al., 2010). Technical PCR duplicates from catfish embryo samples were merged prior to assigning taxonomy to all ASVs using the SILVA-132 database (Quast et al., 2013). Chloroplast and mitochondrial sequences were removed from all datasets. Next, all samples were rarefied to 24,000 sequences corresponding to the lowest sequencing depth among embryo and water samples. Taxa that were identified in blanks and negative controls at a higher average relative abundance than in the catfish embryo and water samples were excluded from

analyses (Altman-Kurosaki et al., 2025). All raw sequences are publicly available through the NCBI's SRA database under PRJNA1446894.

Statistical analyses

Statistical analyses were conducted using RStudio (v2023.03.0+386; (Team, 2023)) to test for microbiome differences among catfish pairings (spawn ID) and between catfish embryos vs. water, catfish strain DS vs. DC, and pre- vs. post betadine treatment for both embryo and water samples. To evaluate the effect of catfish strain on alpha diversity in pre- and post-betadine embryo microbiomes, a one-way Kruskal–Wallis (*H*) test (Kruskal & Wallis, 1952) was performed separately for each treatment. To assess the effect of spawn ID, analyses could not be conducted separately for pre- and post-betadine samples due to insufficient sample size within groups, and therefore, a one-way Kruskal–Wallis (*H*) test was done with all embryo samples to evaluate differences in alpha diversity across spawn ID. Pairwise differences in alpha diversity were assessed using Wilcoxon rank-sum tests calculated from rarefied data and implemented with ggpubr (v0.6.0; (Kassambara, 2025)) and microbiomeutilities (v1.00.17; (Shetty, 2012-2019)) R packages, with comparisons visualized using boxplots. Beta diversity was assessed using Bray-Curits dissimilarity matrix calculated from rarefied data and visualized via NMDS (non-metric multidimensional scaling) using the phyloseq (v1.42.0; (McMurdie & Holmes, 2013)) and ggplot2 (v3.5.2; (Wickham, 2016)) R packages. Differences in community composition between sample types were tested using permutational multivariate analysis of variance (PERMANOVA, adonis2 from the vegan package (v2.6-4; (Oksanen, 2022)) with 999 permutations, and pairwise comparisons were conducted using the pairewiseAdonis package (v0.4.1; (Arbizu, 2017)) with Bonferroni-adjusted p-values. Homogeneity of group dispersions was evaluated using permutational multivariate analysis of dispersion (PERMDISP, betadisper

and permutest from vegan (Oksanen, 2022)) followed by pairwise comparisons with Tukey'sHSD test.

The ANCOM-BC, R package (v2.0.2; (Lin & Peddada, 2020)) was used to identify ASVs with relative abundances differing significantly between embryo and water microbiomes, and between pre- and post-betadine embryo microbiomes. This analysis focused only on ASVs meeting criteria of 1) being detected in at least 65% of all samples, 2) representing at least 5% of sequences in each sample in which they were detected, and 3) exhibiting a log fold change > 2. Analyses were conducted using an adjusted p-value threshold of 0.05 and a log-fold change threshold of 2. ASVs were first compared between all embryo and water microbiomes, where water was the intercept, followed by comparisons between pre- and post-betadine microbiomes, where post-betadine was the intercept.

Metagenomic Data Processing and Analysis

Metagenomic reads from five catfish embryo samples with the highest DNA yields were quality filtered to retain sequences ≥100 bp with a Phred score ≥25 (Trim Galore! (v0.6.10; (Martin, 2011)). To remove host-derived sequences, reads were mapped to the channel catfish genome (*I. punctatus*; NCBI Accession number NC_030416) using Bowtie2 (v2-2.5.1; (Langmead & Salzberg, 2012)), with these sequences then removed from further analysis. Remaining non-host reads were normalized with BBnorm (https://sourceforge.net/projects/bbmap/) and assembled into contigs using metaSPADES (v3.15.3; (Nurk et al., 2017)) with default parameters.

Contigs were quantified using CoverM (v0.7.0; Aroney 2025) to calculate gene abundance using TPM normalization, accounting for sequencing depth and contig length. Unbinned contigs were screened to analyze the metabolic potential of embryo-associated

microbiomes. Contigs were quantified using coverM contig (v0.7.0; (Samuel T N Aroney 2025)) to calculate gene abundance using transcripts per million (TPM) normalization, accounting for sequencing depth and contig length. Additionally, with coverM, the median depth (reads per contig) and mediant coverage (fraction of contig covered by at least one read) were determined for each of the five datasets. Open reading frames (ORFs) were predicted from unbinned contigs using Prodigal (v2.6.3; (Hyatt et al., 2010)), and protein sequences were functionally annotated with eggNOG-mapper (v5.0.2; (Huerta-Cepas et al., 2018)) using DIAMOND (v2.1.8; (Buchfink et al., 2021)) against the eggnog database using default parameters. Genes assigned to functional categories related to antibiotic synthesis (biosynthesis of secondary metabolites) and antibiotic production (defense) from the Database of Cluster of Orthologous Genes (COG; (Galperin et al., 2025)) were selected for further analyses. These annotated genes were mapped to contig-level gene-estimates estimates using locus tags to assess their relative abundance in embryo metagenomes, and abundances were visualized using bar plots in ggplot2. All analyses were performed using default parameters unless otherwise stated.

To complement gene-level analyses of unbinned contigs, contigs were next binned into metagenome-assembled genomes (MAGs), which were used to assess taxon-specific contributions to metabolic defense functions. MAGs were assembled with MetaWRAP (v1.3.2; (Uritskiy et al., 2018)), dereplicated at 99% similarity with dRep (v3.4.2; (Olm et al., 2017)), and taxonomically classified using GTDB-Tk (v2.4.0; (Chaumeil et al., 2019)) using the Genome Taxonomy Database, all with default parameters. This workflow resulted in three MAGs: a Burkholderiales MAG and two Rhizobiales MAGs (one identified only to the order Rhizobiales, and one identified to the Rhizobiales family Rhizobiaceae). To quantify sequencing depth and coverage for each MAG, reads from each sample were mapped to the MAGs using Bowtie 2,

with the very sensitive local setting, and processed with SAMtools (v1.17; (Li et al., 2009)) to generate sorted, indexed BAM files. CoverM genome was then used to calculate mean coverage and fraction of the genome covered for each MAG per sample. Depth and coverage analyses were performed using default parameters unless otherwise stated.

Potential antibiotic resistance genes were identified by running unbinned contigs and MAGs through the Comprehensive Antibiotic Resistance Database (CARD) using the Resistance Gene Identifier (RGI v6.0.5; (Alcock et al., 2023)) with default parameters, reporting only strict hits. Biosynthetic gene clusters (BGCs) in each MAG were identified using antiSMASH (v8.0.4; (Medema et al., 2011)) with default parameters. Unbinned contigs were not analyzed with antiSMASH due to sequence length limitations. Counts of potential antibiotic biosynthesis BGCs and resistance genes were visualized as heatmaps using ggplot2.

All computation was performed on the Tempest High Performance Computing System, operated and supported by University Information Technology Research Cyberinfrastructure (RRID:SCR_026229) at Montana State University, Bozeman, MT, USA (https://www.montana.edu/uit/rci/tempest/). All raw sequences are publicly available through the NCBI's SRA database under PRJNA1446894.

Results

The final 16S rRNA gene amplicon dataset included 76 samples, including 19 pre-betadine and 23 post-betadine embryo samples and 9 pre-betadine and 25 post-betadine water samples. A subset of the pre-betadine water samples was excluded due to loss or compromised sample integrity prior to sequencing; however, the remaining sample size was sufficient to support the statistical comparisons performed in this study. Embryo microbiome datasets

represented 23 catfish spawns all of which had two replicates (pre- and post-betadine treatments) except for spawns 51, 55, 77, and 91, which had only one replicate each. The total embryo microbiome dataset also included 28 and 14 datasets from catfish strains DS and DC, respectively (Table S1). The total shotgun metagenomic dataset included five datasets generated from embryos with high DNA yields (two pre-betadine and three post-betadine).

Neither catfish strain (DS versus DC) nor spawning group significantly affected how microbiome alpha diversity or taxonomic composition responded to betadine treatment. For catfish strain, Shannon diversity did not differ between strains in either pre-betadine (Kruskal–Wallis test, H(1) = 0.03, $P = 0.86$) or post-betadine samples (Kruskal–Wallis test, H(1) = 0, $P = 0.10$). Similarly, taxonomic composition between catfish strains was not significantly different for pre-betadine (PERMANOVA, Bray–Curtis, pseudo-F = 1.6, df = 1, $P = 0.06$) or post-betadine samples (PERMANOVA, Bray–Curtis, pseudo-F = 1.6, df = 1, $P = 0.19$). Spawning group also had no significant effect on Shannon diversity (Kruskal–Wallis test, H(23) = 21.5, $P = 0.55$) or taxonomic composition (PERMANOVA, Bray–Curtis, pseudo-F = 0.9, df = 22, P = 0.77). Consequently, the results below are based on analyses combining data from both catfish strains and all spawning groups.

Microbiome alpha diversity differed between catfish embryo-associated and water microbiomes and in response to betadine treatment. Shannon diversity of embryo-associated microbiomes (both pre- and post-betadine) was significantly lower compared to diversity in pre-betadine water microbiomes (Figure 6a and Table B2). However, betadine treatment strongly affected water microbiomes, reducing median diversity by ~30% from pre- to post-treatment (Figure 6a). In contrast, betadine treatment did not significantly affect alpha diversity in embryo-associated microbiomes (Figure 6a**)**.

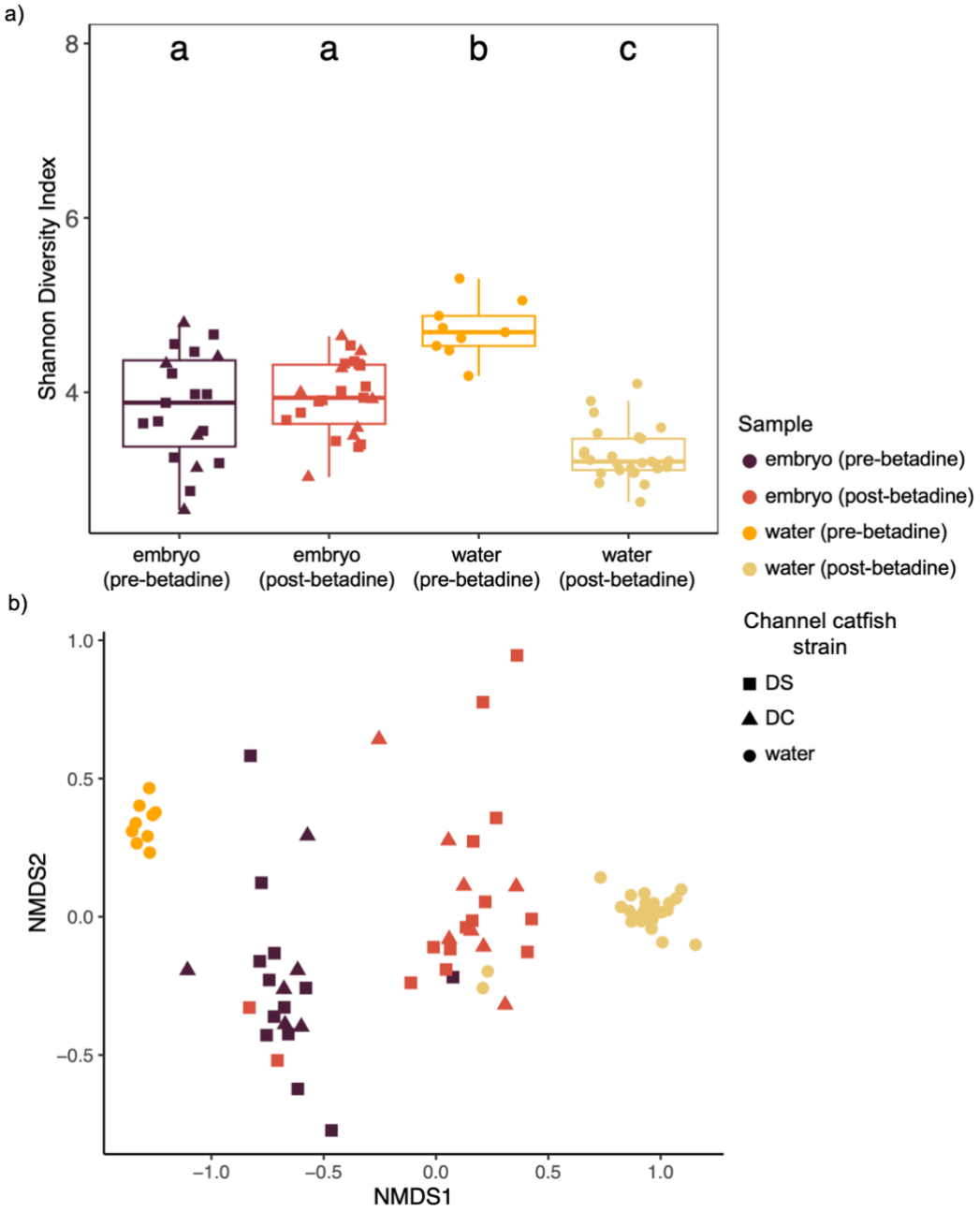

Figure 6: Catfish pre- and post-betadine embryo microbiomes differ significantly from each other and water microbiomes. a) Alpha diversity measures using Shannon diversity index is similar for all embryo microbiomes with differences between embryos and water microbiomes before and after betadine treatment. Box plots show median (center line), 25–75% quartiles (area within the box), minimum and maximum values (whiskers), outliers (large circular data points) and jittered data points by channel catfish strain (DS = Delta Select, DC = Delta Control). Letters represent significantly distinct groups. Pair-wise Wilcoxon-rank sum test values are in Table B2. b) Beta diversity measures using NMDS (with Bray-Curtis) show all microbiomes are distinct from another with no influence from channel catfish strain (DS = Delta Select, DC = Delta Control). Pair-wise PERMANOVA values are in Table B3.

Taxonomic composition assessed by Bray-Curtis dissimilarity differed between embryo and water microbiomes and in response to betadine treatment in both embryo and water microbiomes (PERMANOVA, pseudo-F = 27.58, df = 1, $P = 0.001$). Pre- and post-betadine embryo microbiomes clustered apart from one other in NMDS analysis, as did pre- and post-betadine water microbiomes, with all pairwise comparisons being statistically significant (all pairwise PERMANOVA $P = 0.006$; Figure 6b; Table B3). While dispersion differed significantly between embryo and water microbiomes, dispersion did not change pre- to post-betadine in either embryo or water samples (Table B3).

Pre- and post-betadine embryo microbiomes primarily consisted of Proteobacteria belonging to the families Comamonadaceae (averaging 22% and 12% in pre- and post-betadine samples, respectively), Flavobacteriaceae (20% and 13%), and Rhizobiaceae (5% and 6%; Figure 7a). Representation of the methanotrophic/methylotrophic families Methylomonadaceae and Methylophilaceae increased from 1% (the two families combined) in pre-betadine to 17% in post-betadine samples. Water microbiomes (pre- and post-betadine) were represented by Flavobacteriaceae (averaging 11% and 6% in pre- and post-betadine samples, respectively) and Comamonadaceae (3% and 4%; Figure 7a). Similar to what was observed in the embryo microbiomes, representation of the methanotrophic/methylotrophic families Methylomonadaceae and Methylophilaceae increased from 1% (the two families combined) in pre-betadine to 40% in post-betadine samples. In contrast, cyanobacteria of the Cyanobiaceae decreased substantially with betadine, from 27 % to >1% pre- to post-treatment.

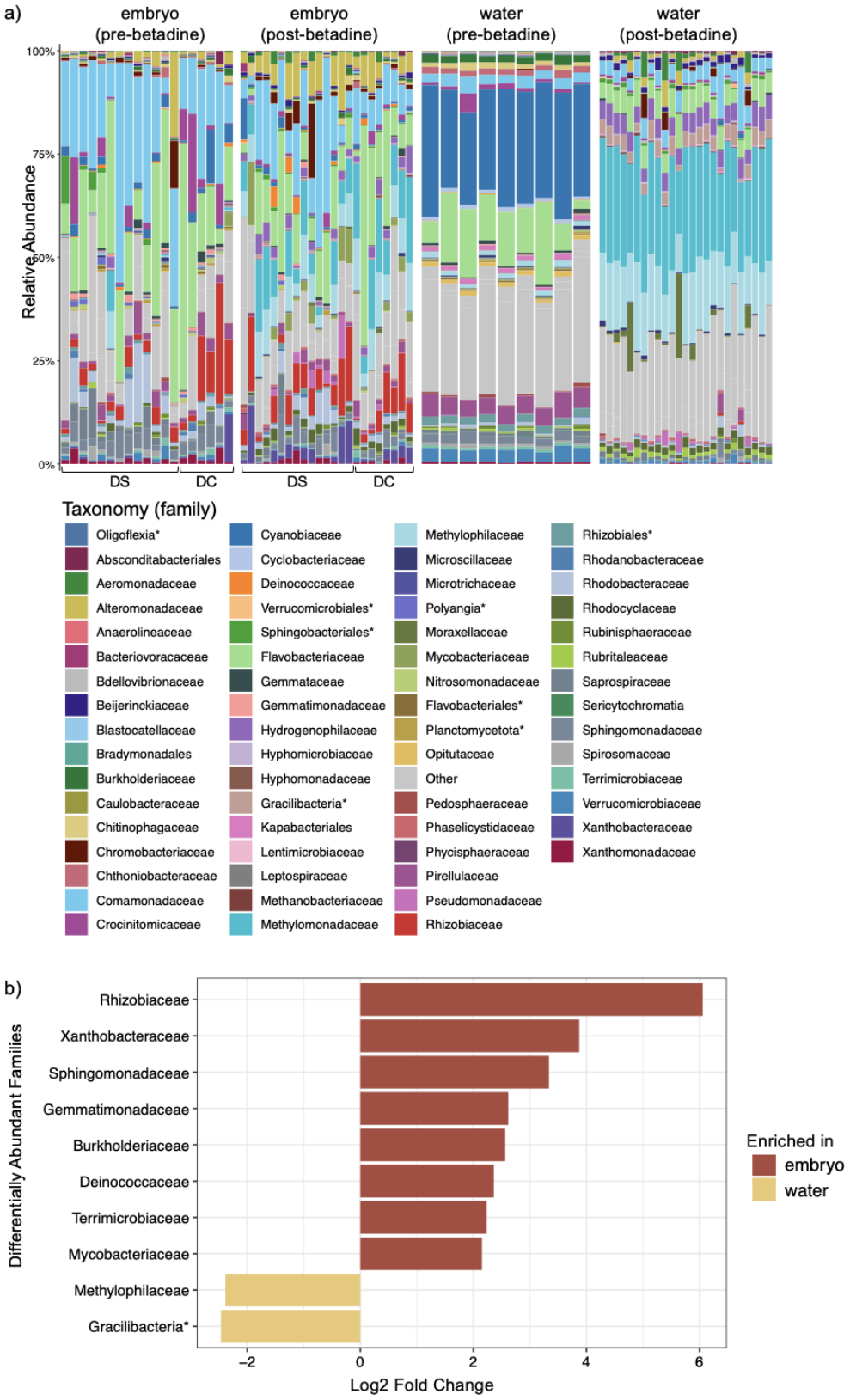
a)
embryo (pre-betadine)
embryo (post-betadine)
water (pre-betadine)
water (post-betadine)
100%
75%
50%
25%
0%
Relative Abundance
DS
DC
DS
DC
Taxonomy (family)
Oligoflexia*
Absconditabacteriales
Aeromonadaceae
Alteromonadaceae
Anaerolineaceae
Bacteriovoracaceae
Bdellovibrionaceae
Beijerinckiaceae
Blastocatellaceae
Bradymonadales
Burkholderiaceae
Caulobacteraceae
Chitinophagaceae
Chromobacteriaceae
Chthoniobacteraceae
Comamonadaceae
Crocinitomicaceae
Cyanobiaceae
Cyclobacteriaceae
Deinococcaceae
Verrucomicrobiales*
Sphingobacteriales*
Flavobacteriaceae
Gemmataceae
Gemmatimonadaceae
Hydrogenophilaceae
Hyphomicrobiaceae
Hyphomonadaceae
Gracilibacteria*
Kapabacteriales
Lentimicrobiaceae
Leptospiraceae
Methanobacteriaceae
Methylomonadaceae
Methylophilaceae
Microscillaceae
Microtrichaceae
Polyangia*
Moraxellaceae
Mycobacteriaceae
Nitrosomonadaceae
Flavobacteriales*
Planctomycetota*
Opitutaceae
Other
Pedosphaeraceae
Phaselicystidaceae
Phycisphaeraceae
Pirellulaceae
Pseudomonadaceae
Rhizobiaceae
Rhizobiales*
Rhodanobacteraceae
Rhodobacteraceae
Rhodocyclaceae
Rubinisphaeraceae
Rubritaleaceae
Saprospiraceae
Sericytochromatia
Sphingomonadaceae
Spirosomaceae
Terrimicrobiaceae
Verrucomicrobiaceae
Xanthobacteraceae
Xanthomonadaceae
b)
Differentially Abundant Families
Rhizobiaceae
Xanthobacteraceae
Sphingomonadaceae
Gemmatimonadaceae
Burkholderiaceae
Deinococcaceae
Terrimicrobiaceae
Mycobacteriaceae
Methylophilaceae
Gracilibacteria*
−2
0
2
4
6
Log2 Fold Change
Enriched in
embryo
water

Figure 7: Embryo microbiomes (pre- and post-betadine) have differential enrichment of bacterial families compared to water microbiomes. a) Taxonomic bar chart of family level bacteria of embryo and water microbiomes with a minimum detection of 5% relative abundance in 65% of all samples (prevalence). Embryo samples are grouped by channel catfish strain (DS = Delta Select, DC = Delta Control). b) Differentially abundant ASVs (5% detection and 65% prevalence) represented as bacterial families, or next closest taxonomic identification, between all embryo and water microbiomes, with an adjusted p-value > 0.05, and a log fold change > 2. Water microbiomes were used as the intercept. Asterisks indicate taxa not resolved at the family level; the nearest assigned taxonomic rank is shown.

We identified a small subset of ASVs differing significantly in percentage abundance between water versus embryo microbiomes and between pre- versus post-betadine embryo microbiomes. This analysis focused only on prevalent and abundant ASVs meeting criteria of 1) being detected in at least 65% of all samples, 2) representing at least 5% of a sample's sequences when detected, and 3) exhibiting a log fold change > 2. Under these strict thresholds, eight ASVs were significantly enriched in embryos versus water (adjusted $P < 0.05$). These eight ASVs belonged to the families Rhizobiaceae ($log_2FC = 6.1$), Xanthobacteraceae ($log_2FC = 3.9$), Sphingomonadaceae ($log_2FC = 3.9$), Gemmatimonadaceae ($log_2FC = 2.6$), Burkholderiaceae ($log_2FC = 2.6$), Deinococcaceae ($log_2FC = 2.4$), Terrimicrobiaceae ($log_2FC = 2.2$), and Mycobacteriaceae ($log_2FC = 2.2$) families (Figure 7b). Conversely, only two ASVs, classified as Gracilibacteria and Methylophilaceae**,** were enriched in water versus embryos ($log_2FC = -2.5$ and -2.4, respectively; Figure 7b). Only one ASV, belonging to the family Spirosomaceae, was enriched in pre- versus post-betadine embryos ($log_2FC = 2.1$). In contrast, seven ASVs were enriched in post-betadine embryos and consisted of ASVs also identified in water microbiomes, including Methylomonadaceae ($log_2FC = -4.4$), Hydrogenophilaceae ($log_2FC = -4.3$),

Methylophilaceae ($log_2FC = -4.3$), Phaselicystidaceae ($log_2FC = -2.6$), Gracilibacteria (class; $log_2FC = -2.6$), and Rhodocyclaceae ($log_2FC = -2.2$; Figure B1).

Metagenome sequencing and assembly generated 89,398 contigs. For all unbinned contigs, median depth values ranged from 0.79x to 1.17x, and median coverage values ranged from 0.51 to 0.65 across the five embryo samples. We first used these unbinned contigs to assess metabolic potential by annotating open reading frames into COG functional categories, focusing on categories related to antibiotic biosynthesis (biosynthesis of secondary metabolites) and antibiotic resistance (defense), which represented 3% and 2% of the total category representation, respectively. The biosynthesis of secondary metabolites category was represented by 16 functional groups, with the topmost abundant containing methyltransferases, ABC transporters, isochorismatase, fumarylacetoacetate hydrolase (FAA), and thioesterase (Figure 8a). The COG defense category was represented primarily by six functional groups (each with relative abundance > 0.1%), including ABC transporters, multidrug resistance efflux pumps, beta-lactamases, peptidases, drug transport systems, and type IV TA systems (Figure 8a). Analyses specifically targeting antibiotic resistance genes (CARD) identified three *vanW* genes associated with vancomycin resistance and three multidrug resistance efflux genes (two *qacJ* and one *qacG*) associated with benzalkonium chloride resistance across all unbinned contigs (Figure 8b).

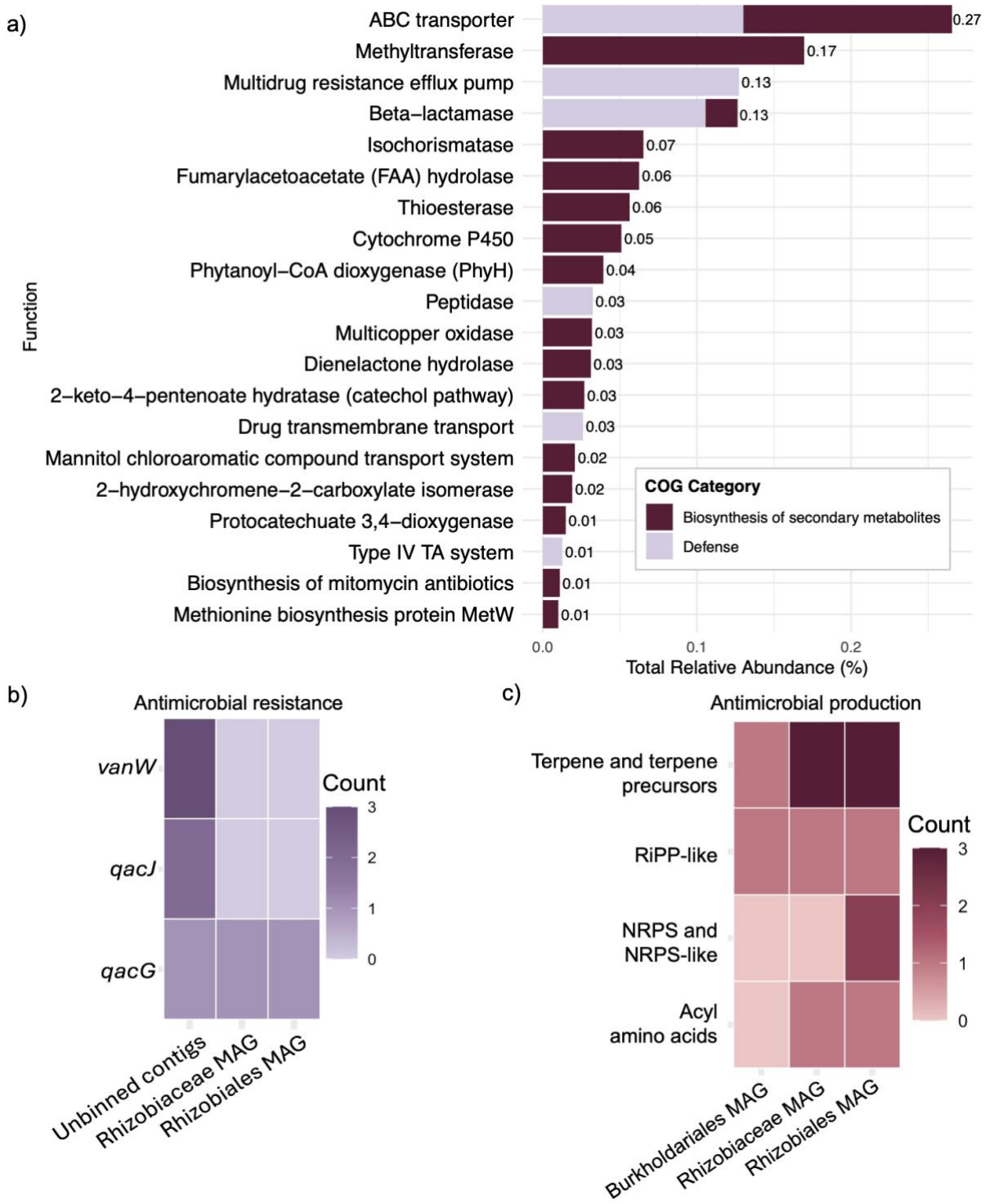

Figure 8: Catfish embryo metagenomes have antimicrobial biosynthetic and resistance potential. a) Total relative abundance of all COG defense and biosynthesis of secondary metabolite pathways in the contig data with a total relative abundance > 0.01%. b) Abundance (count) of antibiotic resistance genes identified in MAG and contig data. c) Abundance (count) of BGCs associated with antimicrobial production in MAGs.

Binning of contigs from all embryo metagenomes generated three metagenome-assembled genomes (MAGs). Reads from all metagenomic samples mapped to the assembled MAGs, with mean coverage ranging from 29.3x to 38.5x across samples. Genome coverage fraction varied among samples (0.12–0.88), with two samples exhibiting high coverage of assembled MAGs (0.65 and 0.88), while the remaining three samples showed lower genome coverage (0.12–0.17). One of the MAGs (52% completeness, 1% contamination) was classified to the order Burkholderiales and shared 90% average nucleotide identity (ANI) to a member of the genus *Rubrivivax*. The other two MAGs (44–46% completeness, 1–2% contamination) were both classified to the genus *Gellertiella* in the family Rhizobiaceae (order Rhizobiales, recently renamed to Hyphomicrobiales). One of these shared 90% ANI to a member of *Gellertiella*, while an ANI score was not available for the second *Gellertiella*-classified MAG. Both the Burkholderiales and Rhizobiales were at notable representation in the 16S rRNA gene amplicon datasets. With Burkholderiales being the most abundant order across embryo and water microbiomes and averaging 26% and 31% of sequences in pre- and post-betadine embryo datasets, respectively (Figure B2). Rhizobiales was also abundant in these embryo datasets (7% and 10%, respectively; Figure B2), as was the Rhizobiaceae specifically (5% and 6%, respectively), although the Rhizobiales order was largely absent from water microbiomes (Figure 7).

Across all MAGs, we identified BGCs related to antibiotic biosynthesis, containing genes linked to synthesis of terpenes and terpene precursors, ribosomally synthesized and post translationally modified proteins (RiPPs), non-ribosomal peptide synthetase (NRPS), and acyl amino acids. Terpene and terpene precursor synthesis genes were detected in three BGCs in each of the two Rhizobiales MAGs and in a single BGC in the Burkholderiales MAG. A single RiPP-

like BGC was detected in each of the three MAGs. An acyl amino acid BGC was detected in each Rhizobiales MAG, while NRPS and NRPS-like BGCs were detected in one but not both of the Rhizobiales MAGs (Figure 8c). We also identified one antibiotic resistance gene, *qacG*, associated with resistance to quaternary ammonium compounds. The *qacG* gene was detected in both Rhizobiales MAGs (one hit each) but absent from the Burkholderiales MAG (Figure 8b). We did not screen the unbinned contigs for genes related to antibiotic biosynthesis, as these contigs were too short to detect complete BGCs.

Discussion

Colonization of externally brooded fish embryos by aquatic bacteria and other microbes likely occurs immediately after embryo deposition into the water. Many of these microbes likely have no effect on embryo health and development. But others may be beneficial or detrimental to the host (Fujimoto et al., 2018). Indeed, embryo mortality in aquaculture is frequently driven by microbial infection, particularly by *Saprolegnia* fungal species that affect a range of farmed fish (Bruno et al., 2011; Liu et al., 2014). Bacterial pathogens from the genera *Flavobacterium, Lactococcus*, and *Aeromonas* also contribute substantially to embryo mortality (Vendrell et al., 2006; Boutin et al., 2012; Meyburgh et al., 2017; Abdul Razak et al., 2019; Shahin, Veek, et al., 2022). Although embryo-associated microbiomes are hypothesized to protect hosts from such pathogens through antagonism, the specific taxa involved and mechanisms underlying these protective interactions remain poorly understood. The current study shows that channel catfish embryos associate with microbiomes distinct from those of the surrounding water and that betadine treatment shifts community structure (Figure 6), suggesting that both host-associated factors and management decisions (e.g., disinfection) shape these microbial communities.

Finally, metagenomic analysis identifies genes for antimicrobial biosynthesis, raising the possibility that embryo-associated microbiomes engage in antagonistic interactions, potentially against pathogenic microbes that could threaten embryo health.

The five most abundant embryo-associated ASVs belong to the Comamonadaceae, Flavobacteriaceae, Methylomonadaceae, Methylophilaceae, and Rhizobiaceae (Figure 7a). Comamonadaceae and Flavobacteriaceae are commonly reported in freshwater fish microbiomes, including those of brown trout (Wilkins et al., 2015), rainbow trout (Chiasson et al., 2023), and coho salmon (Romero & Navarrete, 2006). Comamonadaceae, the most prevalent and proportionately abundant family across all embryo samples, has been associated with healthy fish embryos following exposure to *Saprolegnia* fungi (Liu et al., 2014). In contrast, several members of the Flavobacteriaceae, particularly within the genus *Flavobacterium*, are well-known pathogens of fish and fish embryos, although this widespread family also contains non-pathogenic members (Verner-Jeffreys et al., 2006; Fujimoto et al., 2018; Tarnecki et al., 2019). Although betadine treatment shifted the composition of embryo microbiomes, the relative abundances of Comamonadaceae and Flavobacteriaceae stayed relatively constant with betadine exposure. This suggests that betadine does not substantially reduce the representation of potentially commensal or beneficial Comamonadaceae associated with embryos and may also be ineffective at decreasing potentially pathogenic Flavobacteriaceae. These results also indicate that shifts in other taxa, but not in Comamonadaceae and Flavobacteriaceae, drive the significant change in community-level composition from before to after betadine treatment.

