Marine Biofilm Ecology: Inferring habitat quality in artificial and natural marine ecosystems

Elaine Shen

I. PROJECT SUMMARY

The objective of this project is to determine if marine biofilms on novel and established manmade and natural surfaces are predictors of habitat quality and future colonization events by sessile marine invertebrates.

Source-sink population dynamic theories arise from the fact that not all habitats are of equal quality, which varies the demographic rates (growth, reproduction, and survival) of macro-organisms in particular patches of habitat[1]. Sources exhibit net population growth and export individuals to surrounding areas, while sinks do not exhibit net growth of their own and import individuals from sources[1]. Understanding the varying contributors to habitat quality thus helps predict the niches particular habitat patches play in natural communities.

In marine ecosystems, favorable biotic habitat characteristics for macrofauna like fish are largely determined by benthic sessile invertebrate communities, including their physical complexity, algal cover, and coral cover[2]. These sessile marine invertebrates largely rely on a planktonic larval stage that moves with ocean currents and are highly complex rather than non-random[3]. Biofilms, which are comprised of microbial communities – mainly bacteria – are major facilitators of the larval settlement of sessile invertebrates through the production of chemical compounds that induce invertebrate larval settlement and metamorphosis[3]. These biofilms undergo community successions, with bacteria as the initial colonizers and later incorporating more diverse microbial communities, which has implications for subsequent invertebrate colonization success[4]. Generally speaking, the older a biofilm community, the higher the colonization success of marine invertebrates[5].

While the biofilms of different surfaces in marine communities have been established for microplastics, mammal carcasses, detritus, and naturally-occurring marine living systems, they have not been studied for artificial surfaces that are used for artificial reefs[6-9]. Understanding the biofilm dynamics in artificial reefs in comparison to natural reefs will help determine if marine biofilms can indicate habitat quality and ultimately, if artificial reefs serve as sources or sinks in marine ecosystems.

The following proposed project will test the **hypothesis that biofilms communities form in predictable ways on novel and established natural and manmade surfaces, making them indicators of habitat quality and subsequent colonization events.** To our knowledge, this project is the first to use whole-community sampling in-situ to understand biofilm community composition in manmade and natural marine ecosystems and relate them to macro-scale ecological patterns of invertebrate succession.

Specific Aim 1: Determine if marine surfaces (manmade and natural) differ in their biofilm communities. Manmade materials that are characteristic of artificial reefs will be placed in a natural marine ecosystem and sampled over time to observe changes in microbial and invertebrate communities through shotgun sequencing and visual identification.

Specific Aim 2: Determine if biofilm communities are indicators of habitat quality. Genomic data collected from Aim 1 will be further analyzed for community functions using metatranscriptomics and metaproteomics. In addition, the photos collected in Aim 1 will be scored for habitat quality using standardized habitat assessment scores. These additional analyses will allow for comparisons between diversity (Aim 1), function, structure, and habitat quality.

II. BACKGROUND

Marine biofilms are ubiquitous thin microbial aggregates that develop after a novel surface becomes submerged in the water[10-12]. Within seconds of submerging, the surface will accumulate dissolved organic matter, receive new physiochemical properties, and recruit primary colonizers dominated by bacteria[12-15]. The succession of biofilm colonizers is affected by qurorum sensing (the chemical signals that facilitate cell-to-cell communication between bacteria) and changes in surface physiology, biology, and microbial interactions facilitated by the primary colonizers[16-18]. In addition, differences in early-stage biofilm competition dynamics and ecophysiology is highly dependent on environmental conditions like nutrient status, temperature, pH, nutrient availability, hydrodynamics and water chemistry[19,20]. Over time, it has been reported in multiple studies that certain bacterial communities tend to evolve to a similar pattern on artificial surfaces like glass slides, stainless steel, polycarbonate, and polystyrene, suggesting that bacteria have evolved mechanisms that allow them to colonize surfaces with diverse physicochemical properties[20-23]. However, biofilm composition and larval settlement is still affected by physical properties of the artificial substrate, including color, surface roughness, wettability, and topography[24-27]. Recent surveys of marine biofilms have also revealed that these communities are drastically different than their surrounding environment, harboring communities that were previously largely unknown and undetectable in seawater analyses alone [28].