Community-level compositional change with betadine was driven in part by shifts in ASVs assigned to Methylomonadaceae and Methylophilaceae. These families were enriched in water compared to embryo microbiomes but increased in embryo microbiomes from before to

after betadine treatment (Figure 7; Figure B1). Members of the Methylomonadaceae are methane-oxidizing bacteria that produce methanol, a substrate that can serve as a carbon and energy source for certain members of the Methylophilaceae (Deng et al., 2024), including some strains that oxidize methanol coupled to denitrification (Kalyuhznaya et al., 2009). These families have been detected in aquaculture systems (e.g., (Bruno et al., 2023)) but remain sparsely studied to determine their potential contribution to methane cycling in either the water or in association with embryos. Their enrichment post-betadine raises the possibility that antiseptic treatment may create opportunities, at least initially, for colonization of embryos by taxa common in the surrounding water.

ASVs belonging to the Rhizobiaceae, Xanthobacteraceae, and Sphingomonadaceae were enriched in all embryo microbiomes compared to water microbiomes (Figure 7b). The family Rhizobiaceae (order Rhizobiales) was the fifth most abundant family detected across all of our samples and is best known for its nitrogen-fixing members that engage in symbiosis with terrestrial plants (Wang et al., 2020). In contrast, the functional contributions of aquatic Rhizobiaceae are largely uncharacterized despite frequent detection of this family in diverse habitats, including marine water (Aoki et al., 2022) and host-associated microbiomes of corals (Quigley et al., 2020; Rosales et al., 2020; Dube et al., 2021) and freshwater fish (Ghosh et al., 2025). Rhizobiaceae prevalence in association with aquatic animals suggests potential roles in symbiosis, although the nature of these roles remains undetermined. The family Xanthobacteraceae also belongs to the order Rhizobiales and has been linked to organic matter degradation and partial denitrification in soil systems (Zhou et al., 2023; Martínez-Núñez & Orozco-Ramírez, 2024), although little is known of Xanthobacteraceae physiology in aquatic environments. The family Sphingomonadaceae is widespread, commonly occurring in

freshwater, marine, and engineered systems, including recirculating aquaculture systems (Bartelme et al., 2019) and the microbiomes of farmed *Cynoglossus semilaevis* (Chinese tongue sole) and *Litopenaeus vannamei* (Whiteleg shrimp), as well as those of marine teleost fish gills (Pratte et al., 2018; Moschos et al., 2022). The consistent presence of these ASVs before and after betadine treatment suggests that these taxa may be capable of rapid regrowth or colonization or are overall more resilient to disturbance compared to other microbiome members. Their presence post-betadine may be due to competitive advantages linked to biochemical changes in the embryo-associated biofilm after treatment, or other unexplored factors that promote persistence. Additional ASVs enriched in embryos compared to water included members of Gemmatimonadaceae, Burkholderiaceae, Deinococcaceae, Terrimicrobiaceae, and Mycobacteriaceae, suggesting broad diversity in a potential embryo-specific niche (Figure 7b).

We hypothesize that externally brooded embryos could benefit from chemical transport or antipathogen functions encoded by microbiomes. This motivated us to screen metagenome contigs (~90,000 total unbinned contigs) for genes linked to resistance to or production of potentially harmful chemicals. We detected a diversity of genes encoding ABC transporters, a broad class of membrane proteins that import and export a wide array of molecules such as sugars, lipids, xenobiotics, and antimicrobial compounds (Greene et al., 2018; Ahmad et al., 2020), as well as multidrug efflux pumps known to remove toxins, including antibiotics (Figure 8; (Du et al., 2018)). Screening of unbinned contigs also detected genes linked to antimicrobial functions, including genes encoding methyltransferases, β-lactamases, and isochorismatases (Figure 8). Methyltransferases are known to participate in both antibiotic synthesis and resistance (Atkinson et al., 2013; Niikura et al., 2018). For example, the methyltransferase *GenN* is required for synthesis of the aminoglycoside antibiotic gentamicin, whereas other methyltransferases

confer resistance by modifying rRNA to block antibiotic binding, an important defense against bacteriostatic macrolides and aminoglycosides (S. Li et al., 2018). β-lactamases also confer resistance, specifically by hydrolyzing β-lactam antibiotics (Botts et al., 2023; Lin et al., 2025). Isochorismatases are also hydrolytic, exhibiting γ-lactamase activity that can protect cells from γ-lactam antibiotics (Guo et al., 2022). Detection of these enzyme classes suggests that embryo-associated microbiomes harbor antibiotic synthesis and resistance capabilities, potentially facilitating the persistence of a protective microbiome.

Our metagenomic analysis also involved binning contigs into metagenome-assembled genomes (MAGs), followed by screening of MAGs for antimicrobial synthesis or resistance genes. Binning recovered two MAGs from the order Rhizobiales and one from the order Burkholderiales, all of which contained biosynthetic gene clusters (BGCs) for producing terpene and terpene-precursors (Figure 8c). Terpenes are made by microbes and plants and have antimicrobial functions such as disrupting cell membranes, inhibiting efflux pumps, and enhancing the effects of existing antibiotics (Mahizan et al., 2019; Dias et al., 2022). While some plant-derived terpenes are known to inhibit growth of the fungal fish pathogen *Saprolegnia* spp. (Mostafa et al., 2020), evidence for bacterially produced terpenes with activity against fish pathogens is lacking. However, members of the bacterial family Sphingomonadaceae, which were enriched in embryo compared to water microbiomes (Figure 7), are known terpene producers (Wang et al., 2022). This family was not recovered in our MAG data but may warrant further attention, along with Rhizobiales and Burkholderiales, as potential contributors to terpene synthesis in embryo microbiomes. All three MAGs also contained BGCs for synthesizing RiPPs (Figure 8c), a class of peptides with diverse antimicrobial activities (Arnison et al., 2013). Many common and well characterized bacterial genera, including soil-associated *Streptomyces* and

Bacillus and gut-associated *Streptococcus* and *Enterococcus*, are capable of producing RiPPs (Skinnider et al., 2016); deeper sequencing of the embryo microbiome may therefore recover other taxa linked to RiPP synthesis.

Other genes associated with chemical-based antagonism were also detected in the MAG-based analysis. BGCs for acyl amino acids were detected in both of the Rhizobiales MAGs. Acyl amino acids—composed of a fatty acid linked to an amino acid—are produced by diverse microbes and associated with biofilm formation, immune evasion and antibiotic resistance (Figure 8b; (Brady & Clardy, 2000; Krishnamurthy et al., 2023)). Further, one of the Rhizobiales MAGs contained BGCs for non-ribosomal peptide synthetase (NRPS) or NRPS-like complexes. NRPSs are known to produce diverse bioactive molecules including well known antibiotics and antifungals such as penicillin, cyclosporine, and vancomycin (Figure 8b; (Yu et al., 2017; Iacovelli et al., 2021)). Notably, vancomycin is considered a "last resort" antibiotic that can enter aquatic environments through agricultural and pharmaceutical runoff (Boneca & Chiosis, 2003; Hricová et al., 2021; Shen et al., 2021). Consistent with our MAG-based finding of NPRS BGCs, the unbinned embryo metagenome contigs contained three genes encoding vancomycin resistance (*vanW*; Figure 8b), indicating that while some microbes may produce NRPS-derived antibiotics like vancomycin, others in the community possess the genetic capacity to withstand NRPS attack. Similarly, well characterized genes benzalkonium chloride resistance, specifically *qacJG* and *qacG*, were detected in both of the Rhizobiales MAGs, as well as in the unbinned contigs (Figure 8b**)**. Benzalkonium chloride is a widely used disinfectant in aquaculture, with exposure linked to impaired embryogenesis and developmental abnormalities in farm-raised fish (Mougin et al., 2023; Mukai et al., 2024). The ability of embryo-associated microorganisms to mitigate or withstand these chemical stressors may help protect developing embryos.

The occurrence of diverse genes involved in chemical transport, antimicrobial resistance, and antimicrobial synthesis within the catfish embryo metagenome suggests potential mechanisms for microbially mediated host protection. Mechanisms of antibiotic resistance and chemical transport may allow the microbiome to withstand attack from toxins, potentially including those produced by pathogens or entering the system from anthropogenic sources. In contrast, antimicrobial production by the embryo microbiome could directly inhibit fish pathogens or otherwise shape the make-up of the community so as to favor the persistence and growth of beneficial taxa. Our data indicate that antagonistic microbe-microbe interactions may be intrinsic to the embryo-associated microbiome, as is true for most host-associated and biofilm microbial communities. The exact participants in and outcomes of these interactions remain to be determined. However, our survey identifies taxa enriched in the embryo-associated niche and stable in response to chemical (betadine) stress. Additional study is needed to test if and how these taxa influence the health of externally brooded catfish embryos.

Limitations

This preliminary study has limitations. We were not able to obtain metagenomes from water samples or other aquatic biofilms, thereby preventing comparison to determine if defense-related functions in embryo-associated microbiomes are proportionately enriched relative to surrounding microbiomes or other biofilm systems. Further, the metagenomic dataset includes a low number of embryo samples, all of which contained high proportions of catfish-derived sequences (roughly 80% of total sequences). This relative lack of microbial data made it challenging to recover microbial MAGs with high completeness. Because embryos burst during the DNA extraction process, we cannot rule out the possibility that microbial DNA originated from within the embryos themselves. However, we have no evidence that microbes reside internally in fish embryos, and we consider this possibility to be unlikely.

Further, our sampling did not include non-betadine treated embryos sampled at the exact same developmental state as the embryos we sampled after betadine treatment. We therefore cannot rule out the possibility that microbiome changes observed pre-to-post betadine were not driven by factors other than betadine treatment (e.g., physiological changes associated with host development over time). However, we consider betadine to be the major and most likely cause of microbiome change. Betadine disinfection is a standard biosecurity practice in commercial and research hatcheries to prevent the introduction and spread of fungal and bacterial pathogens. Untreated eggs are not introduced into hatchery facilities due to the associated biosecurity risks and are therefore not representative of production systems. Accordingly, this study was not designed to robustly evaluate the effects of betadine treatment on embryo-associated microbiomes, but rather to characterize microbial communities under conditions that reflect standard hatchery operations.

While our questions relating to microbial functions focused primarily on those related to antagonistic microbe-microbe interactions, other microbial processes (e.g., nutrient cycling, waste removal, and mucosal maintenance) may also be of benefit to developing embryos and should be explored in future studies. Such studies could evaluate the extent to which microbiome functions are expressed and influence the host system, for example by comparing embryo development, growth, and pathogen resistance in the presence versus absence of a microbiome (e.g., using antibiotics to suppress or remove the microbiome).

Conclusions

Embryo-associated microbiomes remain relatively underexplored in vertebrate systems, including those of fishes with external brooding behavior. In such systems, embryos are continuously in contact with microorganisms and potential chemical stressors from surrounding water. We show that channel catfish embryo microbiomes are distinct from those of surrounding water and potentially susceptible to disturbance by antiseptic application. All embryo microbiomes shared dominant taxa Comamonadaceae and Flavobacteriaceae, with members of the Rhizobiaceae, Xanthobacteraceae and Sphingomonadaceae appearing to be stable components of the microbiome before and after betadine treatment. Preliminary metagenomic analysis identified genetic potential for diverse antagonistic interactions in the embryo microbiome, raising the hypothesis that such interactions could influence the success of microbiome members, potentially including pathogens. It remains to be determined if and how the embryo-associated microbiome contributes to fish health, with the current results identifying taxa and defense-related functions that may be targets for future study.

CHAPTER FOUR

HOST IDENTITY SHAPES ENDOZOICOMONAS COMPOSITION IN CORAL MICROBIOMES ACROSS DEPTHS AND MARINE PROTECTION AREAS AT LITTLE CAYMAN ISLAND

Contribution of authors and co-authors

Manuscript in Chapter 4
Author: Madelaine L. Brown
Contributions: devised and performed all bioinformatic analyses, wrote manuscript

Co-author: Andrew J. Maritan
Contributions: performed sampled collections, performed sample extraction and preparation, contributed to manuscript

Co-author: Gretchen Goodbody-Gringley
Contributions: designed field collections, collected samples, contributed to manuscript

Co-author: Matthew L. Doherty
Contributions: designed field collections, performed sampled collections, contributed to manuscript

Co-author: Alex D. Chequer
Contributions: designed field collections, performed sampled collections, contributed to manuscript

Co-author: Frank J. Stewart
Contributions: advised on bioinformatic analyses, wrote manuscript

Co-author: Zoe A. Pratte
Contributions: formulated guiding hypothesis, designed field collections, performed sampled collections, performed sample extraction and preparation, advised on bioinformatic analyses, wrote manuscript

Manuscript Information

Madelaine L. Brown, Andrew J. Maritan, Gretchen Goodbody-Gringley, Matthew L. Doherty, Alex D. Chequer, Frank J. Stewart, Zoe A. Pratte

Coral Reefs
Status of Manuscript: [Double click to put an X in one of the options below, then delete instruction in brackets. If you cannot double click to add an X, search for "Check Box Content Control."]
☐ Prepared for submission to a peer-reviewed journal
☐ Officially submitted to a peer-reviewed journal
☒ Accepted by a peer-reviewed journal
☐ Published in a peer-reviewed journal

Coral Reefs

Abstract

The bacterial genus *Endozoicomonas* is prevalent and abundant in the microbiome of many corals, but the combination of environmental and host factors shaping its distribution remains uncertain. In this study, we used 16S rRNA gene sequencing to examine the distribution of *Endozoicomonas* across hard (*Porites astreoides*, *Montastraea cavernosa*, *Agaricia* spp.) and soft coral (*Muricea* spp., Gorgoniidae, Cladiellidae, Malacalyconacea) microbiomes across a depth gradient from shallow (4.5 m) to upper mesophotic zones (36.6 m) at four sites around the Little Cayman Island, including two marine protected areas. *Endozoicomonas* was abundant across all coral microbiomes, with soft corals exhibiting near-total dominance (relative abundance range 0.1–99%, median 55.3%) by this genus and notable abundances (range 0–96%, median 22.2%) in hard corals. *Endozoicomonas* composition was strongly structured by coral host identity across sites and depths, with each coral type harboring two to four unique *Endozoicomonas* amplicon sequence variants. Site and depth also contributed to *Endozoicomonas* abundance in soft and hard corals, respectively, where relative abundances were highest at unprotected sites in soft corals and in shallow to mid photic zones in hard corals. These findings suggest that *Endozoicomonas* abundance is shaped by both coral host identity and local environmental conditions.

Introduction

Corals rely on relationships with a microbiome of bacteria, protists, fungi, archaea, and viruses to maintain health and support growth (Bayer et al., 2013; Pollock et al., 2018; Caughman et al., 2021; Beatty et al., 2022). Coral microbiomes can influence host immune function, provide protection against pathogens, maintain mucus, and affect host nutrient

absorption (Louis & Flint, 2009; Neish, 2014; Scully et al., 2014; Futo et al., 2015; King et al., 2016; Zheng et al., 2020; Zhu et al., 2021). Specifically, coral microbiomes have been shown to aid in nitrogen fixation, carbon cycling, and the production of antibiotics and vitamins (Bayer et al., 2013; Neave et al., 2017; Pogoreutz et al., 2018). Some coral microbiomes can be dominated by a single bacterial genus, *Endozoicomonas*, from the class Gammaproteobacteria (Morrow et al., 2012; Bayer et al., 2013; Apprill A, 2016; Brener-Raffalli et al., 2018; Pogoreutz et al., 2018; McCauley et al., 2020), which may comprise over 90% of 16S rRNA gene sequences (Vezzulli et al., 2013). While dominance by a single microbe is often associated with disease and dysbiosis in other systems (Vangay et al., 2015; Levy et al., 2017; Kriss et al., 2018), this association is not universal (Lozupone et al., 2013; Srinivasan et al., 2015; Williams et al., 2016; Fernandes et al., 2023) and may not apply to coral microbiomes. In corals, *Endozoicomonas* is present across the coral's mucus, tissue, and skeletal layers, has a strong association with healthy coral microbiomes, and is hypothesized to be a symbiont that provides B vitamins, amino acids, and carbohydrates to the host, or may remove toxic organic sulfur compounds (Bythell, 2011; Bayer et al., 2013; Meyer et al., 2014; Neave et al., 2016; Neave et al., 2017; Pollock et al., 2018; Sweet M, 2021; Pogoreutz et al., 2022). While the exact mechanisms underlying the coral-*Endozoicomonas* relationship remain unclear and potentially variable among hosts and environments, studies repeatedly report high relative abundances of *Endozoicomonas* in healthy corals, and in some cases, in diseased or stressed corals, indicating they likely play important roles in coral health (Bayer et al., 2013; Meyer et al., 2014; Neave et al., 2016; Beatty et al., 2018). The relationship between hard corals and *Endozoicomonas* is better characterized than in soft corals, underscoring a gap in our understanding of the diversity and distribution of this symbiont within soft coral microbiomes and its impact on coral persistence. Moreover, it remains

unclear whether distinct *Endozoicomonas* strains are host-specific or whether a core set of strains is shared broadly across both hard and soft corals.

Distinct shifts in coral reef communities occur between shallow and mesophotic waters. Mesophotic reefs experience cooler temperatures, reduced light penetration, and altered oxygen and nutrient conditions compared to shallow systems (Kahng et al., 2017; Carpenter et al., 2022; Doherty et al., 2024; Slattery et al., 2024; Goodbody-Gringley & Chequer, 2025). These mesophotic coral ecosystems have gained increasing attention as potential refugia for shallow-water corals facing ocean warming and other climate stressors (Doherty et al., 2024; Goodbody-Gringley & Chequer, 2025). Coral disease prevalence, including bleaching, often decreases with increasing depth (Pérez-Rosales et al., 2022; Goodbody-Gringley & Chequer, 2025). Although several coral species span both shallow and mesophotic zones, the factors driving their success at depth remain uncertain. One potential determinant is the coral's symbiotic relationship with *Endozoicomonas*, which is best characterized in shallow, warm-water corals (Morrow et al., 2012; Bayer et al., 2013; Vezzulli et al., 2013; Meyer et al., 2014; Neave et al., 2016; Pogoreutz et al., 2018; McCauley et al., 2020). In contrast, the abundance and function of *Endozoicomonas* in deeper, low-light environments remains poorly understood. Additionally, it is unclear how *Endozoicomonas* strain diversity changes along reef gradients, and how host-specification plays a role. Corals near or below the euphotic zone may host fewer or no symbiotic zooxanthellae and are subject to markedly different light , oxygen , temperature, and nutrient conditions than those in near-surface waters (Lesser et al., 2009; Laverick et al., 2017; Goodbody-Gringley, 2018; Hebbeln et al., 2020; Kellogg & Pratte, 2021; Carpenter, 2022; Candy et al., 2023; Doherty et al., 2024). These environmental gradients can shape the composition and function of coral-associated microorganisms (Franco et al., 2021; Aalto et al., 2022; Pratte et al., 2023),

including *Endozoicomonas* (Galand et al., 2020). However, studies examining *Endozoicomonas* in deep-water corals are limited. For example, a single study detected *Endozoicomonas* in deep-water corals, with relative abundances ranging from 13–88% of 16S rRNA gene sequences in *Desmophyllum pertusum* to just 0.13% in *D. dianthus* at 1300 m (Kellogg & Pratte, 2021). These results suggest a role for *Endozoicomonas* in deep-water corals but also suggest that this role is dynamic across individuals and hosts. The factors shaping this dynamism are unclear.

Variability in *Endozoicomonas* abundance and prevalence may also be shaped by reef protection status. Efforts to mitigate climate change-driven impacts on coral reef ecosystems include the establishment of marine protected areas (MPAs), through fishing restrictions, no-take zones, and limits on destructive gear use to promote reef recovery and resilience (Soler et al., 2015; Cinner et al., 2016; Strain et al., 2019). While MPAs can reduce direct anthropogenic pressures, their effectiveness in restoring coral health remains mixed. Some studies report no measurable increase in coral biomass between protected and unprotected reefs (Coelho & Manfrino, 2007; Toth et al., 2014; Cox et al., 2017), whereas others document notable gains in coral cover within no-take zones (Bellwood et al., 2004; Mumby et al., 2006; Wright, 2022). Furthermore, coral microbiomes within MPAs have exhibited enhanced resistance to pathogens such as *Vibrio coralliilyticus* (Beatty et al., 2019), likely reflecting greater environmental stability, reduced macroalgal overgrowth, and fewer physical injuries from fishing gear (Mumby et al., 2007; Mumby & Harborne, 2010; Lamb et al., 2015). Notably, *Endozoicomonas*, a dominant and potentially beneficial coral symbiont, has been found at high abundances in corals from both protected and unprotected reefs (van de Water et al., 2017; Beatty et al., 2018; Kellogg & Pratte, 2021; Beatty et al., 2022). Whether protection status consistently influences

Endozoicomonas abundance or strain diversity remains unresolved, highlighting the need to clarify its role in coral microbiome stability and host resilience.

This study provides a preliminary exploration of how *Endozoicomonas* is structured within the microbiomes of hard and soft corals across a depth gradient and varying levels of marine protection along the coast of Little Cayman Island, Caribbean Sea. Little Cayman is characteristically less populated than many tropical reefs, limiting influence from coastal tourism and preserving more natural reef communities (Doherty et al., 2025). Four sites around Little Cayman Island were selected based upon protection status and accessibility: two MPAs (Crystal Palace Wall and McCoy's Wall) and two non-MPAs (Ron's Rock and The Edge). While neither Ron's Rock nor The Edge is protected, Ron's Rock does not permit fishing, making it more protected than The Edge, which is a designated line-fishing zone. At each site, hard and soft corals were sampled along depth gradients from shallow (4.5 m) to upper mesophotic (36.6 m) reefs at 4.5 m intervals. Using 16S rRNA gene sequencing, we analyzed the microbiomes of three hard corals (*Porites astreoides*, *Montastraea cavernosa*, and *Agaricia* spp.) and four soft corals (*Muricea* spp., Gorgoniidae, Cladiellidae, and Malacalyconacea). We hypothesize that *Endozoicomonas* will be abundant across all coral microbiomes, but that relative abundance and strain composition will differ between hard and soft corals. We further predict that both abundance and composition may vary with reef protection status and depth.

Methods

Study Site

The Cayman Islands lie in the western Caribbean, about 200 miles northwest of Jamaica and 150 miles south of Cuba, and comprise Grand Cayman, Cayman Brac and Little Cayman. Little Cayman (Figure 9a) is the smallest and least developed, ~80 miles northeast of Grand Cayman, with an area of ~26 km² and ~160 residents. The shoreline is 45 km and 74.2% of it is designated as marine protected areas, including ~57% under full no take protection. The island is encircled by a fringing reef that transitions from spur and groove to a fore reef wall exceeding 2000 m (Doherty et al., 2024; Doherty et al., 2025).

Mapping Methods

The basemap was sourced from the Cayman Islands Department of Environment's *Little Cayman Public Mooring Map* (Cayman Islands Department of Environment 2023). Coastline, bathymetry, and sample sites were traced in Photoshop (Adobe, California) and finalized in Illustrator (Adobe, California; Figure 9a).

Sampling and Data Collection

To characterize the taxonomic diversity and percentage abundance of *Endozoicomonas* in coral-associated microbiomes spanning a range of environmental protection zones and water depths, we sampled hard and soft corals from Little Cayman Island over ten days between 17 August to 6 September 2022 (samples were not taken over consecutive days; Figure 9). The sites included two MPAs, Crystal Palace Wall and McCoy's Wall, and two non-MPA sites, Ron's Rock and The Edge (Figure 9a). At each site, coral sampling occurred along a depth gradient from 4.5 to 36.6 meters at about 4.6-meter intervals resulting in eight different depths (Figure

9b). Hard corals collected were *Montastraea cavernosa*, *Porites astreoides,* and *Agaricia* spp. (Figure 9c). Because *Agaricia* species in the Caribbean are particularly difficult to distinguish visually, it is not uncommon to group them to avoid inaccuracies as we did for this study (Crandall et al., 2016; Doherty et al., 2025). Due to lack of species descriptions as well as distinguishing characteristics, soft corals are also difficult to identify visually in the field. Therefore, we collected visually similar soft corals from each site and molecularly resolved the taxonomy into groups post collection (see "Coral identification" below). Soft coral groups collected included *Muricea* spp., Gorgoniidae, Cladiellidae, and Malacalyconacea. Not all hard coral and soft corals were found at every depth across all four sites, and not all samples passed quality control (sampling sites, coordinates, and depths summarized in Tables 1, and Tables C1 and C2).

All samples were collected via SCUBA. Hard coral mucus was sampled by gently abrading coral tissue with the tip of a 5 mL syringe and capturing released mucus with the syringe, representing the coral mucus-associated microbiome. Soft coral polyps were collected in a sterile wire-closure sample bag (Whirlpak, Madison, WI, USA), representing coral tissue- and mucus-associated microbiomes. Seawater (125 mL) was collected in a sterile needleless syringe and filtered upon surfacing on all but one collection days. Water samples were collected from all four sites between August 17 and September 6, 2022. Water samples from Crystal Palace Wall (n = 5) were taken on August 22, 29, and September 6; from McCoy's Wall (n = 5) on August 18, 30, and September 6; from Ron's Rock (n = 4) on August 17 and September 1; and from The Edge (n = 4) on August 23 and September 2. At each site, water samples were collected at depths of 9.1, 18.3, 22.7, and 36.6 m, except at The Edge, where the shallowest depth sampled was 4.5 m.

All samples were returned to the boat upon surfacing, where coral samples were placed on ice, and water samples were immediately filtered on to a 0.2 µm pore size isopore filter (Millipore). Samples were preserved in an RNA/DNA preservation buffer (25 mM sodium citrate, 10 mM EDTA, 5.3 M ammonium sulfate, pH 5.2 (Pratte Z.A., 2017)) within 20 minutes of returning to the boat. At the field station, all samples were frozen at -20˚C.

DNA Extractions and Sequencing

Genomic DNA was extracted from soft coral tissue and mucus, hard coral mucus, and filters with the DNeasy PowerSoil Pro extraction kit (Qiagen, Hidalgen, Germany). Briefly, coral samples or filters were placed in the provided PowerSoil bead beating tubes and homogenized. The sequential extraction steps followed the manufacturer's protocol. Three extraction blanks (samples with RNA/DNA preservation buffer and no sample) were processed using the same protocol. DNA was quantified using the Qubit 2.0 (Invitrogen). Polymerase chain reaction (PCR) amplification of the V4 region of the 16S rRNA gene was conducted using primers 515F and 806R with an Illumina adapter overhang for each primer (Caporaso et al., 2011; Altman-Kurosaki et al., 2025). PCR reactions included 2–5 µl template DNA, 0.25 µl of each forward and reverse primer, 0.5 µL bovine serum albumin (BSA, 20 mg/ml; New England BioLabs Inc), 12.5 µl GoTaq Green Master Mix (Promega, USA), and sterile nuclease-free water to bring the total volume to 25 µl per sample. Negative controls (PCR reaction solution but no DNA) were included to represent each mixture of PCR master mix. PCR conditions were as follows: initial denaturation at 94°C for 1 min, 30 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 90 s, and a final extension at 72°C for 10 min. V4 16S rRNA gene amplification was confirmed with gel electrophoresis for all samples, and PCR negative controls displayed no bands. A second PCR amplification was conducted to append Illumina barcodes and adapters to amplicons as

previously described in Altman-Kurosaki et al. 2025. Amplicons were sequenced at the Georgia Genomics and Bioinformatics Core, Athens, GA, on a MiSeq (Illumina Inc., San Diego, CA, USA) using a V2 500-cycle kit (250 X 250 bp) with 5% PhiX to increase read diversity.

Microbiome Data Processing and Analysis

All 16S rRNA gene sequences were analyzed using the DADA2 (v1.22.0; (Callahan et al., 2016)) and QIIME2 (v2-2022.11; (Bolyen et al., 2019)) pipelines. Raw sequences were demultiplexed, quality filtered, trimmed (30 bp removed from the 5` end), truncated (200 bp), and denoised using DADA2, which models and corrects Illumina sequencing errors, removes chimeras, and resolves high-resolution amplicon sequence variants (ASVs). ASVs were then aligned with MAFFT (v7.508; (Price et al., 2010)), and phylogeny was estimated with FastTree (v2.1.11; (Paleczny et al., 2015)). Next, all ASVs were assigned taxonomy using the SILVA-132 database (Quast et al., 2013). ASVs were first filtered to remove any sequences identified as chloroplast and mitochondria. Then, all ASVs that were (on average) more abundant across all blanks and negative controls compared to coral and water samples were also removed (Altman-Kurosaki et al., 2025). After rarefication to 23,000 sequences, the final dataset consisted of 309 (159 hard coral, 132 soft coral and 18 water) samples and 34,885 ASVs. For hard coral types, there were 60 *Agaricia* spp., 60 *M. cavernosa*, and 39 *P. astreoides* represented. For soft coral types, there were 24 Cladiellidae, 23 Gorgoniidae, 20 Malacalyconacea and 65 *Muricea* spp. represented. None of the negative controls passed quality filtering steps, validating our quality control approach.

Coral Identification

The 16S rRNA gene primers used in this study also bind the 12S rRNA gene of the coral mitochondrial genome. While 12S rRNA sequences are discarded for microbiome analysis, they can be used to identify the host coral (Chen et al., 2002; Shinzato et al., 2021). Assuming that host coral 12S rRNA gene amplicons would be abundant, we ranked the top 15 most abundant ASVs per dataset. Among this ASV subset, any ASV not identified as prokaryotic via the QIIME2 pipeline was considered a candidate host sequence and queried against the NCBI nt database using MegaBLAST (Chen et al. 2015). For each sample, the most abundant ASV with a top MegaBLAST match to coral 12S rRNA was assumed to represent the host mitochondrion. The annotation of the top matching reference sequence was then used to assign taxonomy to the host coral. This approach identified four soft coral groups representing one genus (*Muricea*), two families (Gorgoniidae and Cladiellidae), and one order (Malacalyconacea). Not all soft coral samples could be resolved to the genus level, and we note that our approach allows for multiple species per soft coral. Malacalcyonacea, Gorgoniidae, and Muricea all had three unique ASVs per group, two of which were nearly identical. Cladiellidae had four unique ASVs, two of which were nearly identical. This method was also used to validate hard coral field identification; however, we were not able to differentiate among *Agaricia* species using this method.

Statistical Analysis

Experimental variables tested were host identity (hard versus soft corals; different coral taxa within hard and soft corals), protection status (sampling site), coral reef depth (meters), and day of sample collection. To improve statistical power in depth-related analyses, individual sampling depths were grouped into broader depth categories: shallow photic (4.5 and 9.1 m), mid photic (13.7 and 18.3 m), deep (22.8 and 27.5 m), and upper mesophotic (32 and 36.6 m). This

categorical depth variable was used as an additional metadata factor in downstream analyses. Statistical analyses were conducted using RStudio (v2023.03.0+386; (Posit team, 2023)) to test for microbiome differences among hard and soft corals, protection status, and sampling depths (meters and categories). Pairwise differences in alpha diversity between samples were assessed using Wilcoxon rank-sum tests with Benjamini-Hochberg adjustment calculated from rarefied data and implemented with the ggpubr (v0.6.0; (Kassambara, 2025)), microbiomeutilities (v1.00.17; (Shetty & Lahti, 2020)) and phyloseq (v1.42.0; (McMurdie & Holmes, 2013)) R packages, with significant comparisons visualized using boxplots with ggplot2 (v3.5.2; (Wickham, 2016)). Beta diversity of hard and soft coral microbiomes by depth (meters and categorical) and site was assessed using the Bray-Curtis dissimilarity index calculated from rarefied data and visualized via non-metric multidimensional scaling (NMDS) plots using the phyloseq and ggplot2 R packages. Differences in community composition between sample sites and depth (meters and categorical) for hard and soft corals were tested using permutational multivariate analysis of variance (PERMANOVA, adonis2 from the vegan package (v2.6-4; (Oksanen, 2022)) with 999 permutations, and pairwise comparisons were conducted using the pairewiseAdonis package (v0.4.1; (Arbizu, 2017)) with Bonferroni-adjusted p-values. Homogeneity of group dispersions was evaluated using permutational multivariate analysis of dispersion (PERMDISP, betadisper and permutest from vegan) followed by pairwise comparisons with Tukey's HSD test. To evaluate how site and depth affected *Endozoicomonas* relative abundance, Kruskal–Wallis (*H*) tests (Kruskal & Wallis, 1952) were conducted separately for hard and soft corals, testing for effects of site, depth (meters and categorical), and site–depth combinations. Post-hoc pairwise comparisons were conducted using Wilcoxon rank-sum tests with Benjamini–Hochberg adjustment for multiple testing. To assess *Endozoicomonas*

ASV variation, we retained ASVs with a minimum relative abundance of 50% in at least one coral sample for visualization in *ggplot2*. Dominant ASVs were assigned taxonomic classifications using MegaBLAST, and matching *Endozoicomonas* reference sequences from NCBI nt+ were included in phylogenetic analyses. The most abundant ASVs and reference 16S rRNA gene sequences were aligned with MAFFT, trimmed with trimAl (Capella-Gutierrez et al., 2009), and a maximum-likelihood phylogenetic tree was inferred using IQ-TREE2 (Minh et al., 2020), which was visualized in iTOL (Letunic & Bork, 2007). All raw sequences are publicly available through the NCBI's SRA database under BioProject PRJNA1310611.

All computation was performed on the Tempest High Performance Computing System, operated and supported by University Information Technology Research Cyberinfrastructure (RRID:SCR_026229) at Montana State University, Bozeman, MT, USA (https://www.montana.edu/uit/rci/tempest/).

Results

Coral and water samples were collected from four sites at Little Caymen Island, including two MPAs (Crystal Palace Wall and McCoy's Wall), an unprotected zone with permitted line fishing (The Edge), and a zone with no protection designation (Ron's Rock; Figure 9a). At each site, hard and soft corals were sampled from eight depths comprising shallow photic (4.5 & 9.1 m), mid photic (13.7 & 18.3 m), deep photic (22.8 & 27.5 m), and upper mesophotic waters (32 & 36.6 m; Figure 9b). The three hard corals included in this study were *P. astreoides*, *M. cavernosa*, and *Agaricia* spp., while the four soft corals were *Muricea* spp., Gorgoniidae, Cladiellidae, and Malacalyconacea (Figure 9c). We were unable to assess how site and depth influenced microbiome structure within a specific type of hard or soft coral, as not all sampled

coral taxa were found at each site and depth. We therefore compared microbiome variation between the broad categories of hard versus soft corals within each site and depth (Table 1; Table C1 and C2). All water microbiomes differed significantly from coral microbiomes (alpha and beta diversity) and were therefore excluded from analyses (Figure C1).

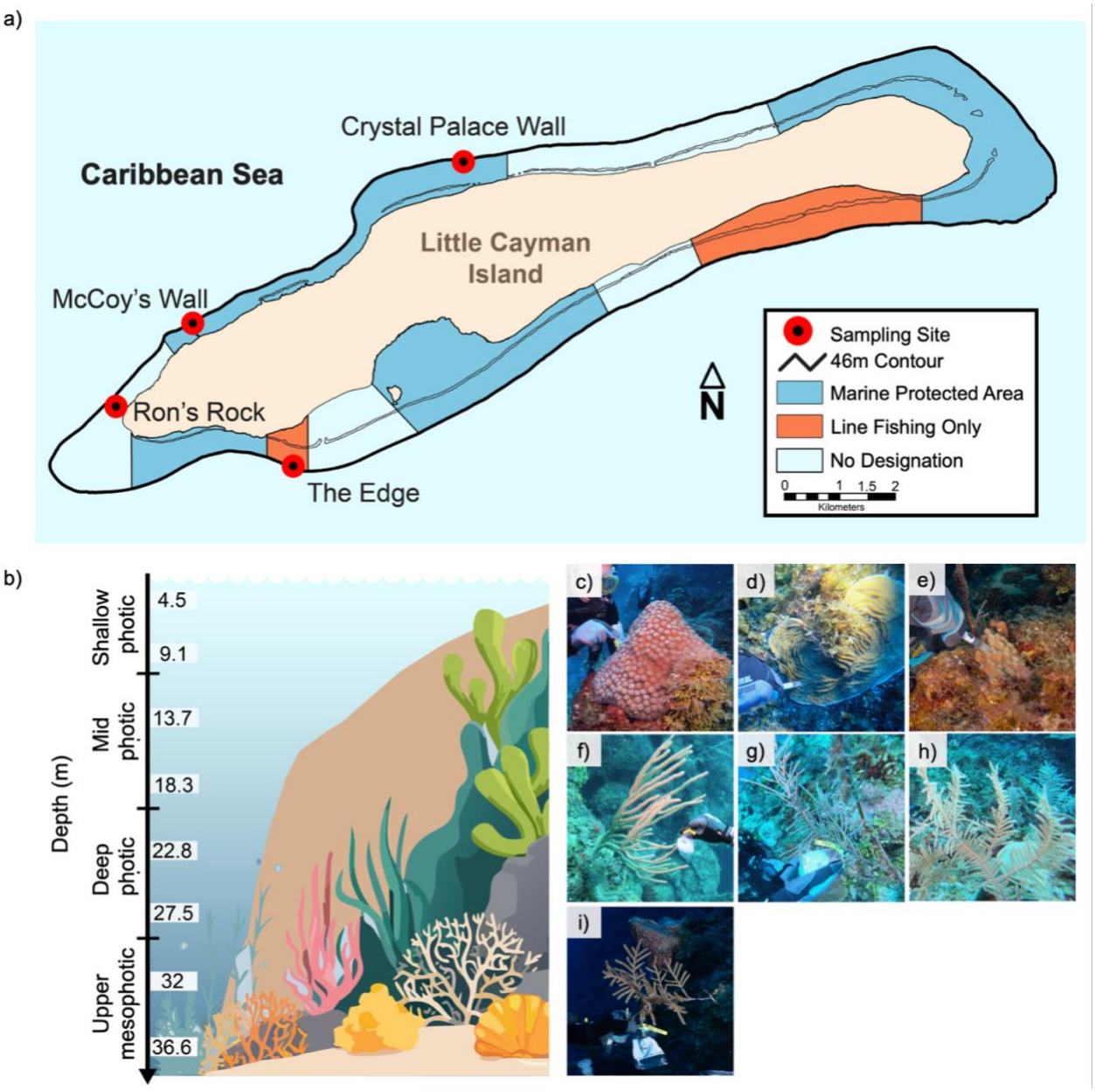

Figure 9: Sampling design for hard and soft coral collections around Little Cayman Island. a) Sampling sites (n = 4) differed in protection status at the time of collection, with Crystal Palace Wall and McCoy's Wall in a Marine Protected Area (MPA), The Edge in a line fishing-only zone, and Ron's Rock in an unprotected but no-fishing zone. The bolded line is the 46 m (~150 ft) depth contour. This map was adapted from the Cayman Islands Government, Departments of the Environment and of Lands and Survey. b) Schematic of sampling depths at all sites, with coral samples collected at 4.6 m (15 ft) intervals from 4.5 to 36.6 m. c-i) Representative hard (c) *Montastraea cavernosa*, d) *Agaricia* spp., e) *Porites astreoides*) and soft (f) *Muricea* spp., g) Cladiellidae, h) Gorgoniidae, i) Malacalcyonacea) corals sampled in this study. Photo Credit: Alex D. Chequer and Andrew J. Maritan.

Table 1: Hard and soft coral microbiome samples by protection status and depth.

Site	Depth (category)	Depth (m)	Number of hard coral samples	Number of soft coral samples
Crystal Palace Wall (MPA)	shallow photic	4.5	3	3
		9.1	3	3
	mid photic	13.7	3	3
		18.3	7	4
	deep photic	22.8	6	6
		27.5	5	4
	upper mesophotic	32	7	4
		36.6	5	4
McCoy's Wall (MPA)	shallow photic	4.5	4	8
		9.1	6	8
	mid photic	13.7	4	3
		18.3	8	4
	deep photic	22.8	7	5
		27.5	5	1
	upper mesophotic	32	9	5
		36.6	5	1
Ron's Rock (unprotected)	shallow photic	4.5	5	4
		9.1	2	5
	mid photic	13.7	6	6
		18.3	5	4
	deep photic	22.8	6	6
		27.5	3	3
	upper mesophotic	32	6	3
		36.6	4	4
The Edge (line fishing only)	shallow photic	4.5	3	3
		9.1	4	6
	mid photic	13.7	4	4
		18.3	5	5
	deep photic	22.8	4	3
		27.5	5	2
	upper mesophotic	32	5	3
		36.6	5	5

Coral Microbiome Structure is Driven by Host Identity

Microbiomes differed between hard and soft corals and among coral taxa within both host types. Overall, soft coral microbiomes had lower alpha diversity than hard corals, as measured by both observed features (ASVs) and Shannon diversity (Figure C1, Table C3 and

C4). Within the different soft corals, alpha diversity was lowest and of similar value in Malacalyconacea and *Muricea* spp., while Cladiellidae and Gorgoniidae had significantly higher alpha diversity (Figure 10a, Table C5 and C6). Microbiomes of all three hard corals harbored similar numbers of observed ASVs, but Shannon diversity was lower in *P. astreoides* and more characteristic of diversity in the soft corals Gorgoniidae and Cladiellidae (Figure 10a, Table C5 and C6). In our beta diversity analysis, all soft coral microbiomes clustered separately from hard coral microbiomes (Figure C1, Table C7) with some separation by host identity within both coral types (Figure 10b). Overall, soft coral microbiomes differed significantly in composition from hard coral microbiomes (PERMANOVA $p = 0.001$, Table C8) with some comparisons also having significant differences in dispersion (PERMDISP $p > 0.05$, Table C8). Specifically, between hard and soft corals, microbiome composition and dispersion differed between *Muricea* spp. and *P. astreoides*, and between Malacalyconacea and both *M. cavernosa* and *Agaricia* spp. (pairwise PERMDISP $p > 0.05$; Table C8). When comparing soft corals, microbiome composition differed significantly between all pairwise comparisons (PERMANOVA $p = 0.001$), with dispersion differing significantly between Malacalyconacea and Cladiellidae, and between Malacalyconacea and *Muricea* spp. (pairwise PERMDISP $p > 0.05$; Table C8). Conversely, all hard coral microbiomes were significantly different from one another, with no differences in dispersion (PERMANOVA $p = 0.001$, PERMDISP $p > 0.05$, Table S8).

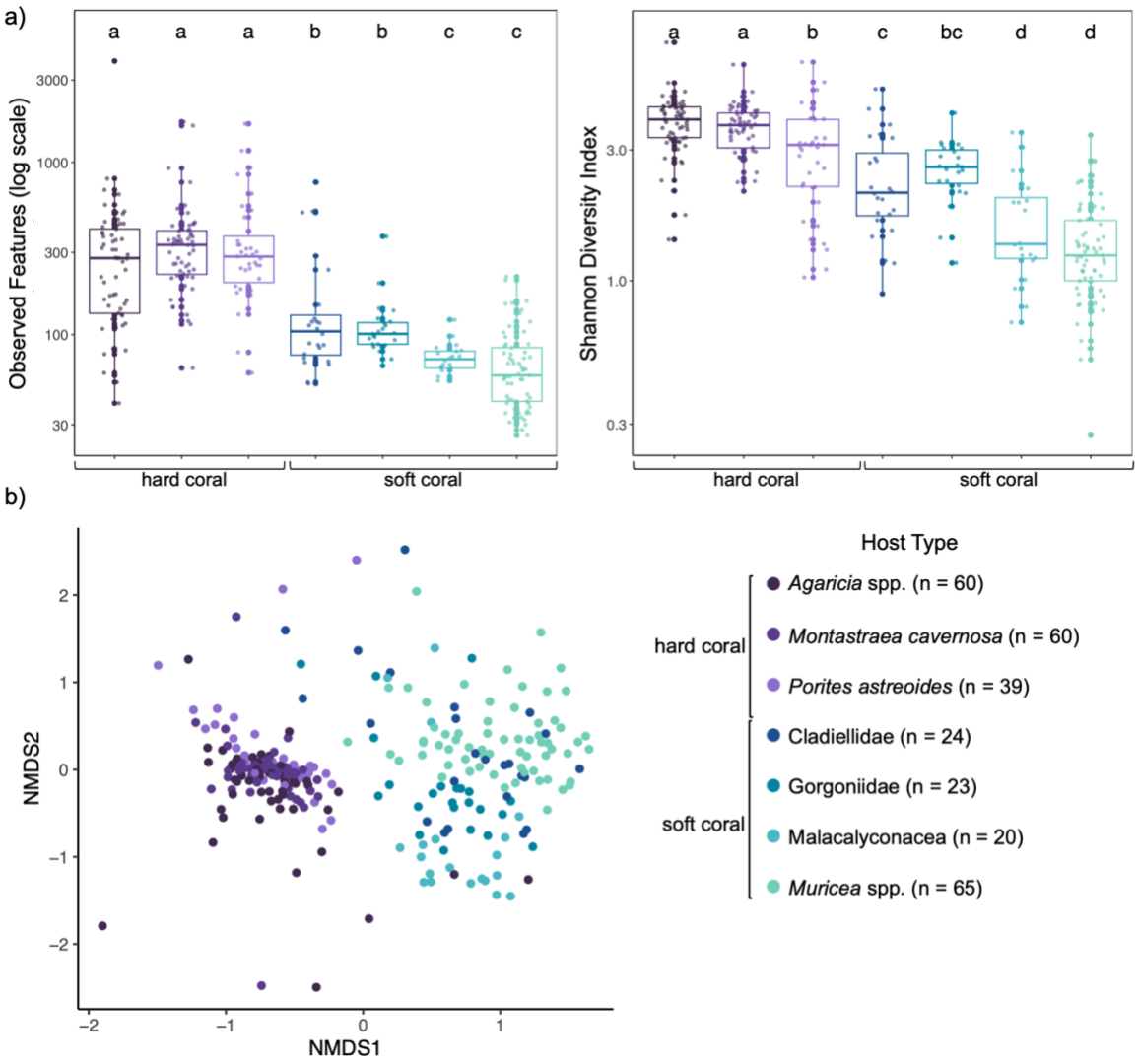

Figure 10: Hard and soft corals host distinct microbiomes. a) Alpha diversity, observed ASVs and Shannon diversity index, is higher in hard versus soft corals. Box plots show median (center line), and 25–75% quartiles (colored area in the box), minimum and maximum values (whiskers) and outliers (larger data points) and jittered data points. b) A NMDS plot (Bray-Curtis) shows differences in microbiome composition between hard and soft corals and between types of hard and soft corals with a stress score of 0.19. Pairwise PERMANOVA analyses are given in Table C8.

Endozoicomonas is More Abundant in Soft Coral Microbiomes

ASVs of the genus *Endozoicomonas* were >90X more common in soft versus hard coral microbiomes (55.3% vs. 0.6% median relative abundance, respectively), although this pattern was not universal across all types of hard and soft corals. For example, within hard corals, median *Endozoicomonas* abundance was 51.8% in *P. astreoides* but fell to 0.2% and 0.3% in *M. cavernosa* and *Agaricia* spp., respectively (Figure 11a). Within soft corals, median *Endozoicomonas* abundance ranged from 45.2% in Gorgoniidae to 76.5% in Malacalcyonacea (Figure 11a). In *M. cavernosa* and *Agaricia* spp., the most abundant bacterial taxon (14.6%–24% median relative abundance) was the family Cyanobiaceae, which was also abundant in water samples (20.6% median) but rare in *P. astreoides* and soft coral microbiomes (<4%; Figure C2).

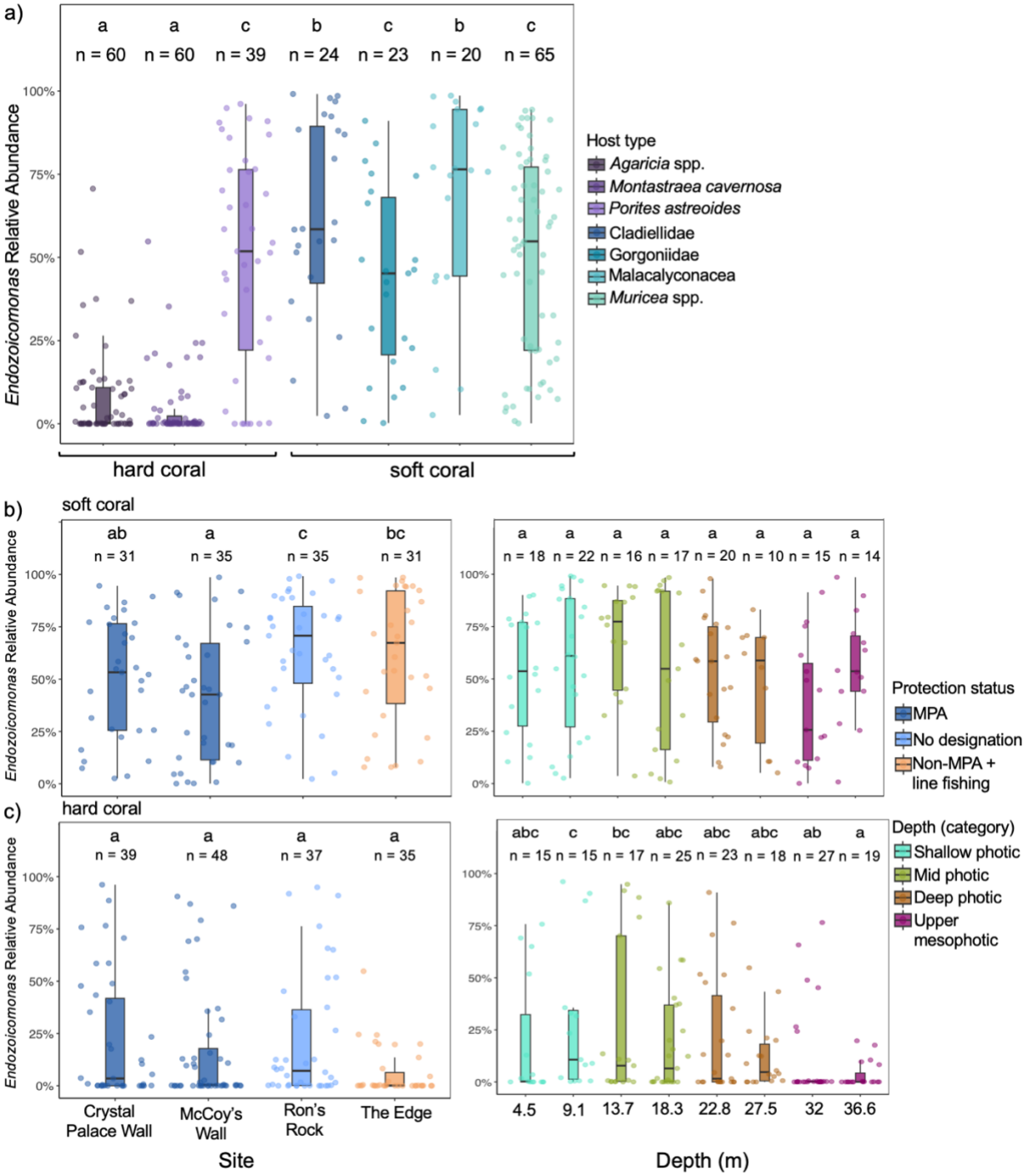

Figure 11: *Endozoicomonas* is most abundant in soft coral microbiomes and varies by site (soft coral) and depth (hard coral). Box plots show median (center line), and 25–75% quartiles (colored area in the box) with minimum and maximum values represented as whisker length and jittered data points. a) *Endozoicomonas* is more abundant in soft corals than in hard coral microbiomes. b) In soft corals, *Endozoicomonas* relative abundance varies significantly by site, but not depth (m). c) Hard coral microbiome *Endozoicomonas* relative abundance varies significantly by depth (m), but not site.

In soft corals, *Endozoicomonas* average relative abundance varied among the four sampling sites and was highest at Ron's Rock in an unprotected but no fishing zone (Figure 11b). Post-hoc pairwise tests revealed significant differences in *Endozoicomonas* relative abundance in soft coral microbiomes between Ron's Rock and Crystal Palace Wall (W = 349, adjusted p = 0.04), Ron's Rock and McCoy's Wall (W = 339, adjusted p = 0.007), and The Edge and McCoy's Wall (W = 363, adjusted p = 0.04; Table C9). *Endozoicomonas* relative abundance in soft corals did not vary when the data from all sites were binned by discrete sampling depth ((Kruskal–Wallis test, $H(7) = 9.27$, $p = 0.23$, n = 132) or by depth category (Kruskal–Wallis test, $H(3) = 5.04$, $p = 0.17$, n = 132; Figure 11b). This was also true when a potential effect of depth was evaluated independently for each site (Kruskal–Wallis test, $H(31) = 42.23$, $p = 0.09$, n = 132; Figure C3). *Endozoicomonas* abundance did not vary with sampling day when evaluated for different depths and sites (Kruskal–Wallis test, $H(34) = 44.0$, $p = 0.12$, n = 132).

In hard corals, *Endozoicomonas* relative abundance did not differ among sites (Kruskal–Wallis test, $H(3) = 6.38$, $p = 0.09$, n = 159) but, in contrast to the pattern in soft corals, differed significantly when the data from all sites were binned by sampling depth (Figure 11c). Abundance was highest in the shallow photic and mid photic depths but dropped considerably in the upper mesophotic hard coral microbiomes. Post-hoc pairwise tests revealed significant differences in *Endozoicomonas* relative abundance between 9.1 and 32 m (W = 69, adjusted p = 0.01), 9.1 and 36.6 m (W = 45, adjusted p = 0.01) and 13.7 and 36.6 m (W = 258, adjusted p = 0.02; Figure 11c, Table C10). Similar patterns were observed when comparing depth categories, with significant differences between shallow photic and upper mesophotic zones and between mid photic and upper mesophotic zones (W = 424.5, adjusted p = 0.013 and W = 557.5, adjusted p = 0.004, respectively; Table C11). However, similar to the pattern in soft corals, depth was not

a significant structuring variable when the data were evaluated independently for each site (Kruskal–Wallis test, $H(31) = 41.21$, $p = 0.10$, n = 159; Figure C3). As in soft corals, *Endozoicomonas* abundance in hard corals did not vary with sampling day when evaluated for different depths and sites (Kruskal–Wallis test, $H(37) = 48.7$, $p = 0.10$, n = 159).

Endozoicomonas ASV Diversity Depends on Host Identity

We detected 1423 *Endozoicomonas* ASVs across all datasets; however, none were present in every sample or host type, even at small relative abundances (≥1%). We, therefore, focused on the 25 ASVs that reached ≥50% relative abundance in at least one sample. ASV composition was structured by host identity with no clear patterns related to sampling site or depth for both hard and soft corals (Figure 12; Figure C4 and C5). Additionally, ASVs partitioned into predominantly hard coral or soft coral-associated variants, with only five ASVs (2, 5, 9, 10, and 14) detected in both coral groups, typically being more prevalent in one coral type than the other. None of these shared ASVs were detected in all samples, and no two samples, within either hard or soft corals, shared the same assemblage of *Endozoicomonas* ASVs (Figure 12).

Figure 12: *Endozoicomonas* diversity is determined by coral identity. ASVs identified as *Endozoicomonas* with a relative abundance ≥50% in at least one coral sample were plotted and organized by host identity. *Endozoicomonas* ASVs 1, 3 and 4 are the most abundant hard coral microbiomes, while ASVs 2, 5–8, and 11 are the most abundant in soft corals. Many ASVs are only present in a single sample with almost 100% relative abundance. This pattern is more common in hard corals than soft corals.

Soft coral microbiomes exhibited higher *Endozoicomonas* diversity than hard corals with double the *Endozoicomonas* ASV Shannon diversity (0.843 vs. 0.403; Wilcoxon rank-sum test, $p < 0.001$) and more than triple the observed ASV richness (15.78 vs. 4.87; Wilcoxon rank-sum test, $p < 0.001$). Each coral group contained characteristic abundant ASVs—such as ASV1 in hard corals and ASV2 in soft corals—and each host type also possessed abundant ASVs largely absent from other hosts (Figure 12). Hard corals frequently showed near-complete dominance by a single ASV, though the dominant variant differed among samples (e.g., ASVs 1, 3, 10, 16, 20 and 21 in *Agaricia* spp., ASVs 1, 2, 4, 10, and 17–19 in *M. cavernosa*, ASVs 1 and 2 in *P. astreoides*), whereas soft corals exhibited single-ASV dominance only rarely (e.g., ASV 5 in

Gorgoniidae, ASV 9 in Malacalcyonacea, and ASVs 2 and 7 in Muricea spp.; yellow bars in Figure 12).

Phylogenetic analysis showed that the 25 most abundant *Endozoicomonas* ASVs revealed partial clustering by host type, with several groups enriched within either hard or soft corals (Figure 13). However, multiple clusters contained ASVs associated with both host types, indicating that phylogenetic relationships are not strictly host specific. The most prevalent ASVs in hard (ASV1) and soft (ASV2) corals were closely related and grouped with *E. euniceicola*, further supporting the presence of shared evolutionary lineages across hosts. Only a subset of ASVs clustered closely with cultured reference strains, including *E. coralli*, *E. acroporae*, *E. ascidiicola*, *E. lisbonensis*, *E. montiporae*, and *E. euniceicola.* The majority of ASVs instead formed distinct groups that were often enriched within a particular host type but not exclusive to it, suggesting that while some *Endozoicomonas* lineages show host-associated patterns, many are shared across coral types (Figure 13).

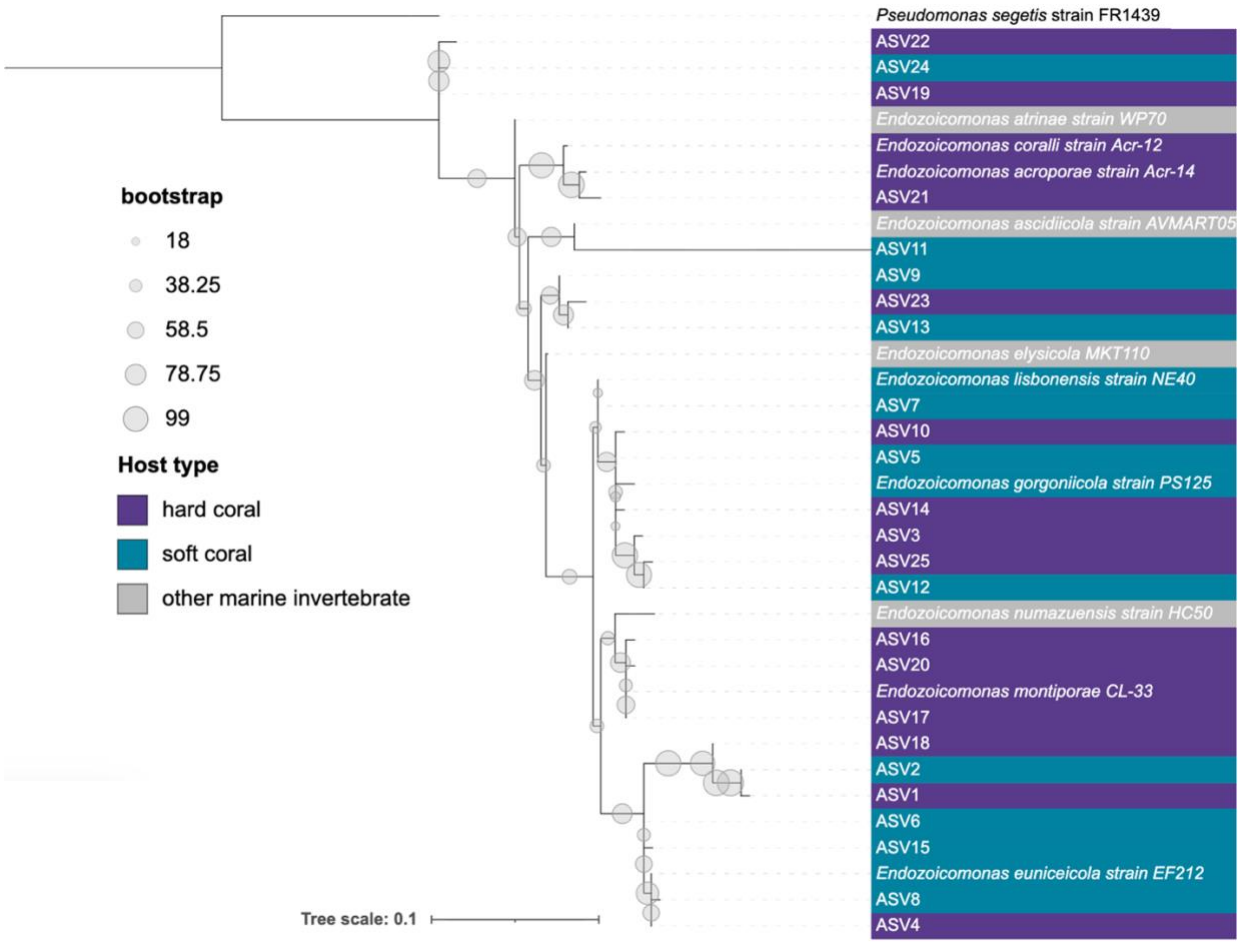

Figure 13: *Endozoicomonas* ASVs cluster based on host identity. The 25 most abundant ASVs were classified with MegaBLAST and analyzed alongside *Pseudomonas segetis* as an outgroup and reference *Endozoicomonas* 16S rRNA gene sequences obtained from NCBI nt+. Sequences were aligned with MAFFT, trimmed with trimAl, and a maximum-likelihood phylogenetic tree was inferred using IQ-TREE. The tree was visualized in iTOL. Branch support values, based on 1,000 ultrafast bootstrap replicates, are represented by circles at the corresponding nodes on a 0–100 scale. Tree scale represents 10% nucleotide sequence divergence. Best (BLASTn) matches for each *Endozoicomonas* ASV can be found in Table C12.

Discussion

Endozoicomonas is broadly regarded as a beneficial coral symbiont. Genomic studies have identified genes involved in nutrient cycling, molecular transport, biosynthesis of vitamins and antimicrobials, and protein secretion, all of which have been hypothesized to directly affect

the host (Price et al., 2014; Neave et al., 2016; Neave et al., 2017; Tandon et al., 2020; Pogoreutz et al., 2022). However, the exact mechanisms by which *Endozoicomonas* affects its host, as well as the ecological nature (benefit or cost) and magnitude of effects, are potentially variable across host types or environmental conditions. Quantifying patterns of *Endozoicomonas* diversity and distribution across natural gradients is a necessary first step in identifying host organisms or conditions in which *Endozoicomonas* may be particularly consequential. Our study analyzes *Endozoicomonas* abundance and prevalence in hard and soft coral microbiomes across sites and depths around Little Cayman Island (Figure 9). Overall, host identity, notably the distinction between hard and soft corals, had the strongest influence on *Endozoicomonas* distribution and diversity, as well as on the diversity of the microbiome as a whole, with site and depth contributing some variability for soft and hard coral microbiomes, respectively.

Endozoicomonas Variation by Host Identity

Hard and soft coral microbiomes differed significantly from one another in both alpha diversity and composition (Figure 10). *Endozoicomonas* was likely a driver of these differences, as it was significantly more abundant, on average, in soft corals (53.8%) than in hard corals (16.8%; Figure 11 and Figure C2), although this pattern was not universal across all hard and soft coral types. An elevated abundance in soft corals may partly reflect compartment-specific colonization, since *Endozoicomonas* is generally more abundant in coral tissues than in surface mucus (Neave et al., 2017; Pollock et al., 2018), and only the mucus-associated microbiome was sampled in hard corals. Mucus-associated microbiomes are often more variable due to frequent mucus turnover and environmental exposure, whereas tissue- and skeletal-associated microbiomes tend to be more stable and tightly host-associated (Sweet et al., 2011; Neave et al., 2017; Pollock et al., 2018; Mohamed et al., 2023). Interestingly, the hard coral *P. astreoides* (a

mucus associated microbiome) showed *Endozoicomonas* levels comparable to those of soft corals (tissue- and mucus-associated microbiomes), suggesting host-specific differences that may override compartment effects (Figure 11). However, the potential for residual tissue in mucus samples complicates interpretation. More broadly, host identity effects on microbiome composition, including variation in *Endozoicomonas* abundance, have been widely documented (Bayer et al., 2013; Pollock et al., 2018; Caughman et al., 2021; Kellogg & Pratte, 2021; Beatty et al., 2022) and provide a likely explanation for the differences observed here between hard and soft corals. However, most prior work has focused on hard corals, limiting firm conclusions about compartment effects in soft corals.

The ecological roles and stress responses of hard corals on Little Cayman reefs may correspond to variation in their *Endozoicomonas* abundance. In Little Cayman, *P. astreoides* is typically only found in shallower waters, while *Agaricia* spp. and *M. cavernosa* can span broader depth ranges (Carpenter et al., 2022; Doherty et al., 2024). *Montastraea cavernosa* serves as a major reef builder, whereas *P. astreoides* and *Agaricia* are considered weedy corals (Darling et al., 2012; Towle et al., 2015; Drury et al., 2020). Long-term surveys in Little Cayman have documented declines in *Montastraea* but increases in *Porites* and *Agaricia*, with *Porites* showing the lowest annual mortality rate (Coelho & Manfrino, 2007). Additionally, following a large bleaching event in Little Cayman in 2023, *Agaricia* spp. and *P. astreoides* suffered significantly higher bleaching and mortality, whereas *M. cavernosa* experienced high bleaching but low mortality (Doherty et al., 2025). Consistent with these ecological roles, we found that *P. astreoides* harbored significantly higher *Endozoicomonas* abundances than both *Agaricia* and *M. cavernosa*. Given the presumed beneficial nature of *Endozoicomonas*, its elevated presence in *P. astreoides* may contribute to this coral's relative survival and dominance in post-disturbance

communities. These results suggest that symbiont abundance and coral ecological strategies may be interconnected and potentially shaping survival and community composition on Little Cayman reefs.

Soft corals, though historically understudied, may be increasing abundance on reefs, a trend potentially supported by their consistently high *Endozoicomonas* abundances. Like hard corals, most soft corals host Symbiodiniaceae and contribute to reef framework and accretion, while also potentially outcompeting hard corals for space and resources (Lasker et al., 2020; Rodriguez et al., 2020; McFadden et al., 2025). In our study, *Muricea* spp., Cladiellidae, and Malacalyconacea microbiomes harbored *Endozoicomonas* at higher relative abundance than in any hard coral, with Gorgoniidae showing intermediate levels. *Muricea* spp., widely distributed on shallow Caribbean reefs (Breedy & Guzman, 2016), consistently maintained high *Endozoicomonas* abundances across depths and sites, mirroring observations from California reefs where *M. californica* hosted simplified microbiomes dominated by *Endozoicomonas* (Holm & Heidelberg, 2016). By contrast, *M. fruticosa* exhibited reduced *Endozoicomonas* and increased *Vibrio* spp., some of which are confirmed coral pathogens or known to be associated with coral disease, highlighting host-specific associations with potential functional consequences (Holm & Heidelberg, 2016). Together, these findings suggest that dominance of *Endozoicomonas* in soft corals may play a role in promoting host health and persistence, paralleling patterns observed in competitive hard corals such as *P. astreoides*.

Endozoicomonas ASV diversity was also largely structured by host identity, with only a few dominant ASVs accounting for most of the diversity. Soft coral microbiomes showed higher ASV diversity than hard corals, with ASVs largely distinct between host types but conserved within groups and showing minimal variation across sites or depths (Figure 12). This aligns with

previous studies identifying coral type as the strongest predictor of *Endozoicomonas* composition (Bayer et al., 2013; Caughman et al., 2021; Kellogg & Pratte, 2021). In our dataset, ASV1 was shared among all hard corals, while ASV2 was consistently found in soft corals (Figure 12 and 13). MegaBLASTN results revealed ASV2 had a 100% match to *Endozoicomonas euniceicola* strain EF212, whereas ASV1 showed 95.7% identity to both *E. euniceicola* EF212 and *E. lisbonensis* NE40. Notably, ASV1 and ASV2 were only 95.7% similar to one another, suggesting that the dominant *Endozoicomonas* strains in hard and soft corals are taxonomically, and potentially functionally, distinct, despite clustering within the same phylogenetic clade (Figure 13). Although little is known about these strains beyond their classification as facultative anaerobes isolated from soft corals (Pike et al., 2013; Chiou et al., 2023; da Silva et al., 2025), the specificity of ASVs by coral type suggests host-driven structuring of *Endozoicomonas* communities**.** Phylogenetic analyses partially supported host specificity, with many ASVs clustering by coral type and showing closest similarity to strains previously isolated from the same host group. However, several ASVs were shared across reference *Endozoicomonas* strains from different coral types, indicating that these bacteria may exhibit a combination of host preference and ecological flexibility (Figure 13). One ASV in particular (ASV11) shows the greatest divergence relative to all other *Endozoicomonas* sequences (both ASVs and reference strains), with the lowest percent identity to a reference strain (*E. ascidiicola*, 92.6%; Table C12). Despite this divergence, ASV11 consistently clustered within the *Endozoicomonas* clade, supporting its classification while highlighting the presence of previously under characterized diversity within coral-associated *Endozoicomonas*. While targeting the V3–V4 region of the 16S rRNA gene is a common approach for microbiome amplicon sequencing, it may provide limited taxonomic resolution, especially for closely related species/strains; definitive identification and

comparison of *Endozoicomonas* ASVs would benefit from sequencing the full length-16S rRNA gene or reconstructing metagenome-assembled genomes. Overall, these findings highlight strong host-driven structuring of *Endozoicomonas* lineages while also suggesting potential divergence between strains associated with coral types.