Biofilms play an important role in marine colonization because they mediate habitat selection (attachment or repulsion) of larger sessile macro-organisms[29]. The surface chemistry, micro-topography, and microbial products that biofilms produce all influence how invertebrate larvae distinguish between favorable and unfavorable surfaces of different origins and physiological qualities[29,30]. There is evidence that biofilms of different bacterial compositions differ in their ability to affect larval and spore settlement, however no predictive relationship between bacteria phylogeny and subsequent invertebrate settlement has been determined[31-34].

There is currently limited information on how marine surfaces, biofilm formation, and larval settlement interact with each other[28,29]. Artificial reefs, which by nature are comprised of artificial surfaces, can help elucidate how marine colonization of biofilms and subsequently, invertebrate larvae, affects the habitat quality of the entire system and attract or repel larger marine organisms. Technical challenges in DNA fingerprinting techniques have made marine biofilm analysis restricted to particular microbial taxa in specific environmental conditions because of limitations in accuracy, sensitivity, and specificity, leading to unsubstantial quantitative analysis[3]. Understanding the settlement effect with respect to the presence, absence, and quantity of mixed microbial communities is a challenge due to primer selection, however whole community sampling of natural biofilms (in contrast to cultured laboratory experiments of monospecies biofilms) and their overall bioactivity can narrow down the search for the relevant bioactive compounds and functions expressed by biofilm microbes[3]. Some attempts have been made to relate biofilm and macrofouling taxa together, however there is no strong relationship. Utilizing novel molecular techniques ("-omic" approaches) however, may illustrate which functions are crucial to biofilm processes. So far, only one metagenomics study has been published on marine biofilms in the Irish Sea to examine the microbial metagenome responsible for colonizing and degrading insoluble polysaccharides[35]. Developing –omics approaches will allow for a better characterization of biofilm development, whole community diversity, and function. Comparing these characteristics to natural communities will help address whether or not artificial reefs are of equal habitat quality to naturally-occurring ecosystems, which will help direct artificial reef design and conservation priorities.

III. RESEARCH QUESTIONS & HYPOTHESES

This proposal will address two outstanding questions concerning how biofilm development affects the (1) successional composition and (2) function of artificial surfaces:

1. *How do manmade and natural marine surfaces differ in their biofilm communities over time?*
2. *What functions and metabolic properties do biofilm communities have, and how do they affect macrofouling settlement and habitat quality?*

The following research approach will test the hypothesis that the biofilm community associated with a marine surface can be used as an indicator of future habitat quality, defined by the subsequent invertebrate colonization on those surfaces. This will be shown through the differences in biofilm and invertebrate colonization communities, functions, and metabolic properties on different marine surfaces (manmade and naturally-occurring).

IV. RESEARCH APPROACH

Specific Aim 1: We will test how artificial reef biofilms change over time in overall community diversity and composition and compare them to those on established artificial reefs and natural ecosystems.

Phase 1: Monitoring of experimental artificial reef biofilms using genomics and visual approaches
Replicates of common artificial reef surfaces (from concrete, tires, streetcars, oil and gas platforms) will be placed in randomly-mixed 5x5 meter quadrants in a sandy patch habitat (less than 10 meters in depth) of the Florida Keys, where an extensive artificial reef program currently exists). One representative replicate each surface type will be collected and scraped of their biofilm community and sequenced using a metgenomic approach every 8-hours for the first week of submersion, then monthly over the course of a year, then twice the following year to measure changes in early biofilm colonization and subsequent stabilization of biofilm and invertebrate communities (n=35 per surface type). A water sample for environmental DNA (eDNA) will be collected at each time point to characterize the surrounding free-floating microbial community as a way to compare available biofilm settlers to successful colonizers. Once fouling (macroinvertebrate) communities begin to emerge, photos will also be taken of these manmade surfaces to monitor macroinvertebrate colonization using image-analysis software.

Phase 2: Genomic and visual sampling of established artificial reefs and natural ecosystems
As a basis of comparison and positive control, established artificial reefs and coral reef ecosystems neighboring the site determined for Phase 1 will be sampled by scraping biofilm/invertebrate communities off of surfaces, collecting water samples for eDNA, and photographed once a month for the two-year sampling period in a similar fashion as Phase 1 protocols. If the biofilm and invertebrate communities from the Phase 1 experiment do not seem to resemble established artificial reef systems, it may be due to the fact that Phase 1 artificial reef biofilm and invertebrate communities have not adequately stabilized during the time period, or that the surface materials have a substantial effect on biofilm production under the same environmental conditions. Negative controls will be collected in sandy bottom habitats that have neither artificial reef materials nor natural ecosystems.