Endozoicomonas Variation by Site

Endozoicomonas relative abundance varied by site in soft coral microbiomes, but not in hard corals, independent from depth. Specifically, *Endozoicomonas* was more abundant in soft corals collected from non-MPA sites, including Ron's Rock and The Edge, compared to MPA, McCoy's Wall (Figure 11b). Ron's Rock, a non-MPA and non-designated fishing zone, exhibited the highest average *Endozoicomonas* abundance in both soft and hard corals, though the difference was not statistically significant for hard corals. These findings are consistent with prior studies on hard corals. However, because approximately 57% of Little Cayman's reefs are designated no-take areas, the overall isolation and extensive protection of the island may reduce large-scale differences between sites (Goodbody-Gringley & Chequer, 2025). This high level of protection could dampen site-level effects that might otherwise be observed in more heavily impacted reef systems. For example, Beatty et al. (2018) found no significant differences in *Endozoicomonas* abundance between MPAs and fished zones in *Pocillopora damicornis* from Fiji. A follow-up study by the same group (2022) reported higher *Endozoicomonas* abundance in MPAs, though ASV-level diversity remained unchanged. However, there are limited data on how MPA status affects *Endozoicomonas* abundance in soft coral microbiomes. Our results suggest that *Endozoicomonas* is more abundant in soft corals from non-MPA sites, regardless of fishing permissions (Figure 11b). Differences in structure and physiology between hard and soft corals may affect their relationship with microbial symbionts such as *Endozoicomonas* to cope

with environmental stress. Coral anatomy has been shown to influence microbial community composition (Sweet et al., 2011; Pollock et al., 2018; Mohamed et al., 2023), and *Endozoicomonas* is often more abundant in coral tissue than in surface mucus, suggesting a potential functional role within the host (Neave et al., 2017). This relationship may be amplified in areas with greater exposure to pollutants or other anthropogenic pressures; however, further studies are needed to confirm this hypothesis.

In addition to differences in protection status, our sampling sites varied geographically. Crystal Palace Wall and McCoy's Wall are located on the north side of Little Cayman, Ron's Rock on the western side, and The Edge on the south side (Figure 9a). The southern reef (The Edge) is windward, meaning it is more strongly impacted by wind and ocean current than leeward northern sites (Crystal Palace Wall and McCoy's Wall; (Coelho & Manfrino, 2007)). While this study does not address the impact of ocean current, or other potential geographical impacts such as temperature, on coral microbiomes or *Endozoicomonas* relative abundance, this distinction may be especially important in Little Cayman, where direct human impacts are relatively low, but reefs remain vulnerable to climate change, hurricanes, and pollutants or potential pathogens transported by ocean currents (Dodge & Gilbert, 1984; Guzmán & García, 2002; McCallum et al., 2003; Kim et al., 2005). Although the majority of Little Cayman's reefs are located within MPAs (75%) and no-take zones (57%; (Goodbody-Gringley & Chequer, 2025)), previous studies have found inconsistent associations between coral health or growth and MPA status (Bellwood et al., 2004; Mumby et al., 2006; Coelho & Manfrino, 2007; Toth et al., 2014; Cox et al., 2017; Wright, 2022), suggesting that other environmental drivers influence coral disease susceptibility. While our study does not directly address coral disease, we did observe distinct differences in *Endozoicomonas* abundance in soft coral microbiomes between

MPA and non-MPA sites. Ron's Rock and The Edge, the only south-facing site sampled, had the highest average relative abundance of *Endozoicomonas*. In particular, *Endozoicomonas* in soft coral microbiomes at The Edge were significantly different from those at the MPA site McCoy's Wall, though not from Crystal Palace Wall (Figure 11b). Greater exposure to wind and ocean currents, potential nutrient influx from the nearby lagoon, and proximity to the island's main population may contribute to shifts in microbial composition at The Edge, including the increased prevalence of *Endozoicomonas* observed in several soft coral hosts. These results suggest that local oceanographic conditions, in addition to protection status, may influence the structure of coral-associated microbial communities.

Endozoicomonas Variation by Depth

Endozoicomonas abundance varied by depth, but only in hard coral microbiomes and independent of site. There were significantly lower relative abundances of *Endozoicomonas* from hard coral microbiomes from upper mesophotic waters (32 and 36.6 m) compared to mid (9.1 m) and deep photic (13.7 m) hard corals (Figure 11c and Table C10). Even though there are no significant differences in *Endozoicomonas* abundance in soft coal microbiomes by sampling depth, upper mesophotic soft corals had the lowest median abundances, consistent with hard corals (Figure 11b). While most studies on *Endozoicomonas* in coral microbiomes have focused on shallow-water taxa, there is increasing evidence for *Endozoicomonas* persistence in deeper habitats. For example, Kellogg and Pratte (2021) reported *Endozoicomonas* at high relative abundances in both hard and soft deep-sea coral (500–1300 m) microbiomes, reaching 88% and 75% in *Desmophyllum pertusum* (hard coral) and *Acanthogorgia* spp. (soft coral), respectively. Although many deep-sea coral species lack detectable levels of *Endozoicomonas*, our findings demonstrate its consistent presence in upper mesophotic corals (32–36.6 m), where it comprised

an average of 7.7% in hard and 44.7% in soft coral microbiomes. Collectively, these findings suggest that *Endozoicomonas* abundance remains consistent across depth and may play an adaptive role in supporting coral host persistence across depth-related environmental gradients.

Coral reef community composition is strongly influenced by light availability, which shifts between shallow and mesophotic zones (Carpenter et al., 2022). Unlike shallow-water reefs, mesophotic systems experience reduced thermal stress and bleaching, but are limited by light. With only 10% of light remaining at 30–40 m, light limitation, not depth itself, has been shown to be a strong factor in shaping coral communities (Lesser et al., 2009; Goodbody-Gringley, 2018; Tamir et al., 2019; Laverick et al., 2020). Around Little Cayman Island, light decreases by about 30% below 25 m, contributing to shifts in hard coral composition (Carpenter et al., 2022). *Agaricia* spp. become more abundant with depth, *P. astreoides* reaches its lower depth limit near 35 m, and *M. cavernosa* maintains stable populations with a peak at 25 m, suggesting that light may not strongly limit the persistence of these corals in our study (Carpenter et al., 2022). It is possible that host genotypic differences exist across sites and depths but could not be resolved with identification methods used in this analysis. Such undetected variation may contribute to observed geographic or depth-related differences in coral microbiome composition and *Endozoicomonas* abundance and diversity. Although our sampling and host identification efforts were uneven across corals, depth-related shifts in *Endozoicomonas* abundance broadly aligned with known coral depth distributions. For example, *P. astreoides* microbiomes showed highest abundances in shallow zones, while *Agaricia* and *M. cavernosa* tended to harbor *Endozoicomonas* at greater depths. In addition to hard corals, soft corals such as *Muricea* spp., Gorgoniidae, and others are known to be dominant members of mesophotic reefs (Kahng et al., 2017; Slattery et al., 2024). In our study, soft corals hosted the highest average

abundances of *Endozoicomonas*, including in upper mesophotic zones, raising questions about whether this putative beneficial symbiont plays a role in coral persistence at depth. While these patterns remain exploratory, they provide valuable context for how microbial associations may shift along environmental gradients and underscore the importance of studying symbiont function in deeper reef systems.

Conclusion

Our study provides a comparative analysis of *Endozoicomonas* across hard and soft coral microbiomes in Little Cayman Island, revealing that host identity is the primary driver of both abundance and diversity. Soft corals were almost entirely dominated by *Endozoicomonas*, whereas hard corals displayed lower but variable abundances, with *P. astreoides* consistently enriched compared to *Agaricia* spp. and *M. cavernosa*. These patterns parallel host-specific ecological roles, suggesting that *Endozoicomonas* may contribute to coral resilience under stressful conditions and disturbances. ASV diversity also reflected host associations, with dominant ASVs conserved within coral groups with some distinction between hard and soft corals, indicating the presence of host-specific lineages with potentially divergent functions. In contrast to host identity, site influenced *Endozoicomonas* relative abundance only in soft coral microbiomes, with higher values observed in non-MPA locations. Depth had an effect only in hard corals, where relative abundance was greatest in shallow and mid photic zones and lowest in upper mesophotic samples. Although no consistent depth-related trends were observed across sites, *Endozoicomonas* was detected in both MPAs and unprotected areas, and across all depth zones sampled, from shallow to upper mesophotic, suggesting its ecological role extends beyond shallow reef environments. Taken together, these findings underscore the dominant influence of

host identity in shaping *Endozoicomonas* communities and highlight the need to better understand the functional diversity of this widespread coral symbiont.

CHAPTER FIVE

CONCLUSION AND SUGGESTIONS

Host-associated microbiomes are important determinants of host health, development, and ecological function, and aquatic systems provide a unique opportunity to study these complex interactions. This thesis demonstrates how host, microbial, and environmental factors shape microbiome composition and function, providing insight into functional potential of host-microbe interactions, microbiome assembly in early life stages, and community structure across environmental gradients. While these factors were investigated independently across chapters, the findings collectively underscore the complexity of host biology, environmental context, and microbial interactions, and highlight the need for rigorous experimental design and genomic resolution to disentangle their effects.

Host Factors

Among these factors, host genetics represents a primary avenue through which microbiome composition can vary (Chapter two). This study revealed clear taxonomic and functional distinctions between hybrid catfish and their parental, channel and blue catfish, gut microbiomes. Hybrid catfish harbored microbial taxa and metabolic pathways associated with host immunity, and nutrient/energy utilization, potentially contributing to hybrid vigor in aquaculture. In particular, hybrid gut microbiomes showed reduced relative abundances of taxa associated with potential pathogens found in channel and blue catfish, consistent with enhanced immune-associated microbial functions. While *Lactococcus*, specifically *Lactococcus lactis*, has been explored for probiotic effects in catfish aquaculture (Huang et al., 2024), *Cetobacterium somerae*, enriched in hybrid catfish and associated with vitamin B_{12} production (Qi et al., 2023;

Zhang et al., 2023), may represent an additional candidate for probiotic development to support host immunity in microbe rich environments. Isolates of both *Lactococcus* and *Cetobacterium* could be cultured from catfish digesta and subsequently used in *in vitro* challenge experiments against common catfish pathogens to evaluate their potential to inhibit or suppress pathogen growth.

Interpretation of functional differences is constrained by the metagenomic sampling scheme. Only samples from the 2022 sampling year were included, which may partially explain inconsistencies in parental association between the amplicon and shotgun metagenomics datasets. Channel catfish in 2022 were initially reared at a different facility than the hybrid and blue catfish were, likely contributing to the observed similarity of hybrid and blue catfish metabolic profiles. In contrast, amplicon data, which included the 2023 sampling year reared in the same facility, show clear differentiation between hybrid and both parental gut microbiomes. These patterns suggest that early rearing environment may have had a strong influence on functional profiles, highlighting the importance and complexity of environmental context in interpreting host-associated microbial patterns.

Additional metadata, such as sex, could further refine our understanding of host-associated microbiome variation. Although intergroup variation was limited in this study, sex has been shown to influence microbiome composition in other aquatic vertebrates and may warrant investigation in future work (Li et al., 2021; Zhao et al., 2023). Although six- to seven-month-old catfish have not yet reached sexual maturity, sex can usually be determined by this stage (Davis, 2009), and hormone systems and size variability between female and male catfish may be associated with microbiome differences. Collectively these findings emphasize that host

hybridization, early-life environment, and their interactions play important roles in shaping the structure and functional potential of gut microbiomes in aquacultured catfish species.

To explore the importance of early life microbial colonization in aquaculture we analyzed the microbiome of channel catfish embryos (Chapter 3). This study provides the first comprehensive characterization of the channel embryo microbiome and reveals distinct community structures compared to surrounding water. Taxa enriched in embryo microbiomes remain largely underexplored in aquatic systems, highlighting the limited knowledge of externally brooded-associated microbes and their potential contribution in nutrient cycling, pathogen resistance, and environmental stress tolerance. In particular, members of the orders Sphingomonadales and Rhizobiales (Alphaproteobacteria) were consistently enriched and represent promising targets for future functional investigation. These taxa have been isolated from channel catfish embryos, with Sphingomonadales isolates showing high similarity to dominant ASVs identified in Chapter 3 and Rhizobiales corresponding to lower-abundance ASVs. Although their roles in aquatic host-associated microbiomes remain poorly defined, Rhizobiales are commonly associated with diverse aquatic animal microbiomes (Quigley et al., 2020; Rosales et al., 2020; Dube et al., 2021), while Sphingomonadales are recognized for their roles in bioremediation and antioxidant activity in aquatic systems(Godoy et al., 2003; Yamatsu et al., 2006; Sharma et al., 2021), suggesting potential contributions to host resilience.

Together, these findings support the importance of early microbiome assembly and raise the possibility of germline-associated microbial contributions. Although embryos were completely lysed during DNA extraction, future work employing spatially resolved methods, such as fluorescence in situ hybridization with confocal laser scanning microscopy (FISH-CLSM), could determine whether microbes reside internally and/or on the surface of the embryo,

and identify potential biofilm structure that may facilitate persistent colonization. Establishing microbial localization and transmission routes would improve understanding of host-microbiome co-evolution and early microbial colonization.

Functional gene analyses indicated that embryo microbiomes encoded antimicrobial biosynthetic gene clusters and resistance genes, suggesting a role in early host defense. While this study focused on direct antagonism, additional protective mechanisms may also be involved. Observations from betadine-treated embryos further suggest that some microbial taxa may be resilient to perturbation, reflecting continuous environmental exposure. To directly test these hypotheses, future studies should employ controlled microbiome perturbation experiments (Figure 14) in which channel catfish embryos are subjected to antibiotic or betadine-based depletion followed by reinoculation with defined taxa and pathogen challenge.

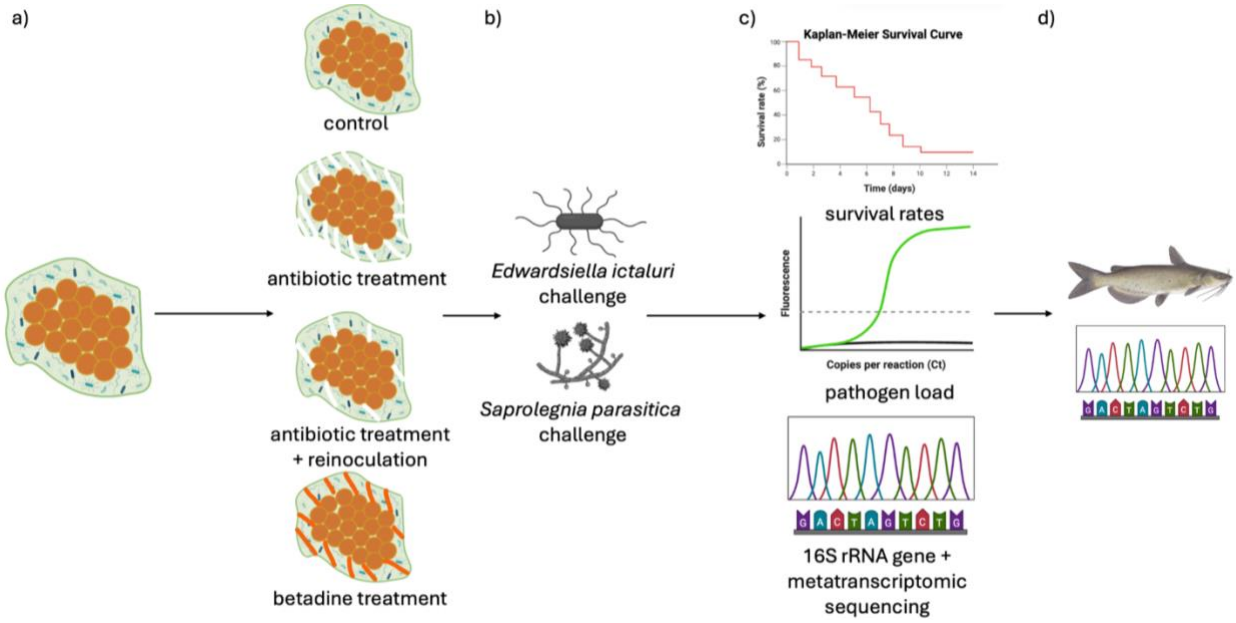

Figure 14: Experimental design to test the role of microbiomes in channel catfish embryos. a) Fertilized embryos are subjected to microbiome perturbations, including antibiotic treatment, antibiotic treatment with reinoculation of dominant bacteria, and betadine disinfection. b) Embryos are challenged with bacterial (E. ictaluri) and fungal (S. parasitica) pathogens. c) Host survival, pathogen load, and microbiome structure/function are quantified using survival rates, qPCR, and sequence-based approaches. d) Longitudinal sampling of survivors is used to determine how early-life microbial perturbations influence microbiome assembly and host health across development.

Specifically, embryos from a single egg mass would be divided by weight into four treatment groups (including a no-treatment control), maintained in separate tanks with technical triplicates, and replicated across three egg masses as biological replicates. After antibiotic depletion, one group would be reinoculated with the Rhizobiales and Spingomonadales isolates, and all groups would then be challenged with known catfish pathogens, *Edwardsiella ictaluri* (Older et al., 2024), and *Saprolegnia parasitica* (Pavic et al., 2022). Host survival would be monitored from fertilization through larval development, and analyzed with Kaplan-Meier survival curves, with mortality defined as loss of transparency and failure to develop over two

consecutive days (cite). Pathogen load would be quantified by quantitative PCR from randomly sampled embryos (~20/group), and microbial community dynamics assessed through 16S rRNA gene sequencing and metatranscriptomics on subsamples collected every two days (~20 embryos/group). This design would test whether embryo-associated microbiomes confer infection resistance and identify taxa associated with host protection.

Extending this framework from embryo to juvenile stages would enable identification of critical windows of microbiome assembly and determine whether early colonizers influence community stability and host fitness. Juvenile survivors (6–7 months) would be analyzed for digesta microbiomes (Chapter 2 methods), and survival to juvenile stage quantified relative to initial embryo numbers. In addition, comparisons across channel, blue, and hybrid catfish would clarify how host genetics and production practices shape microbial succession. Hybrid catfish, produced channel eggs artificially fertilized with blue catfish sperm in non-sterile conditions, display distinct gut microbiomes (Chapter 2), suggesting strong host effects. Parallel embryo-level studies across catfish type would help disentangle host genetics versus production-associated microbial influences. These experiments would need to be conducted in animal laboratory settings, as disruption to standard aquaculture practices would not be permitted due to fish health and pathogen outbreak concerns.

Finally, variation in reproductive behavior among aquatic species likely further shapes microbial colonization dynamics, linking host behavior, environment, and microbiome assembly. For example, male catfish fan and guard their eggs, enhancing aeration and waste removal, while also protecting against predators. Some aquatic species mouthbrood for protection, while others scatter their spawn and provide no protection. These behaviors likely influence microbial assembly, community composition, and function, suggesting that early-life microbial dynamics

are closely linked with both host behavior and environmental exposure. Collectively, these observations highlight the importance of early colonization on shaping microbiome trajectories and functional capabilities, setting the foundation for later host-microbiome interactions.

Environmental Factors

While early colonization highlights the importance of microbials interactions in aquatic hosts, microbial assembly does not occur in isolation. Environmental gradients such as water depth, protections status, and host habitat structure further shape microbial community composition. To examen how environmental conditions are associated with microbiome structure at broader ecological scales, we investigated coral-associated microbiomes across reef gradients. This study demonstrates that both host identity and environmental conditions shape coral microbiome structure and the relative abundance of coral symbiont, *Endozoicomonas*. Soft coral microbiomes had significantly higher relative abundances of *Endozoicomonas* than hard corals, although one hard coral species, *Porites astreoides*, exhibited microbiome characteristics more similar to soft corals, suggesting host identity alone does not fully explain *Endozoicomonas* distribution. Environmental effects were also host-dependent. Marine protection status was only a significant factor in *Endozoicomonas* relative abundance in soft coral, with unprotected sites having the highest abundances. Conversely, depth was only a significant factor in *Endozoicomonas* relative abundances in hard corals, with shallow and mid photic depths having the highest abundances. Regardless of environmental factors, *Endozoicomonas* was persistent across all coral types, even at deeper photic depths, highlighting the importance of their functional diversity that still remains unknown.

Disentangling host and environmental influences will require a more balanced and robust sampling scheme. Increasing replication across coral types, depth, and protection status would improve statistical power and allow for more refined inferences. While this study broadly characterized corals as hard vs soft, variation within groups, particularly the elevated prevalence of *Endozoicomonas* in *P. astreoides*, suggests that species-level resolution is essential for understanding host-specific microbial dynamics. Soft coral taxonomy presents additional challenges, as field identification is difficult and molecular approaches did not resolve all samples to the genus level. These limitations highlight the importance of refined taxonomic resolution for both hosts and microbiomes in future work.

Achieving species-level resolution of *Endozoicomonas* will be critical for elucidating its functional role in coral health across environmental gradients. Advanced omic approaches such as single amplified genomes (SAGs), which isolate and sequence individual microbial cells, could improve recovery of *Endozoicomonas* genomes, which are often masked by host DNA. High-quality genomes would enable more confident taxonomic classification and functional annotation, enabling the investigation into metabolic pathways and stress-response mechanisms that may contribute to coral resilience in protected versus unprotected reefs across depth gradients. Together, the findings from this study emphasize that coral microbiome structure reflects a dynamic relationship between host identity and environmental conditions, and that deeper genomic resolution is necessary to clarify the ecological role of dominant symbionts in reef ecosystems, particularly in deep mesophotic water where coral microbiomes are far less studied.

Concluding remarks

Across catfish and their embryos, and coral reefs, a common pattern emerges, host-associated microbiomes are composed of complex interactions between host, environmental, and microbial factors. Disentangling these interactions requires robust sampling schemes, and significant sequencing and computational power. This thesis provides a foundation for investigating the important roles that microbiomes play in aquatic hosts and how specific taxa and metabolic pathways may contribute to host health and fitness in microbially rich aquatic environments. These findings support broader frameworks in which aquatic microbiomes function as adaptive interfaces between the host and the environment. By integrating ecological, functional, and developmental perspectives across systems, this thesis highlights the importance of viewing aquatic host-associated microbiomes not as static communities, but as dynamic ecological assemblages shaped by multi-factor interactions.

CUMULATIVE REFERENCES CITED

A.-C. Hansen, R. W., G.-I. Hemre. (2015). New B vitamin recommendations in fish when fed plant-based diets. *Aquaculture Nutrition* https://doi.org/https://doi.org/10.1111/anu.12342

Aalto, N. J., Schweitzer, H. D., Krsmanovic, S., Campbell, K., & Bernstein, H. C. (2022). Diversity and Selection of Surface Marine Microbiomes in the Atlantic-Influenced Arctic. *Front Microbiol*, *13*, 892634. https://doi.org/10.3389/fmicb.2022.892634

Abdul Razak, S., Griffin, M. J., Mischke, C. C., Bosworth, B. G., Waldbieser, G. C., Wise, D. J., Marsh, T. L., & Scribner, K. T. (2019). Biotic and abiotic factors influencing channel catfish egg and gut microbiome dynamics during early life stages. *Aquaculture*, *498*, 556-567. https://doi.org/https://doi.org/10.1016/j.aquaculture.2018.08.073

Adekambi, T., Drancourt, M., & Raoult, D. (2009). The rpoB gene as a tool for clinical microbiologists. *Trends Microbiol*, *17*(1), 37-45. https://doi.org/10.1016/j.tim.2008.09.008

Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., & Narasimhan, G. (2016). Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis. *Evol Bioinform Online*, *12*(Suppl 1), 5-16. https://doi.org/10.4137/EBO.S36436

Ahmad, A., Majaz, S., & Nouroz, F. (2020). Two-component systems regulate ABC transporters in antimicrobial peptide production, immunity and resistance. *Microbiology (Reading)*, *166*(1), 4-20. https://doi.org/10.1099/mic.0.000823

Akhila Dharnappa Sannejal, M. D., & Deekshit Vijaya Kumar, M. S. N., and Praveen Rai. (2023). Fish Microbiome and Metagenomics. *Microbiome of Finfish and Shellfish*. https://doi.org/https://doi.org/10.1007/978-981-99-0852-3_4

Alam, K., Mazumder, A., Sikdar, S., Zhao, Y. M., Hao, J., Song, C., Wang, Y., Sarkar, R., Islam, S., Zhang, Y., & Li, A. (2022). Streptomyces: The biofactory of secondary metabolites. *Front Microbiol*, *13*, 968053. https://doi.org/10.3389/fmicb.2022.968053

Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., Edalatmand, A., Petkau, A., Syed, S. A., Tsang, K. K., Baker, S. J. C., Dave, M., McCarthy, M. C., Mukiri, K. M., Nasir, J. A., Golbon, B., Imtiaz, H., Jiang, X., Kaur, K.,

. . . McArthur, A. G. (2023). CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res*, *51*(D1), D690-D699. https://doi.org/10.1093/nar/gkac920

Altman-Kurosaki, N. T., Pratte, Z. A., Stewart, F. J., & Hay, M. E. (2025). Coral–algal competition: allelopathy, temporal variance, and effects on coral microbiomes. *Coral Reefs*, *44*(1), 49-62. https://doi.org/10.1007/s00338-024-02585-7

Amato, K. R., Maurice, C. F., Guillemin, K., & Giles-Vernick, T. (2019). Multidisciplinarity in Microbiome Research: A Challenge and Opportunity to Rethink Causation, Variability, and Scale. *Bioessays*, *41*(10), e1900007. https://doi.org/10.1002/bies.201900007

Amin, A., Khan, M. A., Ehsanullah, M., Haroon, U., Azam, S. M., & Hameed, A. (2012). Production of peptide antibiotics by Bacillus sp. GU 057 indigenously isolated from saline soil. *Braz J Microbiol*, *43*(4), 1340-1346. https://doi.org/10.1590/S1517-838220120004000015

Amy Apprill, S. M., Rachel Parsons, Laura Weber. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquartic Microbial Ecology*, *75*, Article 2. https://doi.org/https://doi.org/10.3354/ame01753

Angela Liu, V. P. T. H. T., Ester Santigosa, Andr´e Dumas, Jose Maria Hernandez. (2022). Vitamin nutrition in salmonid aquaculture: From avoiding deficiencies to enhancing functionalities. *Aquaculture*, *561*. https://doi.org/https://doi.org/10.1016/j.aquaculture.2022.738654

Angoshtari, R., Scribner, K. T., & Marsh, T. L. (2023). The impact of primary colonizers on the community composition of river biofilm. *PLoS One*, *18*(11), e0288040. https://doi.org/10.1371/journal.pone.0288040

Aoki, M., Nakahara, N., Kusube, M., & Syutsubo, K. (2022). Metagenome-Assembled Genome Sequence of Marine Rhizobiaceae sp. Strain MnEN-MB40S, Obtained from Manganese-Oxidizing Enrichment Culture. *Microbiol Resour Announc*, *11*(9), e0064522. https://doi.org/10.1128/mra.00645-22

Apprill A, W. L., Santoro AE. (2016). Distinguishing between Microbial Habitats Unravels Ecological Complexity in Coral Microbiomes. *mSystems*. https://doi.org/https://doi.org/10.1128/msystems.00143-16

Araujo, C., Munoz-Atienza, E., Perez-Sanchez, T., Poeta, P., Igrejas, G., Hernandez, P. E., Herranz, C., Ruiz-Zarzuela, I., & Cintas, L. M. (2015). Nisin Z Production by Lactococcus lactis subsp. cremoris WA2-67 of Aquatic Origin as a Defense Mechanism to Protect Rainbow Trout (Oncorhynchus mykiss, Walbaum) Against Lactococcus garvieae. *Mar Biotechnol (NY)*, *17*(6), 820-830. https://doi.org/10.1007/s10126-015-9660-x

Arbizu, P. M. (2017). Pairwise Multilevel Comparison using Adonis.

Armwood, A. R., Griffin, M. J., Richardson, B. M., Wise, D. J., Ware, C., & Camus, A. C. (2022). Pathology and virulence of Edwardsiella tarda, Edwardsiella piscicida, and Edwardsiella anguillarum in channel (Ictalurus punctatus), blue (Ictalurus furcatus), and channel x blue hybrid catfish. *J Fish Dis*, *45*(11), 1683-1698. https://doi.org/10.1111/jfd.13691

Arnison, P. G., Bibb, M. J., Bierbaum, G., Bowers, A. A., Bugni, T. S., Bulaj, G., Camarero, J. A., Campopiano, D. J., Challis, G. L., Clardy, J., Cotter, P. D., Craik, D. J., Dawson, M., Dittmann, E., Donadio, S., Dorrestein, P. C., Entian, K. D., Fischbach, M. A., Garavelli, J. S., . . . van der Donk, W. A. (2013). Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature. *Nat Prod Rep*, *30*(1), 108-160. https://doi.org/10.1039/c2np20085f

Atkinson, G. C., Hansen, L. H., Tenson, T., Rasmussen, A., Kirpekar, F., & Vester, B. (2013). Distinction between the Cfr Methyltransferase Conferring Antibiotic Resistance and the Housekeeping RlmN Methyltransferase. *Antimicrobial Agents and Chemotherapy*, *57*(8), 4019-4026. https://doi.org/doi:10.1128/aac.00448-13

Bakky, M. A. H., Tran, N. T., Zhang, M., Wang, S., Zhang, Y., & Li, S. (2025). Synergistic effects of butyrate-producing bacteria (Clostridium senegalense I5 or Paraclostridium benzoelyticum G5) and Gracilaria lemaneiformis-originated polysaccharides on the growth and immunity of rabbitfish. *Int J Biol Macromol*, *291*, 138683. https://doi.org/10.1016/j.ijbiomac.2024.138683

Balloux, F., Brønstad Brynildsrud, O., van Dorp, L., Shaw, L. P., Chen, H., Harris, K. A., Wang, H., & Eldholm, V. (2018). From Theory to Practice: Translating Whole-Genome Sequencing (WGS) into the Clinic. *Trends in Microbiology*, *26*(12), 1035-1048. https://doi.org/https://doi.org/10.1016/j.tim.2018.08.004

Bartelme, R. P., Smith, M. C., Sepulveda-Villet, O. J., & Newton, R. J. (2019). Component Microenvironments and System Biogeography Structure Microorganism Distributions in

Recirculating Aquaculture and Aquaponic Systems. *mSphere*, *4*(4). https://doi.org/10.1128/mSphere.00143-19

Bayer, T., Neave, M. J., Alsheikh-Hussain, A., Aranda, M., Yum, L. K., Mincer, T., Hughen, K., Apprill, A., & Voolstra, C. R. (2013). The microbiome of the Red Sea coral Stylophora pistillata is dominated by tissue-associated Endozoicomonas bacteria. *Appl Environ Microbiol*, *79*(15), 4759-4762. https://doi.org/10.1128/AEM.00695-13

Beatty, D. S., Clements, C. S., Stewart, F. J., & Hay, M. E. (2018). Intergenerational effects of macroalgae on a reef coral: major declines in larval survival but subtle changes in microbiomes. *Mar Ecol Prog Ser*, *589*, 97-114. https://doi.org/10.3354/meps12465

Beatty, D. S., Clements, C. S., Valayil, J. M., Jarvis, S. Y., Ritchie, K. B., Stewart, F. J., & Hay, M. E. (2022). Variance of coral anti-pathogen defense in response to transplantation between coral- and macroalgal-dominated reefs. *Coral Reefs*, *41*(5), 1417-1431. https://doi.org/10.1007/s00338-022-02291-2

Beatty, D. S., Valayil, J. M., Clements, C. S., Ritchie, K. B., Stewart, F. J., & Hay, M. E. (2019). Variable effects of local management on coral defenses against a thermally regulated bleaching pathogen. *Sci Adv*, *5*(10), eaay1048. https://doi.org/10.1126/sciadv.aay1048

Beemelmanns, A., Poirier, M., Bayer, T., Kuenzel, S., & Roth, O. (2019). Microbial embryonal colonization during pipefish male pregnancy. *Sci Rep*, *9*(1), 3. https://doi.org/10.1038/s41598-018-37026-3

Bellwood, D. R., Hughes, T. P., Folke, C., & Nyström, M. (2004). Confronting the coral reef crisis. *Nature*, *429*(6994), 827-833. https://doi.org/10.1038/nature02691

Benoit, G. (2015). Simka: fast kmer-based method for estimating the similarity between numerous
metagenomic datasets, RCAM, Oct 2015, Paris, France. ffhal-01231795f.

Benson, A. K., Kelly, S. A., Legge, R., Ma, F., Low, S. J., Kim, J., Zhang, M., Oh, P. L., Nehrenberg, D., Hua, K., Kachman, S. D., Moriyama, E. N., Walter, J., Peterson, D. A., & Pomp, D. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci U S A*, *107*(44), 18933-18938. https://doi.org/10.1073/pnas.1007028107

Bentzon-Tilia, M., Sonnenschein, E. C., & Gram, L. (2016). Monitoring and managing microbes in aquaculture - Towards a sustainable industry. *Microb Biotechnol*, *9*(5), 576-584. https://doi.org/10.1111/1751-7915.12392

Bernardo-Cravo, A. P., Schmeller, D. S., Chatzinotas, A., Vredenburg, V. T., & Loyau, A. (2020). Environmental Factors and Host Microbiomes Shape Host–Pathogen Dynamics. *Trends in Parasitology*, *36*(7), 616-633. https://doi.org/10.1016/j.pt.2020.04.010

Bielen, A., Simatovic, A., Kosic-Vuksic, J., Senta, I., Ahel, M., Babic, S., Jurina, T., Gonzalez Plaza, J. J., Milakovic, M., & Udikovic-Kolic, N. (2017). Negative environmental impacts of antibiotic-contaminated effluents from pharmaceutical industries. *Water Res*, *126*, 79-87. https://doi.org/10.1016/j.watres.2017.09.019

Biju Sam Kamalam, F. M., Stephane Panserat. (2017). Utilisation of dietary carbohydrates in farmed fishes: New insights on influencing factors, biological limitations and future strategies. *Aquaculture*, *467*. https://doi.org/https://doi.org/10.1016/j.aquaculture.2016.02.007

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9

Boneca, I. G., & Chiosis, G. (2003). Vancomycin resistance: occurrence, mechanisms and strategies to combat it. *Expert Opin Ther Targets*, *7*(3), 311-328. https://doi.org/10.1517/14728222.7.3.311

Botts, R. T., Page, D. M., Bravo, J. A., Brown, M. L., Castilleja, C. C., Guzman, V. L., Hall, S., Henderson, J. D., Kenney, S. M., Lensink, M. E., Paternoster, M. V., Pyle, S. L., Ustick, L., Walters-Laird, C. J., Top, E. M., & Cummings, D. E. (2023). Polluted wetlands contain multidrug-resistance plasmids encoding CTX-M-type extended-spectrum beta-lactamases. *Plasmid*, *126*, 102682. https://doi.org/10.1016/j.plasmid.2023.102682

Boutin, S., Bernatchez, L., Audet, C., & Derome, N. (2012). Antagonistic effect of indigenous skin bacteria of brook charr (Salvelinus fontinalis) against Flavobacterium columnare and F. psychrophilum. *Vet Microbiol*, *155*(2-4), 355-361. https://doi.org/10.1016/j.vetmic.2011.09.002

Brady, S. F., & Clardy, J. (2000). Long-Chain N-Acyl Amino Acid Antibiotics Isolated from Heterologously Expressed Environmental DNA. *Journal of the American Chemical Society*, *122*(51), 12903-12904. https://doi.org/10.1021/ja002990u

Breedy, O., & Guzman, H. M. (2016). A new Muricea species (Cnidaria, Anthozoa, Octocorallia) from the eastern tropical Pacific. *Zookeys*(629), 1-10. https://doi.org/10.3897/zookeys.629.10828

Brener-Raffalli, K., Clerissi, C., Vidal-Dupiol, J., Adjeroud, M., Bonhomme, F., Pratlong, M., Aurelle, D., Mitta, G., & Toulza, E. (2018). Thermal regime and host clade, rather than geography, drive Symbiodinium and bacterial assemblages in the scleractinian coral Pocillopora damicornis sensu lato. *Microbiome*, *6*(1), 39. https://doi.org/10.1186/s40168-018-0423-6

Brucker, R. M., & Bordenstein, S. R. (2013). The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus Nasonia. *Science*, *341*(6146), 667-669. https://doi.org/10.1126/science.1240659

Bruno, A., Sandionigi, A., Panio, A., Rimoldi, S., Orizio, F., Agostinetto, G., Hasan, I., Gasco, L., Terova, G., & Labra, M. (2023). Aquaculture ecosystem microbiome at the water-fish interface: the case-study of rainbow trout fed with Tenebrio molitor novel diets. *BMC Microbiol*, *23*(1), 248. https://doi.org/10.1186/s12866-023-02990-y

Bruno, D., West, P. v., & Beakes, G. (2011). Saprolegnia and other oomycetes. *CABI*, 669–720. https://doi.org/10.1079/9781845935542.0669

Buchfink, B., Reuter, K., & Drost, H.-G. (2021). Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nature Methods*, *18*(4), 366-368. https://doi.org/10.1038/s41592-021-01101-x

Bythell, M. J. S. A. C. J. C. (2011). Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs*, *30*. https://doi.org/10.1007/s00338-010-0695-1

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*, *13*(7), 581-583. https://doi.org/10.1038/nmeth.3869

Candy, A. S., Taylor Parkins, S. K., Van Duyl, F. C., Mueller, B., Arts, M. G. I., Barnes, W., Carstensen, M., Scholten, Y. J. H., El-Khaled, Y. C., Wild, C., Wegley Kelly, L., Nelson,

C. E., Sandin, S. A., Vermeij, M. J. A., Rohwer, F. L., Picioreanu, C., Stocchi, P., & Haas, A. F. (2023). Small-scale oxygen distribution patterns in a coral reef [Original Research]. *Frontiers in Marine Science*, *Volume 10 - 2023*. https://doi.org/10.3389/fmars.2023.1135686

Capella-Gutierrez, S., Silla-Martinez, J. M., & Gabaldon, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, *25*(15), 1972-1973. https://doi.org/10.1093/bioinformatics/btp348

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A*, *108 Suppl 1*(Suppl 1), 4516-4522. https://doi.org/10.1073/pnas.1000080107

Carpenter, G. E., Alex D. Chequer, Sabrina Weber, Tali Mass, and Gretchen Goodbody-Gringley. (2022). Light and Photoacclimatization Drive Distinct Differences between Shallow and Mesophotic Coral Communities. *Ecosphere*. https://doi.org/https://doi.org/10.1002/ecs2.4200

Carpenter, G. E., Chequer, A. D., Weber, S., Mass, T., & Goodbody-Gringley, G. (2022). Light and photoacclimatization drive distinct differences between shallow and mesophotic coral communities. *Ecosphere*, *13*(8), e4200. https://doi.org/https://doi.org/10.1002/ecs2.4200

Caughman, A. M., Pratte, Z. A., Patin, N. V., & Stewart, F. J. (2021). Coral microbiome changes over the day–night cycle. *Coral Reefs*, *40*(3), 921-935. https://doi.org/10.1007/s00338-021-02097-8

Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P., & Parks, D. H. (2019). GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics*, *36*(6), 1925-1927. https://doi.org/10.1093/bioinformatics/btz848

Chen, C. A., Wallace, C. C., & Wolstenholme, J. (2002). Analysis of the mitochondrial 12S rRNA gene supports a two-clade hypothesis of the evolutionary history of scleractinian corals. *Mol Phylogenet Evol*, *23*(2), 137-149. https://doi.org/10.1016/S1055-7903(02)00008-8

Chen, Y., Li, D., Dai, Z., Piao, X., Wu, Z., Wang, B., Zhu, Y., & Zeng, Z. (2014). L-methionine supplementation maintains the integrity and barrier function of the small-intestinal

mucosa in post-weaning piglets. *Amino Acids*, *46*(4), 1131-1142. https://doi.org/10.1007/s00726-014-1675-5

Chen, Y., Ye, W., Zhang, Y., & Xu, Y. (2015). High speed BLASTN: an accelerated MegaBLAST search tool. *Nucleic Acids Res*, *43*(16), 7762-7768. https://doi.org/10.1093/nar/gkv784

Chenglong Wu, J. Y., Jun'e Gao, Lian Chen, Zhibin Lu. (2016). The effects of dietary carbohydrate on the growth, antioxidant capacities, innate immune responses and pathogen resistance of juvenile Black carp Mylopharyngodon piceus. *Fish & Shellfish Immunology*, *49*. https://doi.org/https://doi.org/10.1016/j.fsi.2015.12.030

Chiasson, M., Kirk, M., & Huyben, D. (2023). Microbial control during the incubation of rainbow trout (Oncorhynchus mykiss) eggs exposed to humic acid [Original Research]. *Frontiers in Aquaculture*, *Volume 2 - 2023*. https://doi.org/10.3389/faquc.2023.1088072

Chiou, Y. J., Chen, Y. H., Chiang, P. W., Chen, H. J., & Tang, S. L. (2023). High-Quality Genome Sequences of Two Octocoral-Associated Bacteria, Endozoicomonas euniceicola EF212(T) and Endozoicomonas gorgoniicola PS125(T). *Microbiol Resour Announc*, *12*(1), e0087722. https://doi.org/10.1128/mra.00877-22

Cinner, J. E., Huchery, C., MacNeil, M. A., Graham, N. A. J., McClanahan, T. R., Maina, J., Maire, E., Kittinger, J. N., Hicks, C. C., Mora, C., Allison, E. H., D'Agata, S., Hoey, A., Feary, D. A., Crowder, L., Williams, I. D., Kulbicki, M., Vigliola, L., Wantiez, L., . . . Mouillot, D. (2016). Bright spots among the world's coral reefs. *Nature*, *535*(7612), 416-419. https://doi.org/10.1038/nature18607

Coelho, V. R., & Manfrino, C. (2007). Coral community decline at a remote Caribbean island: marine no-take reserves are not enough. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *17*(7), 666-685. https://doi.org/https://doi.org/10.1002/aqc.822

Cox, C., Valdivia, A., McField, M., Castillo, K., & Bruno, J. F. (2017). Establishment of marine protected areas alone does not restore coral reef communities in Belize. *Marine Ecology Progress Series*, *563*, 65-79. https://www.int-res.com/abstracts/meps/v563/meps11984

Crandall, J. B., Teece, M. A., Estes, B. A., Manfrino, C., & Ciesla, J. H. (2016). Nutrient acquisition strategies in mesophotic hard corals using compound specific stable isotope analysis of sterols. *Journal of Experimental Marine Biology and Ecology*, *474*, 133-141. https://doi.org/https://doi.org/10.1016/j.jembe.2015.10.010

da Silva, D. M. G., Marques, M., Couceiro, J. F., Santos, E., Baylina, N., Costa, R., & Keller-Costa, T. (2025). Endozoicomonas lisbonensis sp. nov., a novel marine bacterium isolated from the soft coral Litophyton sp. at Oceanario de Lisboa in Portugal. *Int J Syst Evol Microbiol*, *75*(3). https://doi.org/10.1099/ijsem.0.006696

Darling, E. S., Alvarez-Filip, L., Oliver, T. A., McClanahan, T. R., Cote, I. M., & Bellwood, D. (2012). Evaluating life-history strategies of reef corals from species traits. *Ecol Lett*, *15*(12), 1378-1386. https://doi.org/10.1111/j.1461-0248.2012.01861.x

Davis, K. B. (2009). Age at puberty of channel catfish, Ictalurus punctatus, controlled by thermoperiod. *Aquaculture*, *292*(3), 244-247. https://doi.org/https://doi.org/10.1016/j.aquaculture.2009.04.023

de Bont, L., Donnay, N., Couturier, J., & Rouhier, N. (2022). Redox regulation of enzymes involved in sulfate assimilation and in the synthesis of sulfur-containing amino acids and glutathione in plants. *Front Plant Sci*, *13*, 958490. https://doi.org/10.3389/fpls.2022.958490

Deng, Y., Liang, C., Zhu, X., Zhu, X., Chen, L., Pan, H., Xun, F., Tao, Y., & Xing, P. (2024). Methylomonadaceae was the active and dominant methanotroph in Tibet lake sediments. *ISME Commun*, *4*(1), ycae032. https://doi.org/10.1093/ismeco/ycae032

Dias, K., Miranda, G. M., Bessa, J. R., Araujo, A. C. J., Freitas, P. R., Almeida, R. S., Paulo, C. L. R., Neto, J. B. A., Coutinho, H. D. M., & Ribeiro-Filho, J. (2022). Terpenes as bacterial efflux pump inhibitors: A systematic review. *Front Pharmacol*, *13*, 953982. https://doi.org/10.3389/fphar.2022.953982

Dodge, R. E., & Gilbert, T. R. (1984). Chronology of lead pollution contained in banded coral skeletons. *Marine Biology*, *82*(1), 9-13. https://doi.org/10.1007/BF00392758

Doherty, M. L., Chequer, A. D., Mass, T., & Goodbody-Gringley, G. (2024). Phenotypic variability of Montastraea cavernosa and Porites astreoides along a depth gradient from shallow to mesophotic reefs in the Cayman Islands. *Coral Reefs*, *43*(5), 1173-1187. https://doi.org/10.1007/s00338-024-02524-6

Doherty, M. L., Johnson, J. V., & Goodbody-Gringley, G. (2025). Widespread coral bleaching and mass mortality during the 2023-2024 marine heatwave in Little Cayman. *PLoS One*, *20*(5), e0322636. https://doi.org/10.1371/journal.pone.0322636

Dong, H. T., Techatanakitarnan, C., Jindakittikul, P., Thaiprayoon, A., Taengphu, S., Charoensapsri, W., Khunrae, P., Rattanarojpong, T., & Senapin, S. (2017). Aeromonas jandaei and Aeromonas veronii caused disease and mortality in Nile tilapia, Oreochromis niloticus (L.). *J Fish Dis*, *40*(10), 1395-1403. https://doi.org/10.1111/jfd.12617

Donia, M. S., Cimermancic, P., Schulze, C. J., Wieland Brown, L. C., Martin, J., Mitreva, M., Clardy, J., Linington, R. G., & Fischbach, M. A. (2014). A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. *Cell*, *158*(6), 1402-1414. https://doi.org/10.1016/j.cell.2014.08.032

Drury, C., Pérez Portela, R., Serrano, X. M., Oleksiak, M., & Baker, A. C. (2020). Fine-scale structure among mesophotic populations of the great star coral Montastraea cavernosa revealed by SNP genotyping. *Ecology and Evolution*, *10*(12), 6009-6019. https://doi.org/https://doi.org/10.1002/ece3.6340

Du, D., Wang-Kan, X., Neuberger, A., van Veen, H. W., Pos, K. M., Piddock, L. J. V., & Luisi, B. F. (2018). Multidrug efflux pumps: structure, function and regulation. *Nature Reviews Microbiology*, *16*(9), 523-539. https://doi.org/10.1038/s41579-018-0048-6

Dube, C. E., Ziegler, M., Merciere, A., Boissin, E., Planes, S., Bourmaud, C. A., & Voolstra, C. R. (2021). Naturally occurring fire coral clones demonstrate a genetic and environmental basis of microbiome composition. *Nat Commun*, *12*(1), 6402. https://doi.org/10.1038/s41467-021-26543-x

Dunham, R. A., & Elaswad, A. (2018). Catfish Biology and Farming. *Annual Review of Animal Biosciences*, *6*(Volume 6, 2018), 305-325. https://doi.org/https://doi.org/10.1146/annurev-animal-030117-014646

Estensoro, I., Ballester-Lozano, G., Benedito-Palos, L., Grammes, F., Martos-Sitcha, J. A., Mydland, L. T., Calduch-Giner, J. A., Fuentes, J., Karalazos, V., Ortiz, A., Overland, M., Sitja-Bobadilla, A., & Perez-Sanchez, J. (2016). Dietary Butyrate Helps to Restore the Intestinal Status of a Marine Teleost (Sparus aurata) Fed Extreme Diets Low in Fish Meal and Fish Oil. *PLoS One*, *11*(11), e0166564. https://doi.org/10.1371/journal.pone.0166564

Etyemez, M., & Balcazar, J. L. (2015). Bacterial community structure in the intestinal ecosystem of rainbow trout (Oncorhynchus mykiss) as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Res Vet Sci*, *100*, 8-11. https://doi.org/10.1016/j.rvsc.2015.03.026

Fernandes, K. E., Stanfield, B., Frost, E. A., Shanahan, E. R., Susantio, D., Dong, A. Z., Tran, T. D., Cokcetin, N. N., & Carter, D. A. (2023). Low Levels of Hive Stress Are Associated with Decreased Honey Activity and Changes to the Gut Microbiome of Resident Honey Bees. *Microbiol Spectr*, *11*(4), e0074223. https://doi.org/10.1128/spectrum.00742-23

Fontinha, F., Martins, N., Campos, G., Peres, H., & Oliva-Teles, A. (2024). The Effects of Short-Chain Fatty Acids in Gut Immune and Oxidative Responses of European Sea Bass (Dicentrarchus labrax): An Ex Vivo Approach. *Animals*, *14*(9), 1360. https://www.mdpi.com/2076-2615/14/9/1360

Franco, N. R., Giraldo, M. Á., López-Alvarez, D., Gallo-Franco, J. J., Dueñas, L. F., Puentes, V., & Castillo, A. (2021). Bacterial Composition and Diversity in Deep-Sea Sediments from the Southern Colombian Caribbean Sea. *Diversity*, *13*(1), 10. https://www.mdpi.com/1424-2818/13/1/10

Fujimoto, M., Crossman, J. A., Scribner, K. T., & Marsh, T. L. (2013). Microbial community assembly and succession on lake sturgeon egg surfaces as a function of simulated spawning stream flow rate. *Microb Ecol*, *66*(3), 500-511. https://doi.org/10.1007/s00248-013-0256-6

Fujimoto, M., Lovett, B., Angoshtari, R., Nirenberg, P., Loch, T. P., Scribner, K. T., & Marsh, T. L. (2018). Antagonistic Interactions and Biofilm Forming Capabilities Among Bacterial Strains Isolated from the Egg Surfaces of Lake Sturgeon (Acipenser fulvescens). *Microb Ecol*, *75*(1), 22-37. https://doi.org/10.1007/s00248-017-1013-z

Futo, M., Armitage, S. A., & Kurtz, J. (2015). Microbiota Plays a Role in Oral Immune Priming in Tribolium castaneum. *Front Microbiol*, *6*, 1383. https://doi.org/10.3389/fmicb.2015.01383

Galand, P. E., Remize, M., Meistertzheim, A. L., Pruski, A. M., Peru, E., Suhrhoff, T. J., Le Bris, N., Vetion, G., & Lartaud, F. (2020). Diet shapes cold-water corals bacterial communities. *Environ Microbiol*, *22*(1), 354-368. https://doi.org/10.1111/1462-2920.14852

Galperin, M. Y., Vera Alvarez, R., Karamycheva, S., Makarova, K. S., Wolf, Y. I., Landsman, D., & Koonin, E. V. (2025). COG database update 2024. *Nucleic Acids Res*, *53*(D1), D356-D363. https://doi.org/10.1093/nar/gkae983

Ghosh, S. K., Wong, M. K.-S., Hyodo, S., & Hamasaki, K. (2025). Shifting seas and first feeds: gut microbiome dynamics in juvenile chum salmon (Oncorhynchus keta) and their

climate vulnerability. *Current Research in Microbial Sciences*, *9*, 100452. https://doi.org/https://doi.org/10.1016/j.crmicr.2025.100452

Giatsis, C., Sipkema, D., Ramiro-Garcia, J., Bacanu, G. M., Abernathy, J., Verreth, J., Smidt, H., & Verdegem, M. (2016). Probiotic legacy effects on gut microbial assembly in tilapia larvae. *Sci Rep*, *6*, 33965. https://doi.org/10.1038/srep33965

Godoy, F., Vancanneyt, M., Martinez, M., Steinbuchel, A., Swings, J., & Rehm, B. H. A. (2003). Sphingopyxis chilensis sp. nov., a chlorophenol-degrading bacterium that accumulates polyhydroxyalkanoate, and transfer of Sphingomonas alaskensis to Sphingopyxis alaskensis comb. nov. *Int J Syst Evol Microbiol*, *53*(Pt 2), 473-477. https://doi.org/10.1099/ijs.0.02375-0

Good, J. M., Handel, M. A., & Nachman, M. W. (2008). Asymmetry and polymorphism of hybrid male sterility during the early stages of speciation in house mice. *Evolution*, *62*(1), 50-65. https://doi.org/10.1111/j.1558-5646.2007.00257.x

Goodbody-Gringley, G., & Chequer, A. D. (2025). Mesophotic reefs offer thermal refuge to the 2023 Caribbean mass bleaching event in the Cayman Islands. *Scientific Reports*, *15*(1), 16496. https://doi.org/10.1038/s41598-025-01813-6

Goodbody-Gringley, G. a. W., J. . (2018). Morphological plasticity of the depth generalist coral, Montastraea cavernosa, on mesophotic reefs in Bermuda. *Ecology*, *99*. https://doi.org/https://doi.org/10.1002/ecy.2232

Green, R., Allen, L. H., Bjorke-Monsen, A. L., Brito, A., Gueant, J. L., Miller, J. W., Molloy, A. M., Nexo, E., Stabler, S., Toh, B. H., Ueland, P. M., & Yajnik, C. (2017). Correction: Vitamin B(12) deficiency. *Nat Rev Dis Primers*, *3*, 17054. https://doi.org/10.1038/nrdp.2017.54

Greene, N. P., Kaplan, E., Crow, A., & Koronakis, V. (2018). Corrigendum: Antibiotic Resistance Mediated by the MacB ABC Transporter Family: A Structural and Functional Perspective [Correction]. *Frontiers in Microbiology*, *Volume 9 - 2018*. https://doi.org/10.3389/fmicb.2018.02318

Guo, X., Chang, L., Jin, H., Zhu, J., Tao, Y., Wu, S., & Wang, J. (2022). Converting the E. coli Isochorismatase Nicotinamidase into γ-Lactamase. *Microbiology Spectrum*, *10*(1), e00985-00921. https://doi.org/doi:10.1128/spectrum.00985-21

Guzmán, H. M., & García, E. M. (2002). Mercury levels in coral reefs along the Caribbean coast of Central America. *Marine Pollution Bulletin*, *44*(12), 1415-1420. https://doi.org/https://doi.org/10.1016/S0025-326X(02)00318-1

Hanif, A., Bakopoulos, V., & Dimitriadis, G. J. (2004). Maternal transfer of humoral specific and non-specific immune parameters to sea bream (Sparus aurata) larvae. *Fish Shellfish Immunol*, *17*(5), 411-435. https://doi.org/10.1016/j.fsi.2004.04.013

Harbi, M. A., Amran, R.H. & Satheesh, S. (2025). Importance of biofilms in water quality management in aquaculture systems—a review. https://doi.org/https://doi.org/10.1007/s10499-025-01953-6

He, F., Wu, C., Li, P., Li, N., Zhang, D., Zhu, Q., Ren, W., & Peng, Y. (2018). Functions and Signaling Pathways of Amino Acids in Intestinal Inflammation. *Biomed Res Int*, *2018*, 9171905. https://doi.org/10.1155/2018/9171905

Hebbeln, D., Wienberg, C., Dullo, W.-C., Freiwald, A., Mienis, F., Orejas, C., & Titschack, J. (2020). Cold-water coral reefs thriving under hypoxia. *Coral Reefs*, *39*(4), 853-859. https://doi.org/10.1007/s00338-020-01934-6

Hegde, S., Kumar, G., Engle, C., Hanson, T., Roy, L. A., Cheatham, M., Avery, J., Aarattuthodiyil, S., van Senten, J., Johnson, J., Wise, D., Dahl, S., Dorman, L., & Peterman, M. (2022). Technological progress in the US catfish industry. *Journal of the World Aquaculture Society*, *53*(2), 367-383. https://doi.org/https://doi.org/10.1111/jwas.12877

Hill, J. H., Franzosa, E. A., Huttenhower, C., & Guillemin, K. (2016). A conserved bacterial protein induces pancreatic beta cell expansion during zebrafish development. *eLife*, *5*, e20145. https://doi.org/10.7554/eLife.20145

Holm, J. B., & Heidelberg, K. B. (2016). Microbiomes of Muricea californica and M. fruticosa: Comparative Analyses of Two Co-occurring Eastern Pacific Octocorals. *Front Microbiol*, *7*, 917. https://doi.org/10.3389/fmicb.2016.00917

Hricová, K., Röderová, M., Fryčák, P., Pauk, V., Kurka, O., Mezerová, K., Štosová, T., Bardoň, J., Milde, D., Kučová, P., & Kolář, M. (2021). Prevalence of Vancomycin-Resistant Enterococci and Antimicrobial Residues in Wastewater and Surface Water. *Life*, *11*(12), 1403. https://www.mdpi.com/2075-1729/11/12/1403

Huang, J., Jordan, H. R., Older, C. E., Griffin, M. J., Allen, P. J., Wise, D. J., Goodman, P. M., Reifers, J. G., & Yamamoto, F. Y. (2024). Lactococcus lactis MA5 is a potential autochthonous probiotic for nutrient digestibility enhancement and bacterial pathogen inhibition in hybrid catfish (Ictalurus punctatus x I. furcatus). *J Fish Dis*, e13997. https://doi.org/10.1111/jfd.13997

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., Mende, D. R., Letunic, I., Rattei, T., Jensen, Lars J., von Mering, C., & Bork, P. (2018). eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Research*, *47*(D1), D309-D314. https://doi.org/10.1093/nar/gky1085

Hyatt, D., Chen, G.-L., LoCascio, P. F., Land, M. L., Larimer, F. W., & Hauser, L. J. (2010). Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics*, *11*(1), 119. https://doi.org/10.1186/1471-2105-11-119

Iacovelli, R., Bovenberg, R. A. L., & Driessen, A. J. M. (2021). Nonribosomal peptide synthetases and their biotechnological potential in Penicillium rubens. *J Ind Microbiol Biotechnol*, *48*(7-8). https://doi.org/10.1093/jimb/kuab045

Iijima, S., Washio, K., Okahara, R., & Morikawa, M. (2009). Biofilm formation and proteolytic activities of Pseudoalteromonas bacteria that were isolated from fish farm sediments. *Microb Biotechnol*, *2*(3), 361-369. https://doi.org/10.1111/j.1751-7915.2009.00097.x

Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J Clin Microbiol*, *45*(9), 2761-2764. https://doi.org/10.1128/JCM.01228-07

Ji, L., Zhang, H., Wang, Z., Tian, Y., Tian, W., & Liu, Z. (2024). Temperature alters bacterial community structure in sediment of mountain stream. *Scientific Reports*, *14*(1), 31159. https://doi.org/10.1038/s41598-024-82497-2

Johnson, J. S., Spakowicz, D. J., Hong, B.-Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, *10*(1), 5029. https://doi.org/10.1038/s41467-019-13036-1

Kahng, S., Copus, J. M., & Wagner, D. (2017). Mesophotic Coral Ecosystems. In S. Rossi, L. Bramanti, A. Gori, & C. Orejas (Eds.), *Marine Animal Forests: The Ecology of Benthic*

Biodiversity Hotspots (pp. 185-206). Springer International Publishing. https://doi.org/10.1007/978-3-319-21012-4_4

Kalyuhznaya, M. G., Martens-Habbena, W., Wang, T., Hackett, M., Stolyar, S. M., Stahl, D. A., Lidstrom, M. E., & Chistoserdova, L. (2009). Methylophilaceae link methanol oxidation to denitrification in freshwater lake sediment as suggested by stable isotope probing and pure culture analysis. *Environ Microbiol Rep*, *1*(5), 385-392. https://doi.org/10.1111/j.1758-2229.2009.00046.x

Kanehisa, M. (2019). Toward understanding the origin and evolution of cellular organisms. *Protein Sci*, *28*(11), 1947-1951. https://doi.org/10.1002/pro.3715

Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y., & Ishiguro-Watanabe, M. (2025). KEGG: biological systems database as a model of the real world. *Nucleic Acids Res*, *53*(D1), D672-D677. https://doi.org/10.1093/nar/gkae909

Kanehisa, M., & Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*, *28*(1), 27-30. https://doi.org/10.1093/nar/28.1.27

Kassambara, A. (2025). ggpubr: 'ggplot2' Based Publication Ready Plots. https://doi.org/10.32614/CRAN.package.ggpubr

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res*, *30*(14), 3059-3066. https://doi.org/10.1093/nar/gkf436

Kellogg, C. A., & Pratte, Z. A. (2021). Unexpected diversity of Endozoicomonas in deep-sea corals. *Marine Ecology Progress Series*, *673*, 1-15. https://www.int-res.com/abstracts/meps/v673/meps13844

Kerwin, A. H., & Nyholm, S. V. (2018). Reproductive System Symbiotic Bacteria Are Conserved between Two Distinct Populations of Euprymna scolopes from Oahu, Hawaii. *mSphere*, *3*(2). https://doi.org/10.1128/mSphere.00531-17

Kim, C. J., Kovacs-Nolan, J., Yang, C., Archbold, T., Fan, M. Z., & Mine, Y. (2009). L-cysteine supplementation attenuates local inflammation and restores gut homeostasis in a porcine model of colitis. *Biochim Biophys Acta*, *1790*(10), 1161-1169. https://doi.org/10.1016/j.bbagen.2009.05.018

Kim, K., Dobson, A., Gulland, F., & Harvell, C. (2005). Diseases and the Conservation of Marine Biodiversity. In (pp. 149-168).

King, K. C., Brockhurst, M. A., Vasieva, O., Paterson, S., Betts, A., Ford, S. A., Frost, C. L., Horsburgh, M. J., Haldenby, S., & Hurst, G. D. (2016). Rapid evolution of microbe-mediated protection against pathogens in a worm host. *ISME J*, *10*(8), 1915-1924. https://doi.org/10.1038/ismej.2015.259

Krajmalnik-Brown, R., Ilhan, Z. E., Kang, D. W., & DiBaise, J. K. (2012). Effects of gut microbes on nutrient absorption and energy regulation. *Nutr Clin Pract*, *27*(2), 201-214. https://doi.org/10.1177/0884533611436116

Krishnamurthy, H. K., Pereira, M., Bosco, J., George, J., Jayaraman, V., Krishna, K., Wang, T., Bei, K., & Rajasekaran, J. J. (2023). Gut commensals and their metabolites in health and disease [Review]. *Frontiers in Microbiology*, *Volume 14 - 2023*. https://doi.org/10.3389/fmicb.2023.1244293

Kriss, M., Hazleton, K. Z., Nusbacher, N. M., Martin, C. G., & Lozupone, C. A. (2018). Low diversity gut microbiota dysbiosis: drivers, functional implications and recovery. *Curr Opin Microbiol*, *44*, 34-40. https://doi.org/10.1016/j.mib.2018.07.003

Kruskal, W. H., & Wallis, W. A. (1952). Use of Ranks in One-Criterion Variance Analysis. *Journal of the American Statistical Association*, *47*(260), 583-621. https://doi.org/10.1080/01621459.1952.10483441

Lamb, J. B., Williamson, D. H., Russ, G. R., & Willis, B. L. (2015). Protected areas mitigate diseases of reef-building corals by reducing damage from fishing. *Ecology*, *96*(9), 2555-2567. https://doi.org/10.1890/14-1952.1

Langille, M. G., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., Clemente, J. C., Burkepile, D. E., Vega Thurber, R. L., Knight, R., Beiko, R. G., & Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol*, *31*(9), 814-821. https://doi.org/10.1038/nbt.2676

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods*, *9*(4), 357-359. https://doi.org/10.1038/nmeth.1923

Lasker, H. R., Bramanti, L., Tsounis, G., & Edmunds, P. J. (2020). Chapter Thirteen - The rise of octocoral forests on Caribbean reefs. In B. M. Riegl (Ed.), *Advances in Marine Biology* (Vol. 87, pp. 361-410). Academic Press. https://doi.org/https://doi.org/10.1016/bs.amb.2020.08.009

Laverick, J. H., Andradi-Brown, D. A., & Rogers, A. D. (2017). Using light-dependent scleractinia to define the upper boundary of mesophotic coral ecosystems on the reefs of Utila, Honduras. *PLoS One*, *12*(8), e0183075. https://doi.org/10.1371/journal.pone.0183075

Laverick, J. H., Tamir, R., Eyal, G., & Loya, Y. (2020). A generalized light-driven model of community transitions along coral reef depth gradients. *Global Ecology and Biogeography*, *29*(9), 1554-1564. https://doi.org/https://doi.org/10.1111/geb.13140

Lesser, M. P., Slattery, M., & Leichter, J. J. (2009). Ecology of mesophotic coral reefs. *Journal of Experimental Marine Biology and Ecology*, *375*(1), 1-8. https://doi.org/https://doi.org/10.1016/j.jembe.2009.05.009

Letunic, I., & Bork, P. (2007). Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics*, *23*(1), 127-128. https://doi.org/10.1093/bioinformatics/btl529

Levy, M., Kolodziejczyk, A. A., Thaiss, C. A., & Elinav, E. (2017). Dysbiosis and the immune system. *Nat Rev Immunol*, *17*(4), 219-232. https://doi.org/10.1038/nri.2017.7

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & Subgroup, G. P. D. P. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078-2079. https://doi.org/10.1093/bioinformatics/btp352

Li, H., Lu, L., Chen, R., Li, S., & Xu, D. (2021). Exploring Sexual Dimorphism in the Intestinal Microbiota of the Yellow Drum (Nibea albiflora, Sciaenidae). *Front Microbiol*, *12*, 808285. https://doi.org/10.3389/fmicb.2021.808285

Li, M. H., Robinson, E. H., Bosworth, B. G., & Torrans, E. L. (2014). Growth and Feed Conversion Ratio of Pond-Raised Hybrid Catfish Harvested at Different Sizes. *North American Journal of Aquaculture*, *76*(3), 261-264. https://doi.org/https://doi.org/10.1080/15222055.2014.911225

Li, S., Guo, J., Reva, A., Huang, F., Xiong, B., Liu, Y., Deng, Z., Leadlay, P. F., & Sun, Y. (2018). Methyltransferases of gentamicin biosynthesis. *Proc Natl Acad Sci U S A*, *115*(6), 1340-1345. https://doi.org/10.1073/pnas.1711603115

Li, T., Raza, S. H. A., Yang, B., Sun, Y., Wang, G., Sun, W., Qian, A., Wang, C., Kang, Y., & Shan, X. (2020). Aeromonas veronii Infection in Commercial Freshwater Fish: A Potential Threat to Public Health. *Animals (Basel)*, *10*(4). https://doi.org/10.3390/ani10040608

Li, T. T., Tian, W. L., & Gu, C. T. (2019). Elevation of Lactococcus lactis subsp. cremoris to the species level as Lactococcus cremoris sp. nov. and transfer of Lactococcus lactis subsp. tructae to Lactococcus cremoris as Lactococcus cremoris subsp. tructae comb. nov. *Int J Syst Evol Microbiol*, *71*(3). https://doi.org/10.1099/ijsem.0.004727

Li, W., Liu, J., Tan, H., Yang, C., Ren, L., Liu, Q., Wang, S., Hu, F., Xiao, J., Zhao, R., Tao, M., Zhang, C., Qin, Q., & Liu, S. (2018). Genetic Effects on the Gut Microbiota Assemblages of Hybrid Fish From Parents With Different Feeding Habits. *Front Microbiol*, *9*, 2972. https://doi.org/10.3389/fmicb.2018.02972

Li, Z., Wright, A. G., Si, H., Wang, X., Qian, W., Zhang, Z., & Li, G. (2016). Changes in the rumen microbiome and metabolites reveal the effect of host genetics on hybrid crosses. *Environ Microbiol Rep*, *8*(6), 1016-1023. https://doi.org/10.1111/1758-2229.12482

Lin, H., & Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications*, *11*(1), 3514. https://doi.org/10.1038/s41467-020-17041-7