Phase 3: Next-generation sequencing, morphological analyses, comparison

DNA will be extracted from scraped biofilm for metagenomic sequencing using the culture-independent method of PhyloChip-based 16S rDNA profiling, which will provide not only the presence/absence of low abundance bacteria, but also quantify the relative abundance of bacterial communities within the asasy[36]. After PhyloChip processing, scanning, and probe set scoring, pixel images will be generated and analyzed using microarray analyses software[36]. eDNA samples will be amplified using the V3-V4 regions of 16S rRNA genes for taxonomic profiling of the surrounding seawater. Coral Point Count with Excel extensions (CPCe) will be used to quantify the collected images for macroinvertebrate colonization over time by plotting random points on the images for visual identification to the lowest taxonomic level. Principal coordinates analyses will be used to compare taxon relative abundance data between the different biofilm and invertebrate communities. Changes in overall diversity and invertebrate and biofilm cover by taxa will be plotted over time to see if there is an association between substrate material and biofilm/invertebrate cover. How biofilm and invertebrate communities differ between different artificial reef materials and between manmade and natural ecosystems will be determined by comparing beta diversity indices (like the Jaccard Index). Co-occurrence networks of each biofilm type's microbial communities will be combined with their macrofaunal successional community to generate indicator species in biofilms.

Specific Aim 2: We will use the DNA extracts from Aim 1 to analyze biofilm function and metabolism, and relate it to patterns of diversity and community structure.

Phase 1: Comparative metagenomics
The metagenomics data and taxonomic profiling generated in Specific Aims 1 will be further analyzed by integration with metaproteomic and metatranscriptomic analyses, following the protocol written by Leary et al. (2014). Qualitative liquid chromatography-tandem mass spectrometry metaproteome analyses will allow for identification of unique proteins and allow for further analyses of the complex processes and community composition present in biofilms that no single –omics approach would adequately address [35]. Additionally, metatranscriptomic analyses will help identify the functional structuring and redundancy that contribute to biofilm community composition and succession.

Phase 2: Comparison of biofilm diversity, structure, and function (settlement)
The images collected in Aim 1 will be scored for habitat quality using standardized habitat assessment scores (HAS) using metrics such as structural complexity, refuge space, percent of living cover, and percent of hard substratum[37]. HAS scores between manmade surfaces and natural ecosystems will be compared in order to determine, on a basic level, how habitat complexity differs between artificial reef materials and coral reefs. Then, Pearson's correlations will be made between final biofilm diversity and function, invertebrate diversity and HAS scores, and biofilm diversity and invertebrate diversity to see if there are predictive associations between these different measures. Modeling the different ways in which biofilm diversity correlates with subsequent function, invertebrate diversity, and habitat quality will allow for further analysis of how dominant groups of organisms and functions contribute to the overall patterns observed in ecological succession of marine surfaces. These analyses will help narrow down the groups of biofilm microbes that should be studied further in lab cultures for their metabolite production.

V. INTELLECTUAL MERIT

Artificial reefs are a popular fisheries management and conservation tool, however the mechanisms behind how they attract the invertebrate communities and subsequent macrofauna

populations remains mostly unanswered[34]. Many studies claim that artificial reefs facilitate ecosystem restoration through providing hard substratum, habitat and extra food sources for marine organisms, but have not examined the microbial processes that facilitate these metrics of habitat quality[1,34]. Combining the knowledge gaps in biofilm dynamics with their application to artificial reef materials design has important implications for marine conservation and management of threatened ecosystems.

VI. BROADER IMPACTS

While underrepresented minorities (URMs) are obtaining an increasing percentage of science and engineering degrees at all levels, these strides are not reflected in the diversity of faculty and researchers in senior-level positions at research institutions. Some causes have been identified for the low recruitment of URM undergraduates in marine science fields, including social and financial access to marine ecosystems and internships, though these experiences have been demonstrated to be formative in developing an interest in the field[38,39]. The field, computational, and taxonomic components of this project means that it has the potential to support many URMs from a diverse set of backgrounds and interests to become interested and feel a sense of belonging in the field of marine biology. There will be an emphasis on teaching transparent research skills (data management, reproducible open-source programming, taxonomy, field methods) and professional development (grant-writing, resume building, science communication). Students will be encouraged to lead (and collaborate on) outreach initiatives by utilizing established networks available online, like Skype a Scientist. Through a strong emphasis on inclusive undergraduate mentorship, this project aims to provide the training and opportunities for undergraduates be stewards of their local marine ecosystems to the diverse communities they represent.

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