Lin, J., Wang, T., Zhou, Y., Cheng, T., Liu, H., Chen, X., Luo, W., Liu, Q., Chu, Y., Wang, X., Xie, F., Sha, J., & Song, T. (2025). One-step degradation of 4 classes of β-lactam using β-lactamase enzyme cocktail. *Scientific Reports*, *15*(1), 34701. https://doi.org/10.1038/s41598-025-16947-w

Liu, G., Yu, L., Fang, J., Hu, C. A., Yin, J., Ni, H., Ren, W., Duraipandiyan, V., Chen, S., Al-Dhabi, N. A., & Yin, Y. (2017). Methionine restriction on oxidative stress and immune response in dss-induced colitis mice. *Oncotarget*, *8*(27), 44511-44520. https://doi.org/10.18632/oncotarget.17812

Liu, Y., de Bruijn, I., Jack, A. L., Drynan, K., van den Berg, A. H., Thoen, E., Sandoval-Sierra, V., Skaar, I., van West, P., Dieguez-Uribeondo, J., van der Voort, M., Mendes, R., Mazzola, M., & Raaijmakers, J. M. (2014). Deciphering microbial landscapes of fish

eggs to mitigate emerging diseases. *ISME J*, *8*(10), 2002-2014. https://doi.org/10.1038/ismej.2014.44

Liu, Z., Liu, S., Yao, J., Bao, L., Zhang, J., Li, Y., Jiang, C., Sun, L., Wang, R., Zhang, Y., Zhou, T., Zeng, Q., Fu, Q., Gao, S., Li, N., Koren, S., Jiang, Y., Zimin, A., Xu, P., . . . Waldbieser, G. C. (2016). The channel catfish genome sequence provides insights into the evolution of scale formation in teleosts. *Nature Communications*, *7*(1), 11757. https://doi.org/10.1038/ncomms11757

Lokesh, J., Kiron, V., Sipkema, D., Fernandes, J. M. O., & Moum, T. (2019). Succession of embryonic and the intestinal bacterial communities of Atlantic salmon (Salmo salar) reveals stage-specific microbial signatures. *Microbiologyopen*, *8*(4), e00672. https://doi.org/10.1002/mbo3.672

López-Aladid, R., Fernández-Barat, L., Alcaraz-Serrano, V., Bueno-Freire, L., Vázquez, N., Pastor-Ibáñez, R., Palomeque, A., Oscanoa, P., & Torres, A. (2023). Determining the most accurate 16S rRNA hypervariable region for taxonomic identification from respiratory samples. *Scientific Reports*, *13*(1), 3974. https://doi.org/10.1038/s41598-023-30764-z

Louis, P., & Flint, H. J. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett*, *294*(1), 1-8. https://doi.org/10.1111/j.1574-6968.2009.01514.x

Lozupone, C. A., Li, M., Campbell, T. B., Flores, S. C., Linderman, D., Gebert, M. J., Knight, R., Fontenot, A. P., & Palmer, B. E. (2013). Alterations in the gut microbiota associated with HIV-1 infection. *Cell Host Microbe*, *14*(3), 329-339. https://doi.org/10.1016/j.chom.2013.08.006

Mahizan, N. A., Yang, S. K., Moo, C. L., Song, A. A., Chong, C. M., Chong, C. W., Abushelaibi, A., Lim, S. E., & Lai, K. S. (2019). Terpene Derivatives as a Potential Agent against Antimicrobial Resistance (AMR) Pathogens. *Molecules*, *24*(14). https://doi.org/10.3390/molecules24142631

Maina, J. N. (2002). Structure, function and evolution of the gas exchangers: comparative perspectives. *Journal of Anatomy*, *201*(4), 281-304. https://doi.org/https://doi.org/10.1046/j.1469-7580.2002.00099.x

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnetjournal*. https://doi.org/https://doi.org/10.14806/ej.17.1.200

Martínez-Núñez, M. A., & Orozco-Ramírez, Q. (2024). Characterizing Bacterial Communities in Agroecosystems of the UNESCO Global Geopark Mixteca Alta, Oaxaca. *Agriculture*, *14*(12), 2180. https://www.mdpi.com/2077-0472/14/12/2180

McCallum, H., Harvell, D., & Dobson, A. (2003). Rates of spread of marine pathogens. *Ecology Letters*, *6*(12), 1062-1067. https://doi.org/https://doi.org/10.1046/j.1461-0248.2003.00545.x

McCauley, M., Jackson, C. R., & Goulet, T. L. (2020). Microbiomes of Caribbean Octocorals Vary Over Time but Are Resistant to Environmental Change. *Front Microbiol*, *11*, 1272. https://doi.org/10.3389/fmicb.2020.01272

McDermott, A. J., & Huffnagle, G. B. (2014). The microbiome and regulation of mucosal immunity. *Immunology*, *142*(1), 24-31. https://doi.org/10.1111/imm.12231

McFadden, C. S., Erickson, K. L., Lane, A., Nassongole, B., Aguilar, S., Dunakey, S. K., Durkin, K. M., Lalas, J. A. A., Kushida, Y., Macrina, L., Minor, N. P., Morales-Paredes, M., Nelson, J., Peddada, A., Poole, S., Porto, R., Purow-Ruderman, R., Snyder, K. E., Wismar, T., . . . Benayahu, Y. (2025). Biodiversity and biogeography of zooxanthellate soft corals across the Indo-Pacific. *Sci Rep*, *15*(1), 15461. https://doi.org/10.1038/s41598-025-98790-7

McIlwaine, D. B., Moore, M., Corrigan, A., Niemaseck, B., & Nicoletti, D. (2024). A Comparison of the Microbial Populations in a Culture-Dependent and a Culture-Independent Analysis of Industrial Water Samples. *Applied Microbiology*, *4*(3), 1079-1090. https://www.mdpi.com/2673-8007/4/3/73

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

McMurtrie, J., Bell, A. G., Cable, J., Temperton, B., & Tyler, C. R. (2025). The ecology and plasticity of fish skin and gill microbiomes: seeking what matters in health and disease. *FEMS Microbiology Reviews*, *49*. https://doi.org/10.1093/femsre/fuaf027

Medema, M. H., Blin, K., Cimermancic, P., de Jager, V., Zakrzewski, P., Fischbach, M. A., Weber, T., Takano, E., & Breitling, R. (2011). antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Research*, *39*(suppl_2), W339-W346. https://doi.org/10.1093/nar/gkr466

Meyburgh, C. M., Bragg, R. R., & Boucher, C. E. (2017). Lactococcus garvieae: an emerging bacterial pathogen of fish. *Dis Aquat Organ*, *123*(1), 67-79. https://doi.org/10.3354/dao03083

Meyer, J. L., Paul, V. J., & Teplitski, M. (2014). Community shifts in the surface microbiomes of the coral Porites astreoides with unusual lesions. *PLoS One*, *9*(6), e100316. https://doi.org/10.1371/journal.pone.0100316

Miljkovic, M., Strahinic, I., Tolinacki, M., Zivkovic, M., Kojic, S., Golic, N., & Kojic, M. (2015). AggLb Is the Largest Cell-Aggregation Factor from Lactobacillus paracasei Subsp. paracasei BGNJ1-64, Functions in Collagen Adhesion, and Pathogen Exclusion In Vitro. *PLoS One*, *10*(5), e0126387. https://doi.org/10.1371/journal.pone.0126387

Miller, A. K., Westlake, C. S., Cross, K. L., Leigh, B. A., & Bordenstein, S. R. (2021). The microbiome impacts host hybridization and speciation. *PLoS Biol*, *19*(10), e3001417. https://doi.org/10.1371/journal.pbio.3001417

Miller, W. J., Ehrman, L., & Schneider, D. (2010). Infectious speciation revisited: impact of symbiont-depletion on female fitness and mating behavior of Drosophila paulistorum. *PLoS Pathog*, *6*(12), e1001214. https://doi.org/10.1371/journal.ppat.1001214

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution*, *37*(5), 1530-1534. https://doi.org/10.1093/molbev/msaa015

Mohamed, A. R., Ochsenkuhn, M. A., Kazlak, A. M., Moustafa, A., & Amin, S. A. (2023). The coral microbiome: towards an understanding of the molecular mechanisms of coral-microbiota interactions. *FEMS Microbiol Rev*, *47*(2). https://doi.org/10.1093/femsre/fuad005

Morrow, K. M., Moss, A. G., Chadwick, N. E., & Liles, M. R. (2012). Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. *Appl Environ Microbiol*, *78*(18), 6438-6449. https://doi.org/10.1128/AEM.01162-12

Morshed, S. M., & Lee, T. H. (2023). The role of the microbiome on fish mucosal immunity under changing environments. *Fish Shellfish Immunol*, *139*, 108877. https://doi.org/10.1016/j.fsi.2023.108877

Moschos, S., Kormas, K. A., & Karayanni, H. (2022). Prokaryotic diversity in marine and freshwater recirculating aquaculture systems. *Reviews in Aquaculture*, *14*(4), 1861-1886. https://doi.org/https://doi.org/10.1111/raq.12677

Mostafa, A. A., Al-Askar, A. A., & Taha Yassin, M. (2020). Anti-saprolegnia potency of some plant extracts against Saprolegnia diclina, the causative agent of saprolengiasis. *Saudi J Biol Sci*, *27*(6), 1482-1487. https://doi.org/10.1016/j.sjbs.2020.04.008

Mougin, J., Midelet, G., Leterme, S., Best, G., Ells, T., Joyce, A., Whiley, H., & Brauge, T. (2023). Benzalkonium chloride disinfectant residues stimulate biofilm formation and increase survival of Vibrio bacterial pathogens. *Front Microbiol*, *14*, 1309032. https://doi.org/10.3389/fmicb.2023.1309032

Mukai, K., Mohapatra, S., Matsuyama, M., Ohta, K., & Chakraborty, T. (2024). Exposure effects of benzalkonium chloride (BAC) on gonadal physiology and fertility suppression in medaka (Oryzias latipes). *Environmental Pollution*, *361*, 124778. https://doi.org/https://doi.org/10.1016/j.envpol.2024.124778

Mumby, P. J., Dahlgren, C. P., Harborne, A. R., Kappel, C. V., Micheli, F., Brumbaugh, D. R., Holmes, K. E., Mendes, J. M., Broad, K., Sanchirico, J. N., Buch, K., Box, S., Stoffle, R. W., & Gill, A. B. (2006). Fishing, Trophic Cascades, and the Process of Grazing on Coral Reefs. *Science*, *311*(5757), 98-101. https://doi.org/doi:10.1126/science.1121129

Mumby, P. J., & Harborne, A. R. (2010). Marine reserves enhance the recovery of corals on Caribbean reefs. *PLoS One*, *5*(1), e8657. https://doi.org/10.1371/journal.pone.0008657

Mumby, P. J., Harborne, A. R., Williams, J., Kappel, C. V., Brumbaugh, D. R., Micheli, F., Holmes, K. E., Dahlgren, C. P., Paris, C. B., & Blackwell, P. G. (2007). Trophic cascade facilitates coral recruitment in a marine reserve. *Proc Natl Acad Sci U S A*, *104*(20), 8362-8367. https://doi.org/10.1073/pnas.0702602104

Najafpour, B., Pinto, P. I. S., Moutou, K. A., Canario, A. V. M., & Power, D. M. (2021). Factors Driving Bacterial Microbiota of Eggs from Commercial Hatcheries of European Seabass and Gilthead Seabream. *Microorganisms*, *9*(11). https://doi.org/10.3390/microorganisms9112275

Neave, M. J., Apprill, A., Ferrier-Pages, C., & Voolstra, C. R. (2016). Diversity and function of prevalent symbiotic marine bacteria in the genus Endozoicomonas. *Appl Microbiol Biotechnol*, *100*(19), 8315-8324. https://doi.org/10.1007/s00253-016-7777-0

Neave, M. J., Michell, C. T., Apprill, A., & Voolstra, C. R. (2017). Endozoicomonas genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. *Sci Rep*, *7*, 40579. https://doi.org/10.1038/srep40579

Neish, A. S. (2014). Mucosal immunity and the microbiome. *Ann Am Thorac Soc*, *11 Suppl 1*(Suppl 1), S28-32. https://doi.org/10.1513/AnnalsATS.201306-161MG

Nemergut, D. R., Schmidt, S. K., Fukami, T., O'Neill, S. P., Bilinski, T. M., Stanish, L. F., Knelman, J. E., Darcy, J. L., Lynch, R. C., Wickey, P., & Ferrenberg, S. (2013). Patterns and processes of microbial community assembly. *Microbiol Mol Biol Rev*, *77*(3), 342-356. https://doi.org/10.1128/MMBR.00051-12

Niikura, H., Maruyama, C., Ogasawara, Y., Shin-ya, K., Dairi, T., & Hamano, Y. (2018). Functional analysis of methyltransferases participating in streptothricin-related antibiotic biosynthesis. *Journal of Bioscience and Bioengineering*, *125*(2), 148-154. https://doi.org/https://doi.org/10.1016/j.jbiosc.2017.09.004

Nurk, S., Meleshko, D., Korobeynikov, A., & Pevzner, P. A. (2017). metaSPAdes: a new versatile metagenomic assembler. *Genome Res*, *27*(5), 824-834. https://doi.org/10.1101/gr.213959.116

Nyholm, S. V. (2020). In the beginning: egg-microbe interactions and consequences for animal hosts. *Philos Trans R Soc Lond B Biol Sci*, *375*(1808), 20190593. https://doi.org/10.1098/rstb.2019.0593

Nyholm, S. V., & McFall-Ngai, M. J. (2021). A lasting symbiosis: how the Hawaiian bobtail squid finds and keeps its bioluminescent bacterial partner. *Nat Rev Microbiol*, *19*(10), 666-679. https://doi.org/10.1038/s41579-021-00567-y

Oksanen, J. S., G. L.; Blanchet, F. G.; Kindt, R.;Legendre, P.; Minchin, P. R.; O'Hara, R.B.; Solymos, P. M.; Stevens, H. H.; Szoecs, E.; Wagner, H.; Barbour, M.; Bedward, M.; Bolker, B.; Borcard, D.; et al. . (2022). vegan: Community Ecology Package. https://CRAN.R-project.org/package=vegan

Older, C. E., Griffin, M. J., Richardson, B. M., Waldbieser, G. C., Reifers, J. G., Goodman, P. M., Ware, C., Gatlin, D. M., 3rd, Wise, D. J., & Yamamoto, F. Y. (2024). Influence of probiotic and prebiotic supplementation on intestinal microbiota and resistance to Edwardsiella ictaluri infection in channel catfish (Ictalurus punctatus) following florfenicol administration. *J Fish Dis*, *47*(4), e13910. https://doi.org/10.1111/jfd.13910

Olm, M. R., Brown, C. T., Brooks, B., & Banfield, J. F. (2017). dRep: a tool for fast and accurate genomic comparisons that enables improved genome recovery from metagenomes through de-replication. *The ISME Journal*, *11*(12), 2864-2868. https://doi.org/10.1038/ismej.2017.126

Org, E., Parks, B. W., Joo, J. W., Emert, B., Schwartzman, W., Kang, E. Y., Mehrabian, M., Pan, C., Knight, R., Gunsalus, R., Drake, T. A., Eskin, E., & Lusis, A. J. (2015). Genetic and environmental control of host-gut microbiota interactions. *Genome Res*, *25*(10), 1558-1569. https://doi.org/10.1101/gr.194118.115

Padilla, C. C., Ganesh, S., Gantt, S., Huhman, A., Parris, D. J., Sarode, N., & Stewart, F. J. (2015). Standard filtration practices may significantly distort planktonic microbial diversity estimates. *Front Microbiol*, *6*, 547. https://doi.org/10.3389/fmicb.2015.00547

Paleczny, M., Hammill, E., Karpouzi, V., & Pauly, D. (2015). Population Trend of the World's Monitored Seabirds, 1950-2010. *PLoS One*, *10*(6), e0129342. https://doi.org/10.1371/journal.pone.0129342

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol*, *18*(5), 1403-1414. https://doi.org/10.1111/1462-2920.13023

Paralika, V., & Makridis, P. (2025). Microbial Interactions in Rearing Systems for Marine Fish Larvae. *Microorganisms*, *13*(3), 539. https://www.mdpi.com/2076-2607/13/3/539

Pavic, D., Grbin, D., Hudina, S., Prosenc Zmrzljak, U., Miljanovic, A., Kosir, R., Varga, F., Curko, J., Marcic, Z., & Bielen, A. (2022). Tracing the oomycete pathogen Saprolegnia parasitica in aquaculture and the environment. *Sci Rep*, *12*(1), 16646. https://doi.org/10.1038/s41598-022-16553-0

Penesyan, A., Paulsen, I. T., Kjelleberg, S., & Gillings, M. R. (2021). Three faces of biofilms: a microbial lifestyle, a nascent multicellular organism, and an incubator for diversity. *NPJ Biofilms Microbiomes*, *7*(1), 80. https://doi.org/10.1038/s41522-021-00251-2

Perez, T., Balcazar, J. L., Peix, A., Valverde, A., Velazquez, E., de Blas, I., & Ruiz-Zarzuela, I. (2011). Lactococcus lactis subsp. tructae subsp. nov. isolated from the intestinal mucus of brown trout (Salmo trutta) and rainbow trout (Oncorhynchus mykiss). *Int J Syst Evol Microbiol*, *61*(Pt 8), 1894-1898. https://doi.org/10.1099/ijs.0.023945-0

Pérez-Rosales, G., Pichon, M., Rouzé, H., Villéger, S., Torda, G., Bongaerts, P., Carlot, J., Consortium, U. T. P., Parravicini, V., & Hédouin, L. (2022). Mesophotic coral ecosystems of French Polynesia are hotspots of alpha and beta generic diversity for scleractinian assemblages. *Diversity and Distributions*, *28*(7), 1391-1403. https://doi.org/https://doi.org/10.1111/ddi.13549

Pike, R. E., Haltli, B., & Kerr, R. G. (2013). Description of Endozoicomonas euniceicola sp. nov. and Endozoicomonas gorgoniicola sp. nov., bacteria isolated from the octocorals Eunicea fusca and Plexaura sp., and an emended description of the genus Endozoicomonas. *Int J Syst Evol Microbiol*, *63*(Pt 11), 4294-4302. https://doi.org/10.1099/ijs.0.051490-0

Pindling, S., Azulai, D., Zheng, B., Dahan, D., & Perron, G. G. (2018). Dysbiosis and early mortality in zebrafish larvae exposed to subclinical concentrations of streptomycin. *FEMS Microbiol Lett*, *365*(18). https://doi.org/10.1093/femsle/fny188

Pogoreutz, C., Oakley, C. A., Radecker, N., Cardenas, A., Perna, G., Xiang, N., Peng, L., Davy, S. K., Ngugi, D. K., & Voolstra, C. R. (2022). Coral holobiont cues prime Endozoicomonas for a symbiotic lifestyle. *ISME J*, *16*(8), 1883-1895. https://doi.org/10.1038/s41396-022-01226-7

Pogoreutz, C., Radecker, N., Cardenas, A., Gardes, A., Wild, C., & Voolstra, C. R. (2018). Dominance of Endozoicomonas bacteria throughout coral bleaching and mortality suggests structural inflexibility of the Pocillopora verrucosa microbiome. *Ecol Evol*, *8*(4), 2240-2252. https://doi.org/10.1002/ece3.3830

Pollock, F. J., McMinds, R., Smith, S., Bourne, D. G., Willis, B. L., Medina, M., Thurber, R. V., & Zaneveld, J. R. (2018). Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nat Commun*, *9*(1), 4921. https://doi.org/10.1038/s41467-018-07275-x

Ponnusamy, K., Choi, J. N., Kim, J., Lee, S. Y., & Lee, C. H. (2011). Microbial community and metabolomic comparison of irritable bowel syndrome faeces. *J Med Microbiol*, *60*(Pt 6), 817-827. https://doi.org/10.1099/jmm.0.028126-0

Posit team. (2023). RStudio: Integrated Development Environment for R. http://www.posit.co/

Pratte Z.A., L. G. O., Burns A.S., Hay M.E. & Stewart F.J. . (2017). Contact with turf algae alters the coral microbiome: contact versus systemic impacts. *Coral Reefs*, *37*. https://doi.org/https://doi.org/10.1007/s00338-017-1615-4

Pratte, Z. A., Besson, M., Hollman, R. D., & Stewart, F. J. (2018). The Gills of Reef Fish Support a Distinct Microbiome Influenced by Host-Specific Factors. *Appl Environ Microbiol*, *84*(9). https://doi.org/10.1128/AEM.00063-18

Pratte, Z. A., Stewart, F. J., & Kellogg, C. A. (2023). Functional gene composition and metabolic potential of deep-sea coral-associated microbial communities. *Coral Reefs*, *42*(5), 1011-1023. https://doi.org/10.1007/s00338-023-02409-0

Price, D. R., Feng, H., Baker, J. D., Bavan, S., Luetje, C. W., & Wilson, A. C. (2014). Aphid amino acid transporter regulates glutamine supply to intracellular bacterial symbionts. *Proc Natl Acad Sci U S A*, *111*(1), 320-325. https://doi.org/10.1073/pnas.1306068111

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2--approximately maximum-likelihood trees for large alignments. *PLoS One*, *5*(3), e9490. https://doi.org/10.1371/journal.pone.0009490

Qi, X., Zhang, Y., Zhang, Y., Luo, F., Song, K., Wang, G., & Ling, F. (2023). Vitamin B(12) produced by Cetobacterium somerae improves host resistance against pathogen infection through strengthening the interactions within gut microbiota. *Microbiome*, *11*(1), 135. https://doi.org/10.1186/s40168-023-01574-2

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glockner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*, *41*(Database issue), D590-596. https://doi.org/10.1093/nar/gks1219

Quigley, K. M., Alvarez Roa, C., Torda, G., Bourne, D. G., & Willis, B. L. (2020). Co-dynamics of Symbiodiniaceae and bacterial populations during the first year of symbiosis with Acropora tenuis juveniles. *Microbiologyopen*, *9*(2), e959. https://doi.org/10.1002/mbo3.959

Reichley, S. R., Ware, C., Khoo, L. H., Greenway, T. E., Wise, D. J., Bosworth, B. G., Lawrence, M. L., & Griffin, M. J. (2018). Comparative Susceptibility of Channel Catfish, Ictalurus punctatus; Blue Catfish, Ictalurus furcatus; and Channel (♀) × Blue (♂) Hybrid Catfish to Edwardsiella piscicida, Edwardsiella tarda, and Edwardsiella anguillarum. *Journal of the World Aquaculture Society*, *49*(1), 197-204. https://doi.org/https://doi.org/10.1111/jwas.12467

Reichley, S. R., Ware, C., Steadman, J., Gaunt, P. S., Garcia, J. C., LaFrentz, B. R., Thachil, A., Waldbieser, G. C., Stine, C. B., Bujan, N., Arias, C. R., Loch, T., Welch, T. J., Cipriano,

R. C., Greenway, T. E., Khoo, L. H., Wise, D. J., Lawrence, M. L., & Griffin, M. J. (2017). Comparative Phenotypic and Genotypic Analysis of Edwardsiella Isolates from Different Hosts and Geographic Origins, with Emphasis on Isolates Formerly Classified as E. tarda, and Evaluation of Diagnostic Methods. *J Clin Microbiol*, *55*(12), 3466-3491. https://doi.org/10.1128/JCM.00970-17

Rodriguez, M., Segumalian, C., Lalas, J., & Maningas, J. (2020). Octocorals outcompete scleractinian corals in a degraded reef. IOP Conference Series: Earth and Environmental Science,

Romero, J., & Navarrete, P. (2006). 16S rDNA-based analysis of dominant bacterial populations associated with early life stages of coho salmon (Oncorhynchus kisutch). *Microb Ecol*, *51*(4), 422-430. https://doi.org/10.1007/s00248-006-9037-9

Rosales, S. M., Clark, A. S., Huebner, L. K., Ruzicka, R. R., & Muller, E. M. (2020). Rhodobacterales and Rhizobiales Are Associated With Stony Coral Tissue Loss Disease and Its Suspected Sources of Transmission. *Front Microbiol*, *11*, 681. https://doi.org/10.3389/fmicb.2020.00681

Rubin, M. A., & Leff, L. G. (2007). Nutrients and Other Abiotic Factors Affecting Bacterial Communities in an Ohio River (USA). *Microbial Ecology*, *54*(2), 374-383. https://doi.org/10.1007/s00248-007-9209-2

Samuel T N Aroney , R. J. P. N., Jakob N Nissen , Antonio Pedro Camargo , Gene W Tyson , Ben J Woodcroft. (2025). CoverM: read alignment statistics for metagenomics. *Bioinformatics*, *41*(4). https://doi.org/https://doi.org/10.1093/bioinformatics/btaf147

Sanz-Lazaro, C., Navarrete-Mier, F., & Marin, A. (2011). Biofilm responses to marine fish farm wastes. *Environ Pollut*, *159*(3), 825-832. https://doi.org/10.1016/j.envpol.2010.10.035

Schreiber, L., Kjeldsen, K. U., Obst, M., Funch, P., & Schramm, A. (2016). Description of Endozoicomonas ascidiicola sp. nov., isolated from Scandinavian ascidians. *Syst Appl Microbiol*, *39*(5), 313-318. https://doi.org/10.1016/j.syapm.2016.05.008

Scully, E. D., Geib, S. M., Carlson, J. E., Tien, M., McKenna, D., & Hoover, K. (2014). Functional genomics and microbiome profiling of the Asian longhorned beetle (Anoplophora glabripennis) reveal insights into the digestive physiology and nutritional ecology of wood feeding beetles. *BMC Genomics*, *15*(1), 1096. https://doi.org/10.1186/1471-2164-15-1096

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, *30*(14), 2068-2069. https://doi.org/10.1093/bioinformatics/btu153

Sehnal, L., Brammer-Robbins, E., Wormington, A. M., Blaha, L., Bisesi, J., Larkin, I., Martyniuk, C. J., Simonin, M., & Adamovsky, O. (2021). Microbiome Composition and Function in Aquatic Vertebrates: Small Organisms Making Big Impacts on Aquatic Animal Health. *Front Microbiol*, *12*, 567408. https://doi.org/10.3389/fmicb.2021.567408

Sevellec, M., Laporte, M., Bernatchez, A., Derome, N., & Bernatchez, L. (2019). Evidence for host effect on the intestinal microbiota of whitefish (Coregonus sp.) species pairs and their hybrids. *Ecol Evol*, *9*(20), 11762-11774. https://doi.org/10.1002/ece3.5676

Shahin, K., Mukkatira, K., Yazdi, Z., Richey, C., Kwak, K., Heckman, T. I., Mohammed, H. H., Ortega, C., Avendano-Herrera, R., Keleher, B., Hyatt, M. W., Drennan, J. D., Adkison, M., Griffin, M. J., & Soto, E. (2022). Development of a quantitative polymerase chain reaction assay for detection of the aetiological agents of piscine lactococcosis. *J Fish Dis*, *45*(6), 847-859. https://doi.org/10.1111/jfd.13610

Shahin, K., Veek, T., Heckman, T. I., Littman, E., Mukkatira, K., Adkison, M., Welch, T. J., Imai, D. M., Pastenkos, G., Camus, A., & Soto, E. (2022). Isolation and characterization of Lactococcus garvieae from rainbow trout, Onchorhyncus mykiss, from California, USA. *Transbound Emerg Dis*, *69*(4), 2326-2343. https://doi.org/10.1111/tbed.14250

Shan, J., Tian, X., Guan, C., Zhang, C., Zhang, Y., & Chen, S. (2022). Effect of Copper Ion Sterilization on Bacterial Community in a Freshwater Recirculating Aquaculture System. *Curr Microbiol*, *79*(2), 58. https://doi.org/10.1007/s00284-021-02707-2

Sharma, M., Khurana, H., Singh, D. N., & Negi, R. K. (2021). The genus Sphingopyxis: Systematics, ecology, and bioremediation potential - A review. *Journal of Environmental Management*, *280*, 111744. https://doi.org/https://doi.org/10.1016/j.jenvman.2020.111744

She, Y., Qi, X., Xin, X., He, Y., Wang, W., & Li, Z. (2023). Insights into microbial interactive mechanism regulating dissimilatory nitrate reduction processes in riparian freshwater aquaculture sediments. *Environmental Research*, *216*, 114593. https://doi.org/https://doi.org/10.1016/j.envres.2022.114593

Shen, Q., Zhu, X., Zhao, Q., Li, S., Wang, Y., Xue, J., & Wang, P. (2021). QuEChERS and 96-well plate solid phase extraction for determination of vancomycin and norvancomycin in

fish meat by UPLC–MS/MS. *Food Chemistry*, *342*, 128326. https://doi.org/https://doi.org/10.1016/j.foodchem.2020.128326

Shetty, L. L. S. (2012-2019). microbiome R package. https://doi.org/10.18129/B9.bioc.microbiome

Shetty, S. A., & Lahti, L. (2020). microbiomeutilities: Utilities for Microbiome Analytics. https://microsud.github.io/microbiomeutilities/index.html

Sheu, S. Y., Lin, K. R., Hsu, M. Y., Sheu, D. S., Tang, S. L., & Chen, W. M. (2017). Endozoicomonas acroporae sp. nov., isolated from Acropora coral. *Int J Syst Evol Microbiol*, *67*(10), 3791-3797. https://doi.org/10.1099/ijsem.0.002194

Shinzato, C., Narisoko, H., Nishitsuji, K., Nagata, T., Satoh, N., & Inoue, J. (2021). Novel Mitochondrial DNA Markers for Scleractinian Corals and Generic-Level Environmental DNA Metabarcoding [Original Research]. *Frontiers in Marine Science*, *Volume 8 - 2021*. https://doi.org/10.3389/fmars.2021.758207

Silo-Suh, L. A., Lethbridge, B. J., Raffel, S. J., He, H., Clardy, J., & Handelsman, J. (1994). Biological activities of two fungistatic antibiotics produced by Bacillus cereus UW85. *Appl Environ Microbiol*, *60*(6), 2023-2030. https://doi.org/10.1128/aem.60.6.2023-2030.1994

Skinnider, M. A., Johnston, C. W., Edgar, R. E., Dejong, C. A., Merwin, N. J., Rees, P. N., & Magarvey, N. A. (2016). Genomic charting of ribosomally synthesized natural product chemical space facilitates targeted mining. *Proc Natl Acad Sci U S A*, *113*(42), E6343-E6351. https://doi.org/10.1073/pnas.1609014113

Slattery, M., Lesser, M. P., Rocha, L. A., Spalding, H. L., & Smith, T. B. (2024). Function and stability of mesophotic coral reefs. *Trends in Ecology & Evolution*, *39*(6), 585-598. https://doi.org/https://doi.org/10.1016/j.tree.2024.01.011

Soler, G. A., Edgar, G. J., Thomson, R. J., Kininmonth, S., Campbell, S. J., Dawson, T. P., Barrett, N. S., Bernard, A. T. F., Galván, D. E., Willis, T. J., Alexander, T. J., & Stuart-Smith, R. D. (2015). Reef Fishes at All Trophic Levels Respond Positively to Effective Marine Protected Areas. *PLoS One*, *10*(10), e0140270. https://doi.org/10.1371/journal.pone.0140270

Song, Z., Tong, G., Xiao, K., Jiao le, F., Ke, Y., & Hu, C. (2016). L-cysteine protects intestinal integrity, attenuates intestinal inflammation and oxidant stress, and modulates NF-kappaB and Nrf2 pathways in weaned piglets after LPS challenge. *Innate Immun*, *22*(3), 152-161. https://doi.org/10.1177/1753425916632303

Sonnenburg, E. D., Smits, S. A., Tikhonov, M., Higginbottom, S. K., Wingreen, N. S., & Sonnenburg, J. L. (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature*, *529*(7585), 212-215. https://doi.org/10.1038/nature16504

Srinivasan, S., Morgan, M. T., Fiedler, T. L., Djukovic, D., Hoffman, N. G., Raftery, D., Marrazzo, J. M., & Fredricks, D. N. (2015). Metabolic signatures of bacterial vaginosis. *mBio*, *6*(2). https://doi.org/10.1128/mBio.00204-15

Staley, J. T., & Konopka, A. (1985). Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu Rev Microbiol*, *39*, 321-346. https://doi.org/10.1146/annurev.mi.39.100185.001541

Strain, E. M. A., Edgar, G. J., Ceccarelli, D., Stuart-Smith, R. D., Hosack, G. R., & Thomson, R. J. (2019). A global assessment of the direct and indirect benefits of marine protected areas for coral reef conservation. *Diversity and Distributions*, *25*(1), 9-20. https://doi.org/https://doi.org/10.1111/ddi.12838

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., Djahanschiri, B., Zeller, G., Mende, D. R., Alberti, A., Cornejo-Castillo, F. M., Costea, P. I., Cruaud, C., d'Ovidio, F., Engelen, S., Ferrera, I., Gasol, J. M., Guidi, L., Hildebrand, F., . . . Velayoudon, D. (2015). Structure and function of the global ocean microbiome. *Science*, *348*(6237), 1261359. https://doi.org/doi:10.1126/science.1261359

Sweet M, V. H., Keller-Costa T,Costa R,,Romano S, Bourne DG,Cárdenas A, Huggett MJ,Kerwin AH, Kuek F,Medina M, Meyer JL, Müller M, Pollock FJ,Rappé MS, Sere M, Sharp KH, Voolstra CR, Zaccardi N, Ziegler M, Peixoto R,. (2021). Insights into the Cultured Bacterial Fraction of Corals. *mSystems*, *6*(3). https://doi.org/https://doi.org/10.1128/msystems.01249-20

Sweet, M. J., Croquer, A., & Bythell, J. C. (2011). Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs*, *30*(1), 39-52. https://doi.org/10.1007/s00338-010-0695-1

Tamir, R., Eyal, G., Kramer, N., Laverick, J. H., & Loya, Y. (2019). Light environment drives the shallow-to-mesophotic coral community transition. *Ecosphere*, *10*(9), e02839. https://doi.org/https://doi.org/10.1002/ecs2.2839

Tandon, K., Lu, C. Y., Chiang, P. W., Wada, N., Yang, S. H., Chan, Y. F., Chen, P. Y., Chang, H. Y., Chiou, Y. J., Chou, M. S., Chen, W. M., & Tang, S. L. (2020). Comparative genomics: Dominant coral-bacterium Endozoicomonas acroporae metabolizes dimethylsulfoniopropionate (DMSP). *ISME J*, *14*(5), 1290-1303. https://doi.org/10.1038/s41396-020-0610-x

Tarnecki, A. M., Wafapoor, M., Phillips, R. N., & Rhody, N. R. (2019). Benefits of a Bacillus probiotic to larval fish survival and transport stress resistance. *Scientific Reports*, *9*(1), 4892. https://doi.org/10.1038/s41598-019-39316-w

Team, R. (2023). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Thompson, J. R., Rivera, H. E., Closek, C. J., & Medina, M. (2014). Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. *Front Cell Infect Microbiol*, *4*, 176. https://doi.org/10.3389/fcimb.2014.00176

Tian, L., Zhou, X. Q., Jiang, W. D., Liu, Y., Wu, P., Jiang, J., Kuang, S. Y., Tang, L., Tang, W. N., Zhang, Y. A., Xie, F., & Feng, L. (2017). Sodium butyrate improved intestinal immune function associated with NF-kappaB and p38MAPK signalling pathways in young grass carp (Ctenopharyngodon idella). *Fish Shellfish Immunol*, *66*, 548-563. https://doi.org/10.1016/j.fsi.2017.05.049

Toth, L. T., van Woesik, R., Murdoch, T. J. T., Smith, S. R., Ogden, J. C., Precht, W. F., & Aronson, R. B. (2014). Do no-take reserves benefit Florida's corals? 14 years of change and stasis in the Florida Keys National Marine Sanctuary. *Coral Reefs*, *33*(3), 565-577. https://doi.org/10.1007/s00338-014-1158-x

Towle, E. K., Carlton, R., Langdon, C., & Manzello, D. P. (2015). In-situ measurement of metabolic status in three coral species from the Florida Reef Tract. *Regional Studies in Marine Science*, *2*, 145-153. https://doi.org/https://doi.org/10.1016/j.rsma.2015.09.007

Truong Dinh Hoai, T. T. T., Nguyen Van Tuyen, Nguyen Thi Huong Giang, Kim Van Vana. (2019). Aeromonas veronii caused disease and mortality in channel catfish in Vietnam. *Aquaculture*, *513*. https://doi.org/https://doi.org/10.1016/j.aquaculture.2019.734425

Uritskiy, G. V., DiRuggiero, J., & Taylor, J. (2018). MetaWRAP-a flexible pipeline for genome-resolved metagenomic data analysis. *Microbiome*, *6*(1), 158. https://doi.org/10.1186/s40168-018-0541-1

van de Water, J. A. J. M., Melkonian, R., Voolstra, C. R., Junca, H., Beraud, E., Allemand, D., & Ferrier-Pagès, C. (2017). Comparative Assessment of Mediterranean Gorgonian-Associated Microbial Communities Reveals Conserved Core and Locally Variant Bacteria. *Microbial Ecology*, *73*(2), 466-478. https://doi.org/10.1007/s00248-016-0858-x

Vangay, P., Ward, T., Gerber, J. S., & Knights, D. (2015). Antibiotics, pediatric dysbiosis, and disease. *Cell Host Microbe*, *17*(5), 553-564. https://doi.org/10.1016/j.chom.2015.04.006

Vendrell, D., Balcazar, J. L., Ruiz-Zarzuela, I., de Blas, I., Girones, O., & Muzquiz, J. L. (2006). Lactococcus garvieae in fish: a review. *Comp Immunol Microbiol Infect Dis*, *29*(4), 177-198. https://doi.org/10.1016/j.cimid.2006.06.003

Verner-Jeffreys, D. W., Nakamura, I., & Shields, R. J. (2006). Egg-associated microflora of Pacific threadfin, Polydactylus sexfilis and amberjack, Seriola rivoliana, eggs. Characterisation and properties. *Aquaculture*, *253*(1), 184-196. https://doi.org/https://doi.org/10.1016/j.aquaculture.2005.08.019

Vezzulli, L., Pezzati, E., Huete-Stauffer, C., Pruzzo, C., & Cerrano, C. (2013). 16SrDNA Pyrosequencing of the Mediterranean Gorgonian Paramuricea clavata Reveals a Link among Alterations in Bacterial Holobiont Members, Anthropogenic Influence and Disease Outbreaks. *PLoS One*, *8*(6), e67745. https://doi.org/10.1371/journal.pone.0067745

Wang, F., Wei, Y., Yan, T., Wang, C., Chao, Y., Jia, M., An, L., & Sheng, H. (2022). Sphingomonas sp. Hbc-6 alters physiological metabolism and recruits beneficial rhizosphere bacteria to improve plant growth and drought tolerance. *Front Plant Sci*, *13*, 1002772. https://doi.org/10.3389/fpls.2022.1002772

Wang, S., Meade, A., Lam, H. M., & Luo, H. (2020). Evolutionary Timeline and Genomic Plasticity Underlying the Lifestyle Diversity in Rhizobiales. *mSystems*, *5*(4). https://doi.org/10.1128/mSystems.00438-20

Wellborn, T. L. (1990). Channel Catfish Life History and Biology.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. *Springer-Verlag New York*. https://ggplot2.tidyverse.org

Wilkins, L. G., Fumagalli, L., & Wedekind, C. (2016). Effects of host genetics and environment on egg-associated microbiotas in brown trout (Salmo trutta). *Mol Ecol*, *25*(19), 4930-4945. https://doi.org/10.1111/mec.13798

Wilkins, L. G., Rogivue, A., Schutz, F., Fumagalli, L., & Wedekind, C. (2015). Increased diversity of egg-associated bacteria on brown trout (Salmo trutta) at elevated temperatures. *Sci Rep*, *5*, 17084. https://doi.org/10.1038/srep17084

Williams, C. L., Dill-McFarland, K. A., Vandewege, M. W., Sparks, D. L., Willard, S. T., Kouba, A. J., Suen, G., & Brown, A. E. (2016). Dietary Shifts May Trigger Dysbiosis and Mucous Stools in Giant Pandas (Ailuropoda melanoleuca). *Front Microbiol*, *7*, 661. https://doi.org/10.3389/fmicb.2016.00661

Wolters, W. R., Wise, D. J., & Klesius, P. H. (1996). Survival and Antibody Response of Channel Catfish, Blue Catfish, and Channel Catfish Female × Blue Catfish Male Hybrids after Exposure to Edwardsiella ictaluri. *Journal of Aquatic Animal Health*, *8*(3), 249-254. https://doi.org/https://doi.org/10.1577/1548-8667(1996)008<0249:SAAROC>2.3.CO;2

Wright, L. S. (2022). Small no-take areas benefit hard corals more than regulation through fishing permits. *Aquatic Conservation*, *32*(7). https://doi.org/https://doi.org/10.1002/aqc.3814

Xia, H., Chen, H., Cheng, X., Yin, M., Yao, X., Ma, J., Huang, M., Chen, G., & Liu, H. (2022). Zebrafish: an efficient vertebrate model for understanding role of gut microbiota. *Mol Med*, *28*(1), 161. https://doi.org/10.1186/s10020-022-00579-1

Yamatsu, A., Matsumi, R., Atomi, H., & Imanaka, T. (2006). Isolation and characterization of a novel poly(vinyl alcohol)-degrading bacterium, Sphingopyxis sp. PVA3. *Appl Microbiol Biotechnol*, *72*(4), 804-811. https://doi.org/10.1007/s00253-006-0351-4

Yang, C. S., Chen, M. H., Arun, A. B., Chen, C. A., Wang, J. T., & Chen, W. M. (2010). Endozoicomonas montiporae sp. nov., isolated from the encrusting pore coral Montipora aequituberculata. *Int J Syst Evol Microbiol*, *60*(Pt 5), 1158-1162. https://doi.org/10.1099/ijs.0.014357-0

Yu, D., Xu, F., Zhang, S., & Zhan, J. (2017). Decoding and reprogramming fungal iterative nonribosomal peptide synthetases. *Nature Communications*, *8*(1), 15349. https://doi.org/10.1038/ncomms15349

Zhang, F., Qin, W., Zhang, J. P., & Hu, C. Q. (2015). Antibiotic toxicity and absorption in zebrafish using liquid chromatography-tandem mass spectrometry. *PLoS One*, *10*(5), e0124805. https://doi.org/10.1371/journal.pone.0124805

Zhang, K., Zheng, X., He, Z., Yang, T., Shu, L., Xiao, F., Wu, Y., Wang, B., Li, Z., Chen, P., & Yan, Q. (2020). Fish growth enhances microbial sulfur cycling in aquaculture pond sediments. *Microb Biotechnol*, *13*(5), 1597-1610. https://doi.org/10.1111/1751-7915.13622

Zhang, Y., Qi, X., Zhang, Z., Jin, Z., Wang, G., & Ling, F. (2023). Effects of dietary Cetobacterium somerae on the intestinal health, immune parameters and resistance against Nocardia seriolae of largemouth bass, Micropterus salmoides. *Fish Shellfish Immunol*, *135*, 108693. https://doi.org/10.1016/j.fsi.2023.108693

Zhao, Z., Zhao, H., Zhang, L., Huang, Z., Ke, H., Liu, Y., Duan, Y., Li, H., Wang, X., & Li, Q. (2023). Integrated analysis of how gender and body weight affect the intestinal microbial diversity of Gymnocypris chilianensis. *Scientific Reports*, *13*(1), 8811. https://doi.org/10.1038/s41598-023-35600-y

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Research*, *30*(6), 492-506. https://doi.org/10.1038/s41422-020-0332-7

Zheng, D., Liwinski, T. & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Res*, *30*. https://doi.org/https://doi.org/10.1038/s41422-020-0332-7

Zhou, Y., Wang, C., Xu, X., Jin, W., Liu, L., Meng, F., & Yang, F. (2023). Deciphering the partial denitrification function of companion bacteria in mixotrophic anammox systems under different carbon/nitrogen ratios. *Journal of Environmental Chemical Engineering*, *11*(6), 111232. https://doi.org/https://doi.org/10.1016/j.jece.2023.111232

Zhu, L., Zhang, Z., Chen, H., Lamer, J. T., Wang, J., Wei, W., Fu, L., Tang, M., Wang, C., & Lu, G. (2021). Gut microbiomes of bigheaded carps and hybrids provide insights into invasion: A hologenome perspective. *Evol Appl*, *14*(3), 735-745. https://doi.org/10.1111/eva.13152

Zong, W., Friedman, E. S., Allu, S. R., Firrman, J., Tu, V., Daniel, S. G., Bittinger, K., Liu, L., Vinogradov, S. A., & Wu, G. D. (2024). Disruption of intestinal oxygen balance in acute colitis alters the gut microbiome. *Gut Microbes*, *16*(1), 2361493. https://doi.org/10.1080/19490976.2024.2361493

APPENDICES

APPENDIX A

SUPPLEMENTARY FIGURES AND TABLES FROM CHAPTER TWO

Table A1: Summary of samples collected.

Sample Type	Year	Tank	Number of samples
blue	2023	blue_1	25
	2024	blue_1	20
		blue_2	20
		blue_3	21
channel	2023	channel_1	25
	2024	channel_1	20
		channel_2	18
		channel_3	20
hybrid	2023	hybrid_1	25
	2024	hybrid_1	20
		hybrid_2	20
		hybrid_3	22
water	2023	source	3
		blue	5
		channel	5
		hybrid	5
	2024	source	9
		blue_1	1
		blue_2	1
		blue_3	1
		channel_1	1
		channel_2	1
		channel_3	1
		hybrid_1	1
		hybrid_2	1
		hybrid_3	1

Table A2: Pairwise PERMANOVA Test. All comparisons of beta-diversity between catfish type and water samples are statistically significant (p-value < 0.01).

Group 1	Group 2	Sample size	Pseudo *F*	*P* value	*q* value
blue	channel	164	54.4	0.001	0.001*
blue	hybrid	164	30.0	0.001	0.001*
blue	Water	117	142.5	0.001	0.001*
channel	hybrid	166	5.5	0.009	0.009*
channel	Water	119	104.7	0.001	0.001*
hybrid	Water	119	112.6	0.001	0.001*

* $P < 0.05$

Table A3: Tank does not influence catfish digesta microbiomes. All Pairwise PERMANOVA (beta-diversity) analyses between catfish tanks from 2024 sampling were insignificant (p-value > 0.05).

Tank comparison	SumOfSquares	F.Model	R2	*P* value	*P* adjusted
blue_1 vs blue_2	0.2	9.4	0.2	0.002	0.5
blue_1 vs blue_3	0.2	9.0	0.2	0.002	0.5
blue_2 vs blue_3	0.03	1.8	0.1	0.2	1
channel_1 vs channel_2	0.02	0.7	0.02	0.4	1
channel_1 vs channel_3	0.1	2.9	0.1	0.1	1
channel_2 vs channel_3	0.01	0.7	0.02	0.5	1
hybrid_1 vs hybrid_2	0.04	1.1	0.03	0.4	1
hybrid_1 vs hybrid_3	0.03	0.5	0.01	0.6	1
hybrid_2 vs hybrid_3	0.1	1.3	0.03	0.3	1

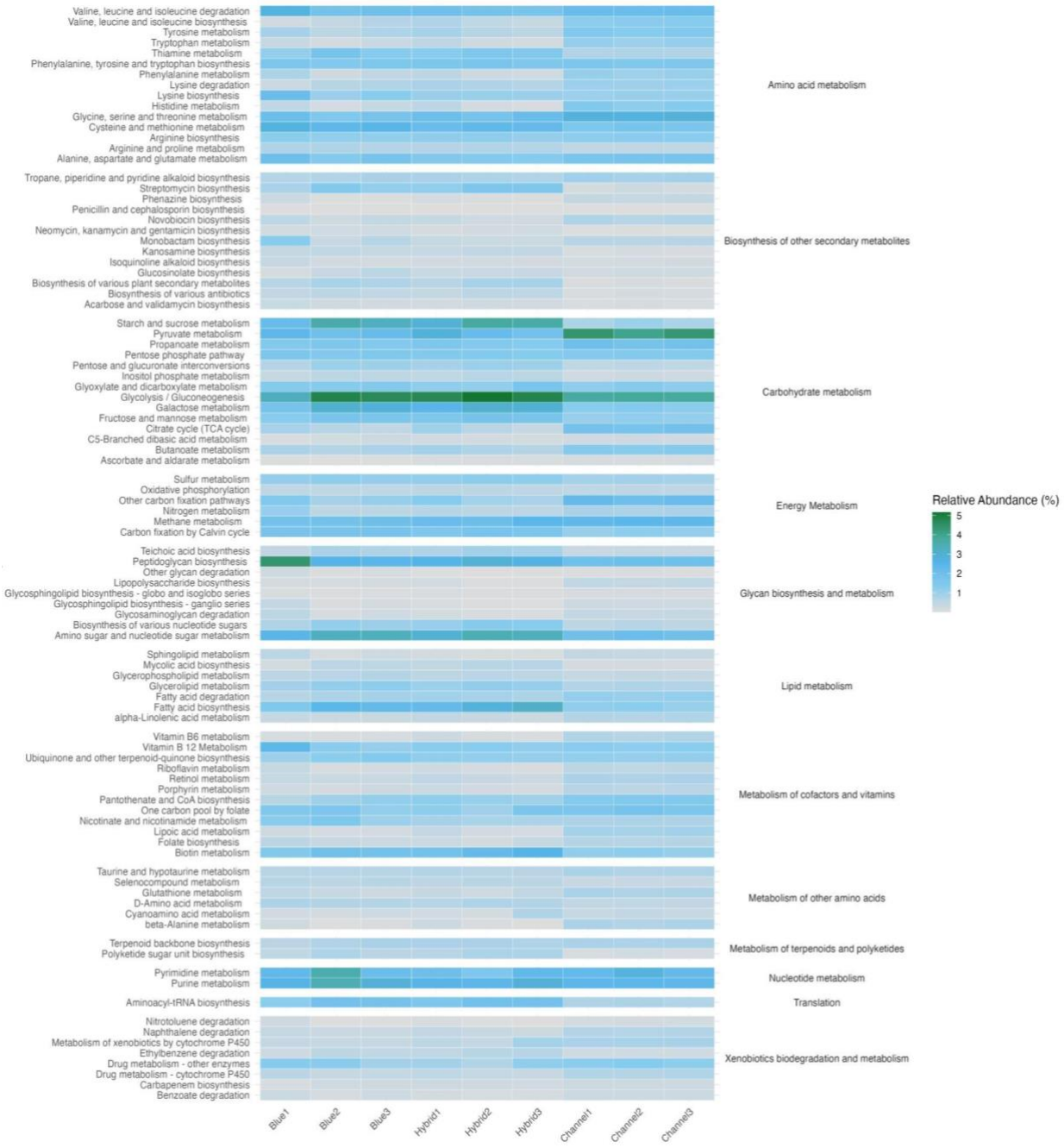

Figure A1: Heat map of all statistically enriched metabolic pathways associated with catfish metagenomes. Relative abundance of all normalized gene counts associated with statistically enriched metabolic pathways (adjusted $P > 0.05$, DESeq2; y-axis) for all catfish digesta samples.

Table A4: Relative abundances (%) of metabolic pathways of interest per microbial metagenomic sample.

KEGG Super-Pathway	Pathway of interest	blue			hybrid			channel		
Biosynthesis of secondary metabolites	Streptomycin	0.8	1.4	1.2	1.6	1.6	1.2	0.2	0.3	0.1
	Various antibiotics	0.4	0.5	0.4	0.5	0.5	0.4	0.1	0.2	0.2
Carbohydrate metabolism	Starch and sucrose	2.2	3.6	3.2	3.7	3.6	2.9	0.8	1.0	0.8
	Pyruvate	1.3	1.1	1.2	1.2	1.1	1.5	2.3	2.1	2.3
	Glycolysis/ Gluconeogenesis	1.9	2.6	2.5	2.8	2.6	2.6	2.2	2.2	2.2
	Galactose	1.2	1.9	1.9	1.9	1.8	1.6	0.9	0.9	0.8
	Fructose and mannose	0.8	1.2	1.2	1.2	1.2	1.0	0.5	0.6	0.6
	TCA Cycle	0.9	0.6	0.5	0.6	0.4	1.0	1.8	1.7	1.9
Energy metabolism	Sulfur	1.1	1.2	1.1	1.2	1.2	1.2	0.9	0.9	0.9
	Oxidative Phosphorylation	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.5	0.5
	Nitrogen	1.1	0.5	0.5	0.6	0.5	0.6	0.8	0.8	0.8
	Methane	1.1	1.2	1.3	1.3	1.3	1.5	1.4	1.4	1.4
	Carbon fixation by Calvin Cycle	1.7	1.7	1.7	1.7	1.6	1.5	1.1	1.2	1.1
Metabolism of cofactors and vitamins	Vitamin B_6	2.4	0.1	0.1	0.1	0.1	0.3	0.7	0.6	0.7
	Vitamin B_{12}	0.1	1.3	1.1	1.3	1.2	1.3	1.3	1.2	1.2
	Vitamin B_7	1.5	1.9	1.9	2.2	2.6	1.9	1.2	1.2	1.1

APPENDIX B
SUPPLEMENTARY FIGURES AND TABLES FROM CHAPTER THREE

Table B1: Channel catfish embryo samples. Embryo microbiomes that passed quality control and what spawn they originated from, whether they were treated with betadine or not, and which catfish strain (IP= industry pool, DS = delta select). There are 19 pre-betadine treatment and 23 post-betadine treatment embryo microbiomes. Across the 23 individual spawns there are 28 embryo microbiomes from the delta select channel catfish strain and 14 from the industry pool strain.

Embryo Sample	Spawn ID	Betadine	Catfish Strain
Embryo1	51	post	IP
Embryo 2	52	post	IP
Embryo 3	52	pre	IP
Embryo 4	53	pre	IP
Embryo 5	53	post	IP
Embryo 6	54	pre	IP
Embryo 7	54	post	IP
Embryo 8	55	post	IP
Embryo 9	56	post	IP
Embryo 10	56	pre	IP
Embryo 11	58	pre	IP
Embryo 12	58	post	IP
Embryo 13	59	pre	IP
Embryo 14	59	post	IP
Embryo 15	64	post	DS
Embryo 16	64	pre	DS
Embryo 17	66	post	DS
Embryo 18	66	pre	DS
Embryo 19	71	pre	DS
Embryo 20	71	post	DS
Embryo 21	73	post	DS
Embryo 22	73	pre	DS
Embryo 23	77	post	DS
Embryo 24	80	post	DS
Embryo 25	80	pre	DS
Embryo 26	83	pre	DS
Embryo 27	83	post	DS
Embryo 28	84	pre	DS
Embryo 29	84	post	DS
Embryo 30	88	post	DS
Embryo 31	88	pre	DS

Table B1 Continued.

Embryo Sample	Spawn ID	Betadine	Catfish Strain
Embryo 32	91	post	DS
Embryo 33	97	post	DS
Embryo 34	97	pre	DS
Embryo 35	98	post	DS
Embryo 36	98	pre	DS
Embryo 37	99	pre	DS
Embryo 38	99	post	DS
Embryo 39	110	pre	DS
Embryo 40	110	post	DS
Embryo 41	111	post	DS
Embryo 42	111	pre	DS

Table B2: Shannon diversity index (alpha diversity) between sample types. Pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg adjustment.

Group1	Group2	n1	n2	statistic	p.adj
water (pre-betadine)	water (post-betadine)	9	25	225	<0.001*
embryo (post-betadine	water (post-betadine)	23	25	509	<0.001*
embryo (post-betadine	water (pre-betadine)	23	9	13	<0.001*
embryo (pre-betadine)	water (pre-betadine)	19	9	17	<0.001*
embryo (pre-betadine)	water (post-betadine)	19	25	367	0.002*
embryo (pre-betadine)	embryo (post-betadine	19	23	198	0.617

* $P < 0.05$

Table B3: Pairwise PERMANOVA of all samples by betadine treatment. All microbiomes are significantly different from one another with dispersion contributing to significant differences between water and embryo microbiomes.

Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
embryo (post-betadine) vs embryo (pre-betadine)	1	2.41	11.05	0.22	0.001	0.006*
embryo (post-betadine) vs water (pre-betadine)	1	4.45	27.95	0.48	0.001	0.006*a
embryo (pre-betadine) vs water (pre-betadine)	1	3.50	21.03	0.45	0.001	0.006*a
embryo (post-betadine) vs water (post-betadine)	1	2.68	21.41	0.32	0.001	0.006*a
embryo (pre-betadine) vs water (post-betadine)	1	6.75	53.48	0.56	0.001	0.006*a
water (pre-betadine) vs water (post-betadine)	1	6.05	143.17	.82	0.001	0.006*

* $P < 0.05$; [a]PERMDISP $P < 0.01$

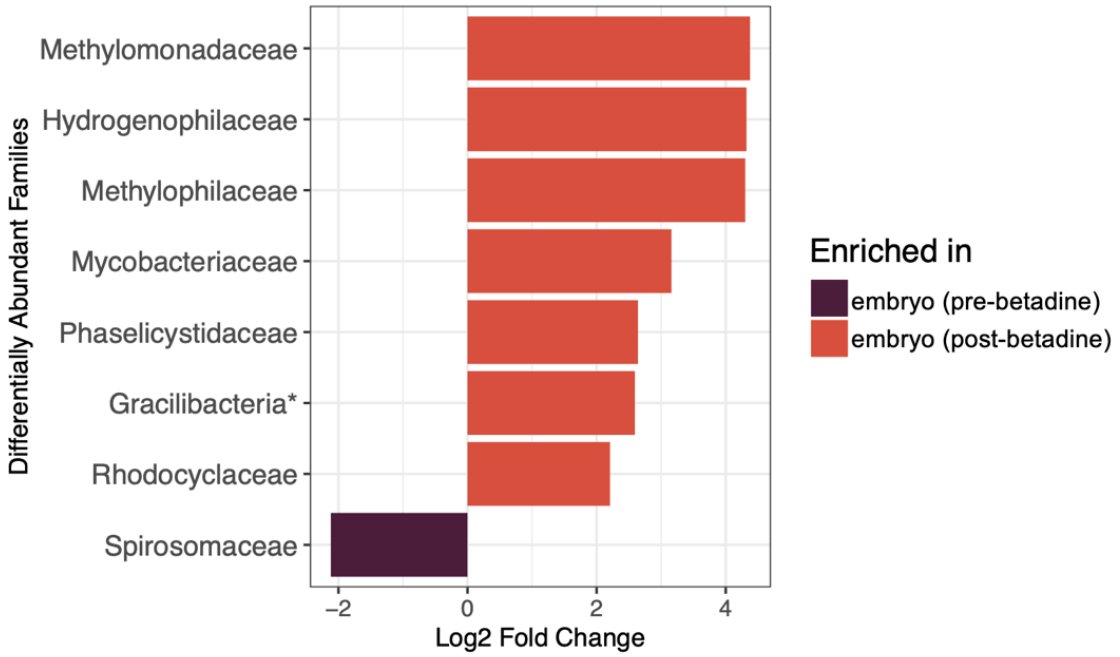

Figure B1: Differential abundance of bacterial families between pre- and post-betadine embryo microbiomes. Taxa reflect ASVs enriched in pre- and post-betadine embryo microbiomes. Enrichment was calculated with differential abundance analysis and visualization only included ASVs represented as bacterial family, with adjusted p-value > 0.05, and log fold changes > 2 and detection and prevalence limits of 5% and 65%, respectively. Pre-betadine embryo microbiomes were used as the intercept. Asterisks indicate taxa not resolved at the family level; the nearest assigned taxonomic rank is shown.

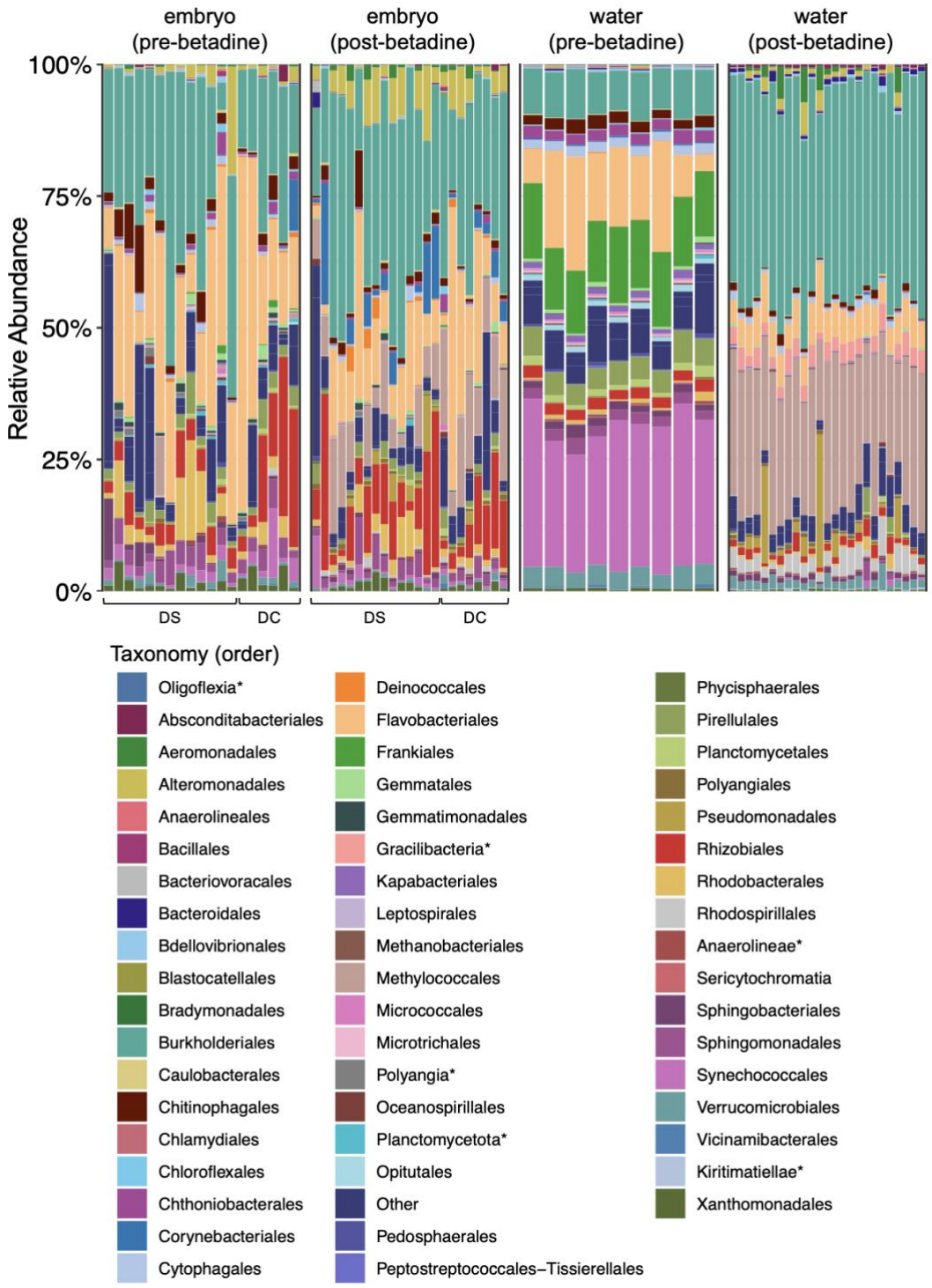

Figure B2: Taxonomic composition of bacterial orders across all microbiomes. Taxonomic bar chart of order level bacteria of embryo and water microbiomes with a minimum detection of 5% relative abundance in 65% of all samples (prevalence). Embryo samples are grouped by channel catfish strain (DS = Delta Select, DC = Delta Control). Asterisks indicate taxa not resolved at the family level; the nearest assigned taxonomic rank is shown.

APPENDIX C

SUPPLEMENTARY FIGURES AND TABLES FROM CHAPTER FOUR

Table C1: Number of hard coral samples. Hard corals collected from each sampling site and depth (m and category). *Agaricia* spp. and *M. cavernosa* samples were collected from all sampling sites at all sampling depths. *Porites astreoides* were collected from all sampling sites, but not all sampling depths (m) for The Edge.

Hard Coral Species	Site	Depth (Category)	Depth (m)	Number of Samples
Agaricia spp.	Crystal Palace Wall	shallow photic	4.5	1
			9.1	1
		mid photic	13.7	1
			18.3	2
		deep photic	22.8	2
			27.5	2
		upper mesophotic	32	2
			36.6	1
	McCoy's Wall	shallow photic	4.5	1
			9.1	2
		mid photic	13.7	1
			18.3	2
		deep photic	22.8	2
			27.5	2
		upper mesophotic	32	3
			36.6	2
	Ron's Rock	shallow photic	4.5	2
			9.1	1
		mid photic	13.7	1
			18.3	3
		deep photic	22.8	2
			27.5	2
		upper mesophotic	32	2
			36.6	2
	The Edge	shallow photic	4.5	2
			9.1	2
		mid photic	13.7	2
			18.3	2
		deep photic	22.8	2
			27.5	2
		upper mesophotic	32	3
			36.6	3
Montastraea covernosa	Crystal Palace Wall	shallow photic	4.5	1
			9.1	1
		mid photic	13.7	1
			18.3	2
		deep photic	22.8	2
			27.5	1
		upper mesophotic	32	3
			36.6	3

Table C1 Continued.

Hard Coral Species	Site	Depth (Category)	Depth (m)	Number of Samples
Montastraea covernosa	McCoy's Wall	shallow photic	4.5	1
			9.1	1
		mid photic	13.7	1
			18.3	3
		deep photic	22.8	2
			27.5	3
		upper mesophotic	32	4
			36.6	2
	Ron's Rock	shallow photic	4.5	1
			9.1	0
		mid photic	13.7	3
			18.3	2
		deep photic	22.8	2
			27.5	1
		upper mesophotic	32	2
			36.6	2
	The Edge	shallow photic	4.5	1
			9.1	2
		mid photic	13.7	2
			18.3	2
		deep photic	22.8	2
			27.5	3
		upper mesophotic	32	2
			36.6	2
Porites astreoides	Crystal Palace Wall	shallow	4.5	1
			9.1	1
		upper mesophotic	13.7	1
			18.3	3
		mid mesophotic	22.8	2
			27.5	2
		deep mesophotic	32	2
			36.6	1
	McCoy's Wall	shallow	4.5	2
			9.1	3
		upper mesophotic	13.7	2
			18.3	3
		mid mesophotic	22.8	3
			27.5	0
		deep mesophotic	32	2
			36.6	1
	Ron's Rock	shallow	4.5	2
			9.1	1
		upper mesophotic	13.7	2
			18.3	0

Table C1 Continued.

Hard Coral Species	Site	Depth (Category)	Depth (m)	Number of Samples
Porites astreoides	Ron's Rock	mid mesophotic	22.8	2
			27.5	0
		deep mesophotic	32	2
			36.6	0
	The Edge	shallow	4.5	0
			9.1	0
		upper mesophotic	13.7	0
			18.3	1
		mid mesophotic	22.8	0
			27.5	0
		deep mesophotic	32	0
			36.6	0

Table C2: Number of soft coral samples. Soft corals collected from each sampling site and depth. All soft coral groups were collected at all sites; however not all groups were collected at all depths.

Soft Coral Group	Site	Depth (Category)	Depth (m)	Number of Samples
Cladiellidae	Crystal Palace Wall	shallow	4.5	0
			9.1	0
		upper mesophotic	13.7	0
			18.3	1
		mid mesophotic	22.8	2
			27.5	0
		deep mesophotic	32	0
			36.6	0
	McCoy's Wall	shallow	4.5	0
			9.1	2
		upper mesophotic	13.7	0
			18.3	1
		mid mesophotic	22.8	0
			27.5	0
		deep mesophotic	32	0
			36.6	0
	Ron's Rock	shallow	4.5	2
			9.1	5
		upper mesophotic	13.7	0
			18.3	3
		mid mesophotic	22.8	2
			27.5	0
		deep mesophotic	32	0
			36.6	0
	The Edge	shallow	4.5	0

Table C2 Continued.

Soft Coral Group	Site	Depth (Category)	Depth (m)	Number of Samples
Cladiellidae	The Edge	shallow	9.1	1
		upper mesophotic	13.7	1
			18.3	1
		mid mesophotic	22.8	0
			27.5	0
		deep mesophotic	32	1
			36.6	2
Gorgoniidae	Crystal Palace Wall	shallow	4.5	0
			9.1	1
		upper mesophotic	13.7	0
			18.3	2
		mid mesophotic	22.8	0
			27.5	1
		deep mesophotic	32	1
			36.6	0
	McCoy's Wall	shallow	4.5	1
			9.1	2
		upper mesophotic	13.7	1
			18.3	2
		mid mesophotic	22.8	2
			27.5	1
		deep mesophotic	32	1
			36.6	0
	Ron's Rock	shallow	4.5	0
			9.1	0
		upper mesophotic	13.7	2
			18.3	1
		mid mesophotic	22.8	1
			27.5	0
		deep mesophotic	32	2
			36.6	1
	The Edge	shallow	4.5	0
			9.1	1
		upper mesophotic	13.7	0
			18.3	0
		mid mesophotic	22.8	0
			27.5	0
		deep mesophotic	32	0
			36.6	0
Malacalcyonacea	Crystal Palace Wall	shallow	4.5	2
			9.1	1
		upper mesophotic	13.7	1
			18.3	1
		mid mesophotic	22.8	1
			27.5	0

Table C2 Continued.

Soft Coral Group	Site	Depth (Category)	Depth (m)	Number of Samples
Malacalcyonacea	Crystal Palace Wall	deep mesophotic	32	0
			36.6	1
	McCoy's Wall	shallow	4.5	3
			9.1	1
		upper mesophotic	13.7	2
			18.3	0
		mid mesophotic	22.8	0
			27.5	0
		deep mesophotic	32	1
			36.6	0
	Ron's Rock	shallow	4.5	0
			9.1	0
		upper mesophotic	13.7	1
			18.3	0
		mid mesophotic	22.8	0
			27.5	0
		deep mesophotic	32	0
			36.6	0
	The Edge	shallow	4.5	1
			9.1	2
		upper mesophotic	13.7	0
			18.3	2
		mid mesophotic	22.8	0
			27.5	0
		deep mesophotic	32	0
			36.6	0
Muricea spp.	Crystal Palace Wall	shallow	4.5	1
			9.1	1
		upper mesophotic	13.7	2
			18.3	0
		mid mesophotic	22.8	3
			27.5	3
		deep mesophotic	32	3
			36.6	3
	McCoy's Wall	shallow	4.5	4
			9.1	3
		upper mesophotic	13.7	0
			18.3	1
		mid mesophotic	22.8	3
			27.5	0
		deep mesophotic	32	3
			36.6	1
	Ron's Rock	shallow	4.5	2
			9.1	0
		upper mesophotic	13.7	3

Table C2 Continued.

Soft Coral Group	Site	Depth (Category)	Depth (m)	Number of Samples
Muricea spp.	Ron's Rock	upper mesophotic	18.3	0
		mid mesophotic	22.8	3
			27.5	3
		deep mesophotic	32	1
			36.6	3
	The Edge	shallow	4.5	2
			9.1	2
		upper mesophotic	13.7	3
			18.3	2
		mid mesophotic	22.8	3
			27.5	2
		deep mesophotic	32	2
			36.6	3

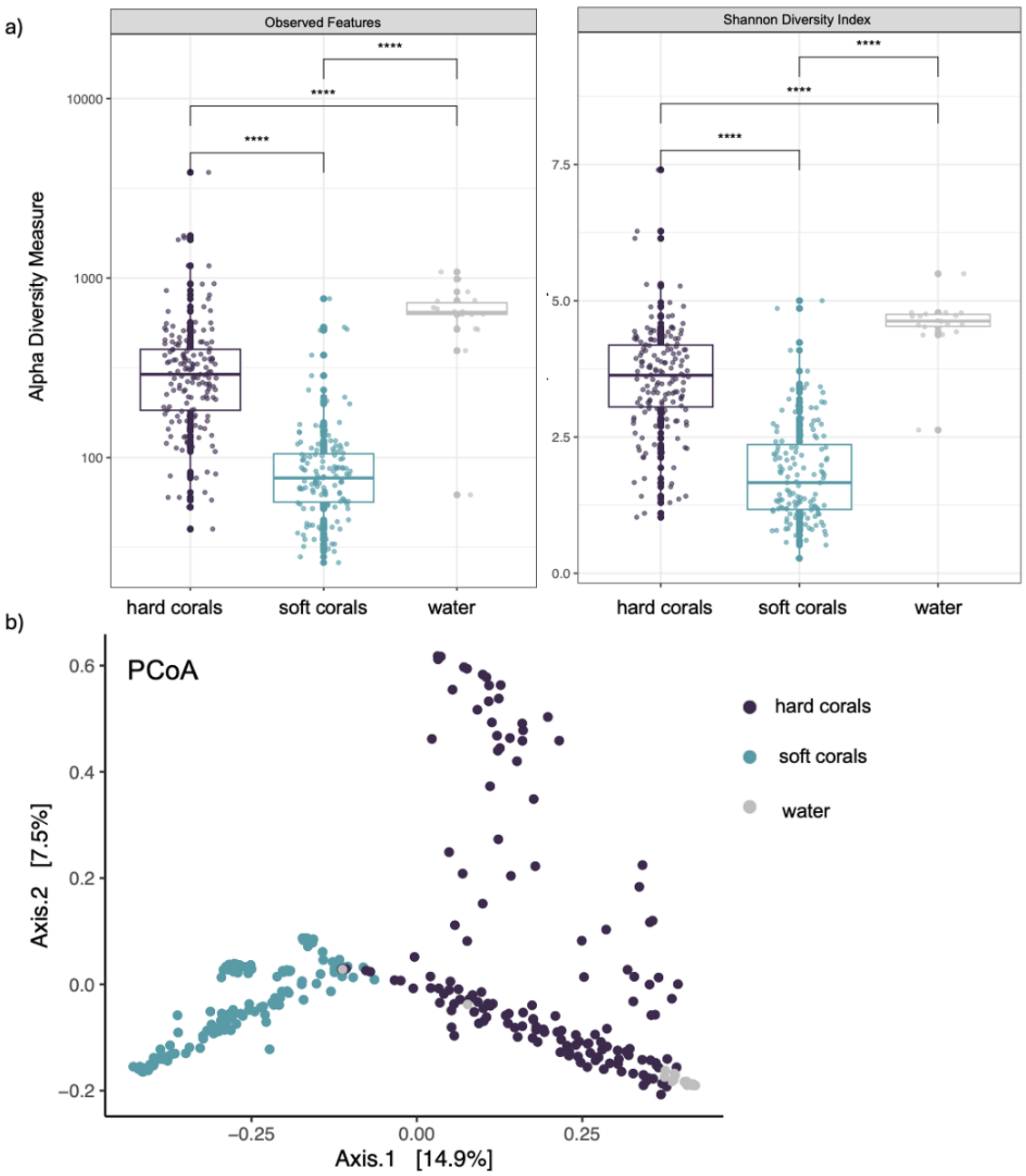

Figure C1: Hard corals have distinct and more diverse microbiomes than soft corals. a) Alpha diversity (observed features in log scale and Shannon diversity index in linear scale) comparing hard corals, soft coral, and water sample microbiomes. Significance: **** $P \leq 0.0001$. b) Beta diversity comparing hard corals, soft coral, and water sample microbiomes plotted using Principal Coordinate Analysis and Bray-Curtis.

Table C3: Pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg adjustment for observed ASV richness. Comparisons were performed among hard corals, soft corals, and water samples.

Group 1	Group 2	n1	n2	Statistic	p.adj
hard coral	soft coral	159	132	19282.5	< 0.001*
hard coral	water	159	18	400	< 0.001*
soft coral	water	132	18	109	< 0.001*

* $P > 0.05$

Table C4: Pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg adjustment for Shannon diversity. Comparisons were performed among hard corals, soft corals, and water samples.

Group 1	Group 2	n1	n2	Statistic	p.adj
hard coral	soft coral	159	132	19100	< 0.001*
hard coral	water	159	18	336	< 0.001*
soft coral	water	132	18	40	< 0.001*

* $P > 0.05$

Table C5: Pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg adjustment for observed ASV richness. Comparisons were performed among coral types.

Group 1	Group 2	n1	n2	Statistic	p.adj
Montastraea cavernosa	*Muricea* spp.	60	65	3823	< 0.001*
Agaricia spp.	*Muricea* spp.	60	65	3635	< 0.001*
Porites astreoides	*Muricea* spp.	39	65	2461.5	< 0.001*
Montastraea cavernosa	Malacalcyonacea	60	20	1182	< 0.001*
Montastraea cavernosa	Gorgoniidae	60	23	1293	< 0.001*
Porites astreoides	Malacalcyonacea	39	20	757	< 0.001*
Agaricia spp.	Malacalcyonacea	60	20	1109.5	< 0.001*
Porites astreoides	Gorgoniidae	39	23	815	< 0.001*
Montastraea cavernosa	Cladiellidae	60	24	1203	< 0.001*
Gorgoniidae	*Muricea* spp.	23	65	1230.5	< 0.001*
Agaricia spp.	Gorgoniidae	60	23	1144.5	< 0.001*
Porites astreoides	Cladiellidae	39	24	777	< 0.001*
Gorgoniidae	Malacalcyonacea	23	20	408	< 0.001*
Cladiellidae	*Muricea* spp.	24	65	1240	< 0.001*
Agaricia spp.	Cladiellidae	60	24	1082	< 0.001*
Cladiellidae	Malacalcyonacea	24	20	373	0.002*
Malacalcyonacea	*Muricea* spp.	20	65	833	0.072
Agaricia spp.	*Montastraea cavernosa*	60	60	1538	0.153
Agaricia spp.	*Porites astreoides*	60	39	1025.5	0.274
Montastraea cavernosa	*Porites astreoides*	60	39	1264.5	0.526
Cladiellidae	Gorgoniidae	24	23	272	0.941

* $P > 0.05$

Table C6: Pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg adjustment for Shannon diversity. Comparisons were performed among coral types.

Group1	Group2	n1	n2	statistic	p.adj
Agaricia spp.	*Muricea* spp.	60	65	3897	< 0.001*
Montastraea cavernosa	*Muricea* spp.	60	65	3855	< 0.001*
Porites astreoides	*Muricea* spp.	39	65	2267	< 0.001*
Agaricia spp.	Malacalcyonacea	60	20	1175	< 0.001*
Montastraea cavernosa	Malacalcyonacea	60	20	1156	< 0.001*
Gorgoniidae	*Muricea* spp.	23	65	1360	< 0.001*
Agaricia spp.	Gorgoniidae	60	23	1262	< 0.001*
Montastraea cavernosa	Gorgoniidae	60	23	1187	< 0.001*
Agaricia spp.	Cladiellidae	60	24	1247	< 0.001*
Montastraea cavernosa	Cladiellidae	60	24	1222	< 0.001*
Cladiellidae	*Muricea* spp.	24	65	1271	< 0.001*
Porites astreoides	Malacalcyonacea	39	20	649	< 0.001*
Gorgoniidae	Malacalcyonacea	23	20	381	< 0.001*
Agaricia spp.	*Porites astreoides*	60	39	1705	< 0.001*
Montastraea cavernosa	*Porites astreoides*	60	39	1554	0.008*
Cladiellidae	Malacalcyonacea	24	20	339	0.025*
Porites astreoides	Cladiellidae	39	24	614	0.048*
Porites astreoides	Gorgoniidae	39	23	569	0.094
Agaricia spp.	*Montastraea cavernosa*	60	60	2146	0.108
Malacalcyonacea	*Muricea* spp.	20	65	808	0.108
Cladiellidae	Gorgoniidae	24	23	212	0.178

* $P > 0.05$

Table C7: Pairwise PERMANOVA (beta diversity) between hard and soft corals, and water samples.

Pairs	Df	SumsOfSqs	F.Model	R2	p.adj
hard coral vs soft coral	1	14.61	37.16	0.13	0.003*[a]
hard coral vs water	1	2.63	7.59	0.04	0.003*[a]
soft coral vs water	1	7.14	18.43	0.11	0.003*[a]

*PERMANOVA $P > 0.05$, [a]PERMDISP $P < 0.05$

Table C8: Pairwise PERMANOVA (beta diversity) of all coral microbiomes by coral groups.

Pairs	Df	SumsOfSqs	F.Model	R2	p.adj
Cladiellidae vs *Muricea* spp.	1	3.36	9.58	0.10	0.02*
Montastraea cavernosa vs Gorgoniidae	1	5.91	18.61	0.19	0.02*
Cladiellidae vs *Agaricia* spp.	1	5.03	14.41	0.15	0.02*
Agaricia spp. vs *Muricea* spp.	1	10.06	29.41	0.19	0.02*
*Montastraea cavernos*a vs *Agaricia* spp.	1	3.26	9.94	0.08	0.02*
Agaricia spp. vs Gorgoniidae	1	5.47	16.23	0.17	0.02*
Cladiellidae vs *Montastraea cavernosa*	1	5.45	16.55	0.17	0.02*
Cladiellidae vs Gorgoniidae	1	1.44	4.12	0.08	0.04*
Muricea spp. vs Gorgoniidae	1	4.78	14.09	0.14	0.02*
Montastraea cavernosa vs *Muricea* spp.	1	10.79	32.76	0.21	0.02*
Porites astreoides vs Malacalcyonacea	1	6.80	26.81	0.32	0.02*
Porites astreoides vs Gorgoniidae	1	5.91	20.44	0.25	0.02*
Porites astreoides vs *Montastraea cavernosa*	1	6.78	22.94	0.19	0.02*
Porites astreoides vs Cladiellidae	1	5.50	18.00	0.23	0.02*
Gorgoniidae vs Malacalcyonacea	1	4.73	16.81	0.29	0.02*
Porites astreoides vs *Agaricia* spp.	1	5.02	16.10	0.14	0.02*
Porites astreoides vs *Muricea* spp.	1	9.65	30.65	0.23	0.02*[a]
Montastraea cavernosa vs Malacalcyonacea	1	6.97	23.78	0.23	0.02*[a]
Cladiellidae vs Malacalcyonacea	1	4.11	13.47	0.24	0.02*[a]
Agaricia spp. vs Malacalcyonacea	1	6.60	21.05	0.21	0.02*[a]
Muricea spp. vs Malacalcyonacea	1	6.62	20.87	0.20	0.02*[a]

*PERMANOVA $P > 0.05$, [a]PERMDISP $P < 0.05$

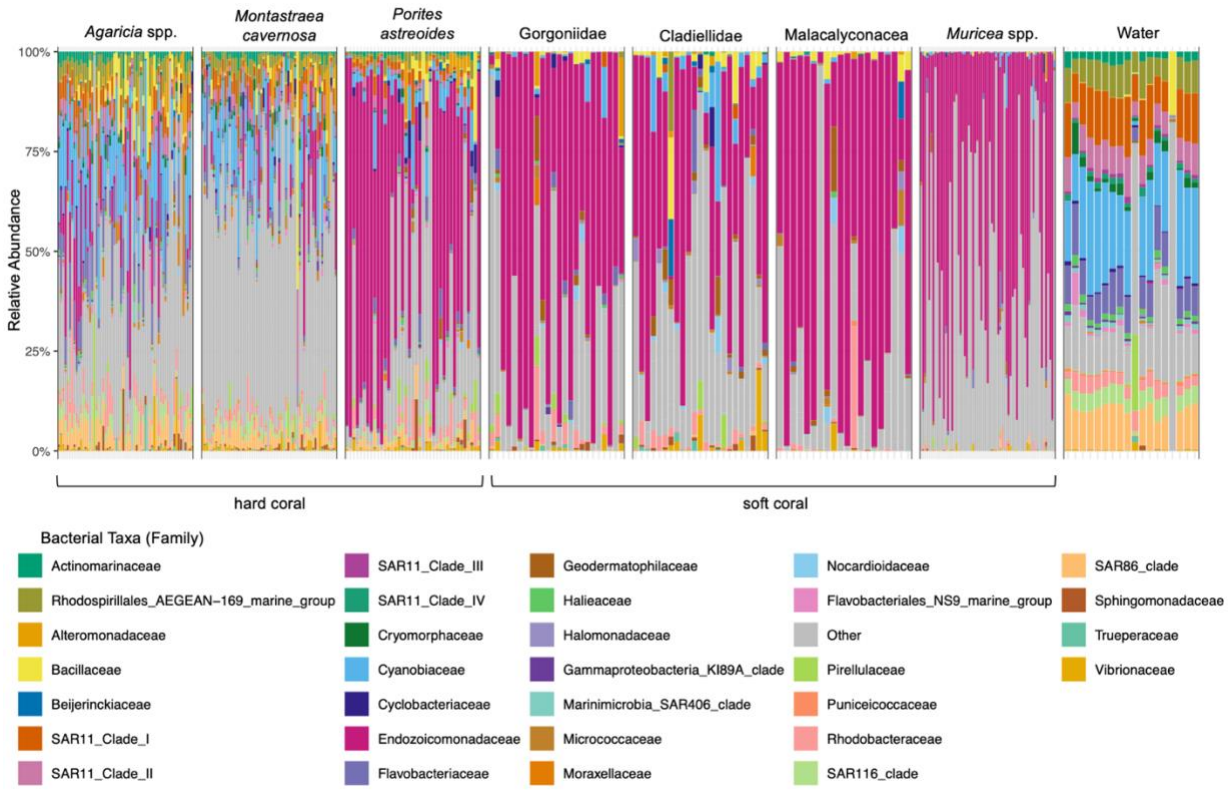

Figure C2: *Porites astreoides*, Gorgoniidae, Cladiellidae, Malacalyconacea and *Muricea* spp. microbiomes are dominated by Endozoicomonadaceae. Microbiome community composition by bacterial family with ASVs detected with a minimum relative abundance of 25% (detection) in 50% of all samples (prevalence). All soft corals, and one hard coral microbiome, *P. astreoides*, were dominated by Endozoicomonadaceae. The other hard corals, *Agaricia* spp. and *M. cavernosa*, microbiomes family with the highest relative abundance was Cyanobiaceae. Water samples had a distinct microbial community compared to corals. Other represents all ASVs that did not reach the detection and prevalence limits.

Table C9: Pairwise Kruskal-Wallis test of soft coral *Endozoicomonas* relative abundance by site. Ron's Rock and The Edge have significantly higher *Endozoicomonas* relative abundance than McCoy's Wall. Ron's Rock also has significantly higher *Endozoicomonas* relative abundance than Crystal Palace Wall.

Group1	Group2	n1	n2	statistic	p.adj
Mccoy's Wall	Ron's Rock	35	35	339	0.01*
Crystal Palace Wall	Ron's Rock	31	35	349	0.04*
Mccoy's Wall	The Edge	35	31	363	0.04*
Crystal Palace Wall	Mccoy's Wall	31	35	643	0.24
Crystal Palace Wall	The Edge	31	31	384	0.24
Ron's Rock	The Edge	35	31	593	0.52

*$P > 0.05$

Table C10: Pairwise Kruskal-Wallis test of hard coral *Endozoicomonas* relative abundance by depth (m). There were significantly higher relative abundances of *Endozoicomonas* at 9.1 m compared to 32 and 26.6 m, and 13.7 m compared to 26.6 m.

Group1	Group2	n1	n2	statistic	p.adj
32	9.1	27	15	69	0.01*
36.6	9.1	19	15	45	0.01*
13.7	36.6	17	19	258	0.02*
13.7	32	17	27	340	0.05*
18.3	36.6	25	19	342	0.07
27.5	36.6	18	19	242	0.14
27.5	32	18	27	325.5	0.19
4.5	9.1	15	15	66	0.19
13.7	4.5	17	15	175	0.21
18.3	32	25	27	434.5	0.21
13.7	22.8	17	23	251	0.3
18.3	9.1	25	15	137	0.3
22.8	36.6	23	19	273	0.3
22.8	9.1	23	15	120	0.3
27.5	9.1	18	15	95	0.3
18.3	4.5	25	15	224.5	0.53
13.7	18.3	17	25	245	0.55
13.7	9.1	17	15	105	0.55
22.8	32	23	27	357	0.55
27.5	4.5	18	15	159.5	0.55
32	36.6	27	19	293	0.55
13.7	27.5	17	18	175	0.58
36.6	4.5	19	15	122	0.58
18.3	22.8	25	23	320	0.59
22.8	4.5	23	15	190	0.67
32	4.5	27	15	188.5	0.77
18.3	27.5	25	18	236.5	0.82
22.8	27.5	23	18	200	0.86

* $P > 0.05$

Table C11: Pairwise Kruskal-Wallis test of hard coral *Endozoicomonas* relative abundance by depth (category). There were significantly higher relative abundances of *Endozoicomonas* at shallow and mid photic depths compared to upper mesophotic.

Group1	Group2	n1	n2	statistic	p.adj
upper mesophotic	mid photic	46	42	557.5	0.004*
upper mesophotic	shallow photic	46	30	424.5	0.01*
upper mesophotic	deep photic	46	41	688.5	0.06
deep photic	mid photic	41	42	739.5	0.4
deep photic	shallow photic	41	30	564.5	0.67
shallow photic	mid photic	30	42	618.5	0.9

* $P > 0.05$

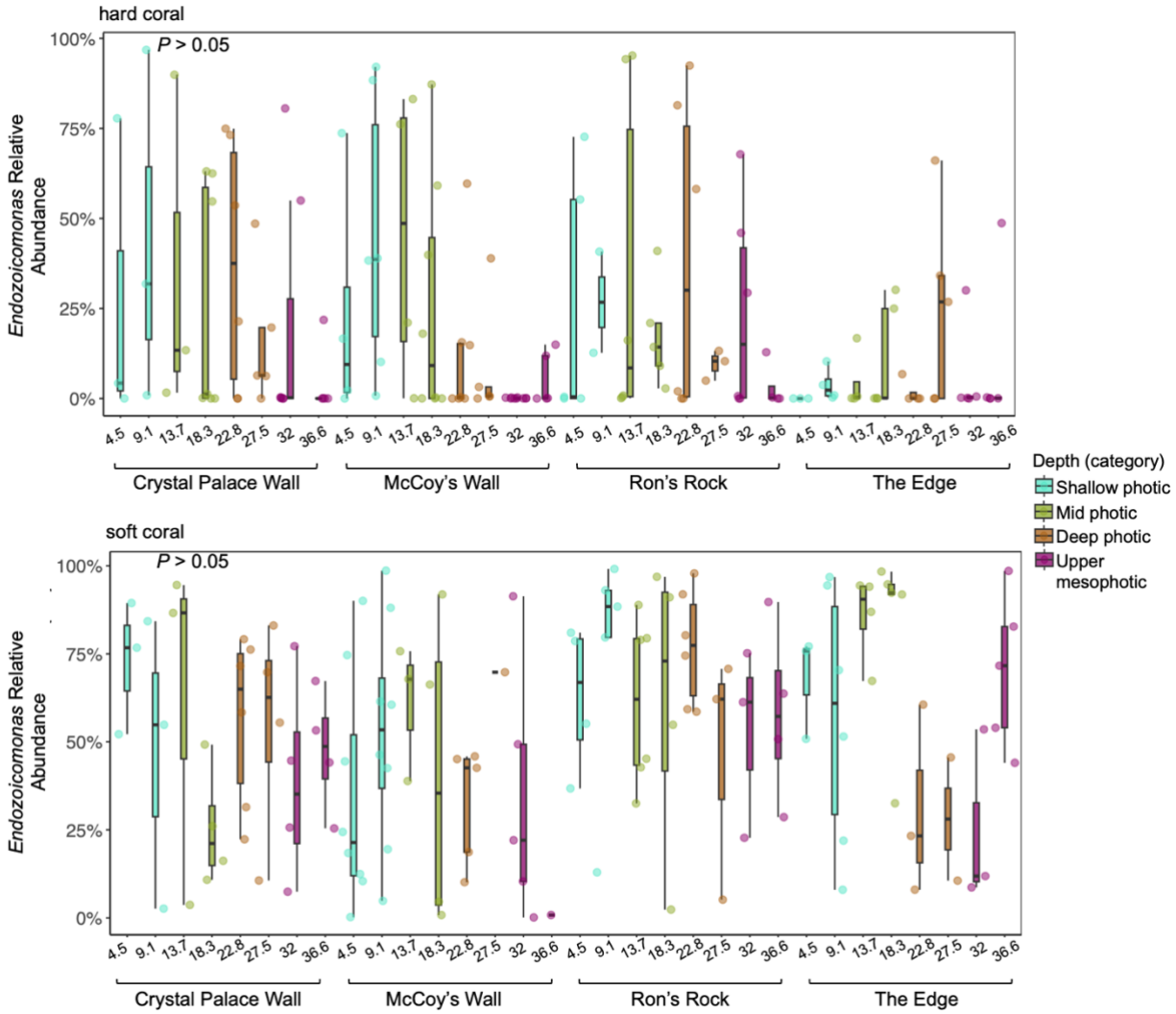

Figure C3: *Endozoicomonas* relative abundance does not vary by depth at any site. Hard and soft coral *Endozoicomonas* relative abundance plotted for every depth (m) at every site shows no significant differences (Kruskal Wallace test, $P > 0.05$). Box plots show median (center line), and 25–75% quartiles (colored area in the box), minimum and maximum values (whiskers) and outliers (larger data points) and jittered data points.

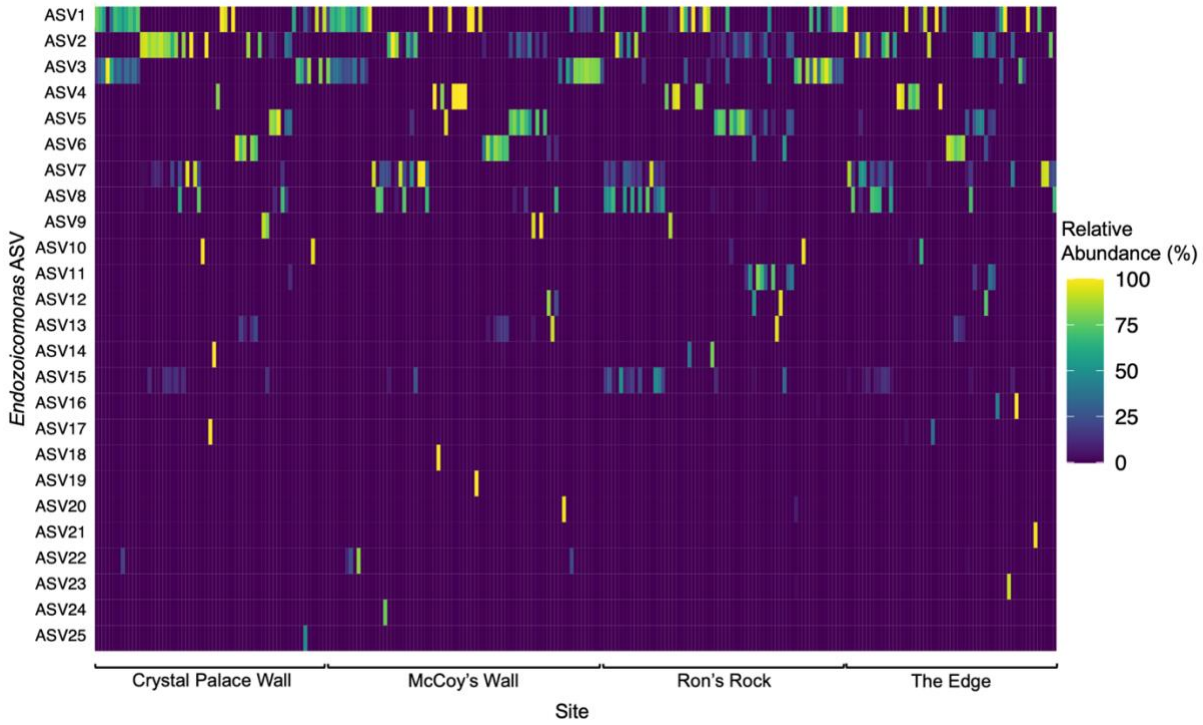

Figure C4: *Endozoicomonas* ASV diversity is not shaped by sample site. ASVs identified as *Endozoicomonas* with a relative abundance ≥50% in at least one coral sample were plotted and organized by sample site. Crystal Palace Wall and McCoy's Wall are MPAs while Ron's Rock and The Edge are not. There are no patterns between *Endozoicomonas* ASV diversity and site.

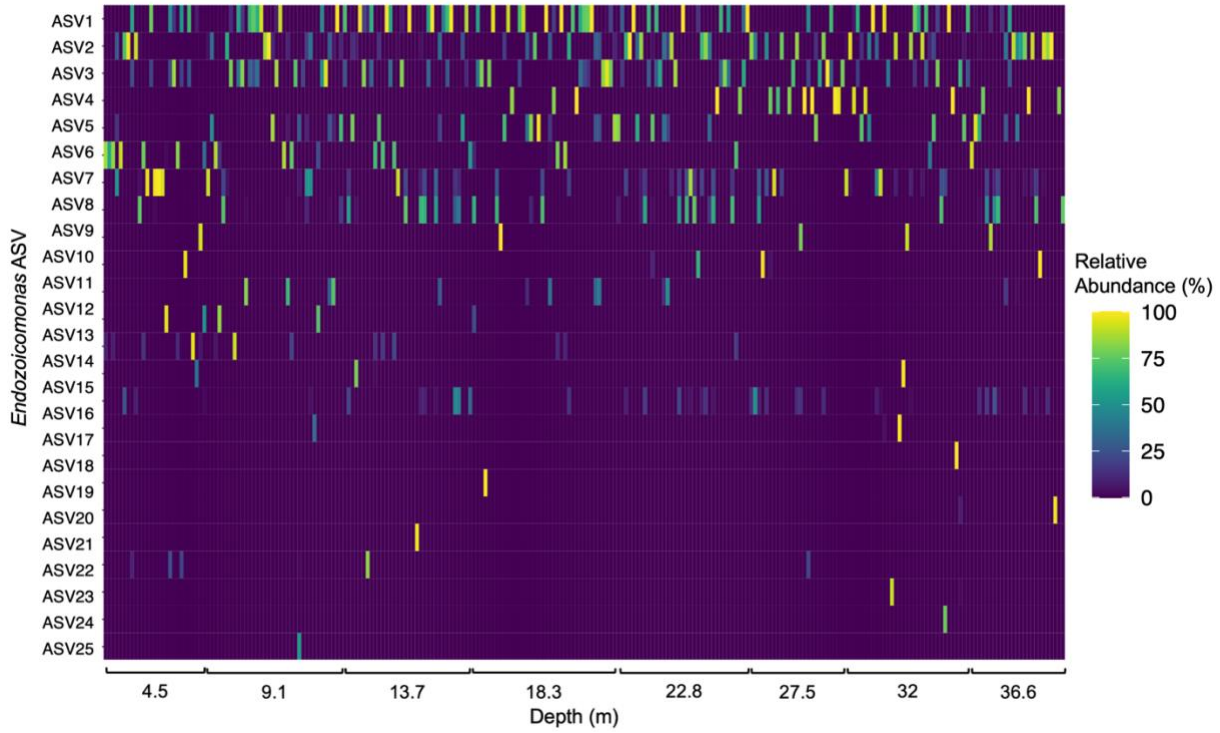

Figure C5: *Endozoicomonas* ASV diversity is not shaped by depth (m). ASVs identified as *Endozoicomonas* with a relative abundance ≥50% in at least one coral sample were plotted and organized by sample cite. Depths (m) 4.5 and 9.1 represent shallow photic reefs, 13.7 and 18.3 mid photic reefs, 22.8 and 27.5 deep photic, and 32 and 36.6 upper mesophotic reefs. There are no patterns between *Endozoicomonas* ASV diversity and depth (m).

Table C12: *Endozoicomonas* ASV reference matches (BLASTn). ASVs identified as *Endozoicomonas* with a relative abundance ≥50% in at least one coral sample and their closest reference match using BLASTn.

ASV	RefSeq	Host type	Host	Per. Identity	Reference
ASV1	NR_199442.1	soft coral	*Litophyton* sp.	96.1%	(da Silva et al., 2025)
ASV2	NR_199442.1	soft coral	*Litophyton* sp.	95.7%	(da Silva et al., 2025)
ASV3	NR_199442.1	soft coral	*Litophyton* sp.	98.7%	(da Silva et al., 2025)
ASV4	NR_109684.2	soft coral	*Eunicea fusca* and *Plexaura* sp.	100%	(Pike et al., 2013)
ASV5	NR_199442.1	soft coral	*Litophyton* sp.	100%	(da Silva et al., 2025)
ASV6	NR_109684.2	soft coral	*Eunicea fusca* and *Plexaura* sp.	99.6%	(Pike et al., 2013)
ASV7	NR_199442.1	soft coral	*Litophyton* sp.	100%	(da Silva et al., 2025)
ASV8	NR_109684.2	soft coral	*Eunicea fusca* and *Plexaura* sp.	100%	(Pike et al., 2013)
ASV9	NR_199442.1	soft coral	*Litophyton* sp.	98.7%	(da Silva et al., 2025)
ASV10	NR_116609.1	hard coral	*Montipora aequituberculata*	100%	(Yang et al., 2010)
ASV11	NR_146692.1	sea squirt	*Scandinavian ascidians*	92.6%	(Schreiber et al., 2016)
ASV12	NR_199442.1	soft coral	*Litophyton* sp.	98.7%	(da Silva et al., 2025)
ASV13	NR_199442.1	soft coral	*Litophyton* sp.	98.7%	(da Silva et al., 2025)
ASV14	NR_199442.1	soft coral	*Litophyton* sp.	99.6%	(da Silva et al., 2025)
ASV15	NR_109684.2	soft coral	*Eunicea fusca* and *Plexaura* sp.	99.6%	(Pike et al., 2013)
ASV16	NR_116609.1	hard coral	*Montipora aequituberculata*	99.6%	(Yang et al., 2010)
ASV17	NR_116609.1	hard coral	*Montipora aequituberculata*	100%	(Yang et al., 2010)
ASV18	NR_199442.1	soft coral	*Litophyton* sp.	96.8%	(da Silva et al., 2025)
ASV19	NR_146692.1	sea squirt	*Scandinavian ascidians*	97.4%	(Schreiber et al., 2016)
ASV20	NR_116609.1	hard coral	*Montipora aequituberculata*	99.6%	(Yang et al., 2010)
ASV21	NR_158127.1	hard coral	*Acropora* sp.	98.7%	(Sheu et al., 2017)
ASV22	NR_146692.1	sea squirt	*Scandinavian ascidians*	96.8%	(Schreiber et al., 2016)
ASV23	NR_199442.1	soft coral	*Litophyton* sp.	97.8%	(da Silva et al., 2025)

Table C12 Continued

ASV	RefSeq	Host type	Host	Per. Identity	Reference
ASV24	NR_146692.1	sea squirt	*Scandinavian ascidians*	96.8%	(Schreiber et al., 2016)
ASV24	NR_146692.1	sea squirt	*Scandinavian ascidians*	96.8%	(Schreiber et al., 2016)