

## Body of Proposal

### *Project Overview*

Regions with high marine biodiversity are often prioritized as focal areas for conservation to manage impacts of anthropogenic disturbances and climate change<sup>1,2</sup>. However, most of these regions are found in resource-poor tropical developing countries which are often understudied and thus lack the necessary data needed to fully represent and describe patterns of biodiversity<sup>3</sup>. For example, comprehensive marine biodiversity information in the Indo-Pacific region, the most prominent marine biodiversity hotspot in the world, mostly comes from marine fishes and corals, while many smaller invertebrate taxa have not been studied as rigorously<sup>4</sup>. Given the global-scale declines in marine biodiversity<sup>2,5</sup>, environmental DNA (eDNA) metabarcoding, a rapid, non-invasive biomonitoring tool that can comprehensively identify species from environmental samples<sup>6</sup>, provides a solution to implement rapid spatial monitoring and management responses to the biodiversity crisis<sup>3</sup>.

We propose to use eDNA metabarcoding to determine the patterns and drivers of marine biodiversity across different marine taxonomic groups at the epicenter of marine biodiversity – the Philippines (Figure 1). Spatiotemporal trends in fish biodiversity are well reported in the Philippines<sup>7–10</sup>, thus providing a good foundation with which hypotheses on other taxonomic groups, including invertebrates, eukaryotic plankton, and microbes, can be tested. We plan to compare the biodiversity of these different taxa within and among selected marine biogeographic regions in the country, and then determine how the patterns of diversity are affected by the following factors: (1) geographical location, (2) oceanographic variables, (3) primary productivity, (4) coastal urbanization, and (5) fishing effort.

### *Context*

Biodiverse regions provide important socio-economic (e.g., food security, source of livelihood) and ecosystem services (ecological resilience, nutrient cycling, carbon sequestration) both at local and global scales<sup>5,9,11</sup>. Identifying the threats brought about by direct and indirect anthropogenic activities, such as climate change, is critical to inform management, conservation, and restoration efforts<sup>3</sup>. However, the highest concentration of global marine biodiversity is located within the Indo-Pacific region in the Indo-Malay-Philippines Archipelago<sup>4,8,12</sup>, which is comprised mostly of resource-poor tropical countries that are most vulnerable to biodiversity threats<sup>3</sup>. Developing and utilizing rapid biomonitoring tools and identifying the factors that greatly influence biodiversity in this region are therefore necessary to address biodiversity crisis.

One of the most vulnerable regions that warrant special attention for conservation efforts is the Philippines, which is characterized as the epicenter of marine biodiversity based on species distribution of coastal fishes, invertebrates (e.g., crustaceans, mollusks), reef-building corals, seagrasses, and mangroves<sup>8,12</sup>. Spatial variation in biodiversity, geomorphology, and oceanography across the Philippine archipelago

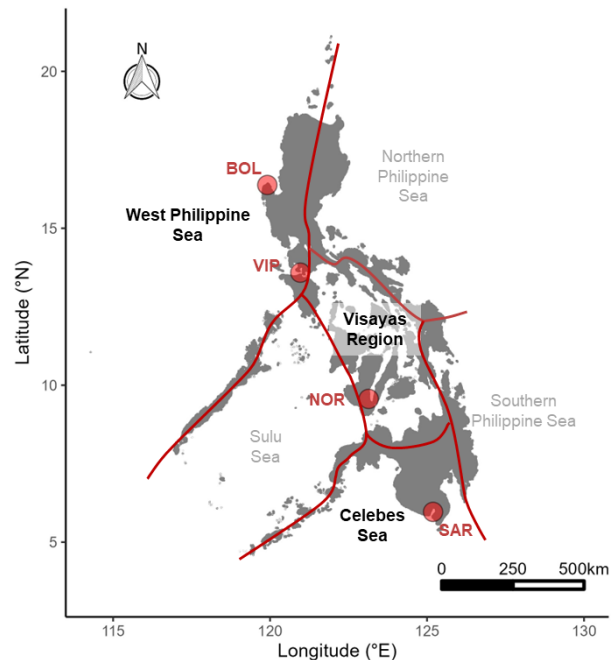


Figure 1. Sampling locations in the Philippines. The different marine biogeographic regions are delineated by the red line. The selected sites (red points) and biogeographic regions (bold text) are (1) BOL = Bolinao, West Philippine Sea; (2) VIP = Verde Island Passage, transition between West Philippine Sea and Visayas Region; (3) NOR = Negros Oriental, Visayas Region; and (4) SAR = Sarangani, Celebes Sea.

characterize its six marine biogeographic regions (Figure 1)<sup>7,13,14</sup>, which provide the geographical framework upon which management and conservation of marine resources are implemented<sup>15,16</sup>. While the geographical information system (GIS) overlay of marine species distribution based on taxonomic records indicated that the greatest marine biodiversity was concentrated centrally within the Philippines in the Visayas Region (VR, Figure 1)<sup>8</sup>, underwater fish visual census done from 1991 - 2008 suggested otherwise<sup>7</sup>. The biodiversity loss in VR underscores the need to refocus management and conservation efforts on areas where diversity has likely declined<sup>7,9,10</sup>. However, there are knowledge gaps on which such recommendations were based on. First, these observations were limited only to commercially important reef fishes. Observing similar patterns across a wide range of taxa, including other marine eukaryotes, bacteria, and archaea would result in a more reliable estimate of ecosystem level biodiversity and thus provide a stronger support in identifying regions of conservation priority<sup>3</sup>. Second, loss of biodiversity was attributed mainly to intense fishing pressure and habitat degradation in the region, but this conclusion was based mostly on observations of fishing practices, fisheries reports, and traditional knowledge in the area<sup>7,17</sup> rather than rigorous statistical analysis. Since marine biodiversity can also be impacted by geographical (e.g., longitude, latitude, depth) and environmental variables (e.g., temperature, primary productivity)<sup>11,18</sup>, revisiting the conclusion by testing statistical correlation between biodiversity and factors that could affect it, namely (1) geographical location, (2) oceanographic variables including temperature, nutrients, oxygen, pH, etc., (3) primary productivity, (4) coastal urbanization, and (5) fishing effort would provide a more rigorous assessment of the drivers of biodiversity. We therefore ask the following questions: (1) Is the low species richness of reef fishes observed in VR relative to other marine biogeographic regions concordant with other taxonomic groups in the food web? (2) Are the patterns of diversity (i.e., community composition) in the different marine biogeographic regions consistent across taxa, namely fishes, other marine eukaryotes, and microbes? (3) Which among the factors of interest most strongly correlate with the geographic patterns in diversity?

Relying on conventional monitoring methods (e.g., underwater visual census, market surveys) to answer our questions would be challenging and resource intensive, but such limitations can be circumvented with the use of environmental DNA (eDNA) metabarcoding. eDNA metabarcoding involves isolating the trace amounts of DNA shed by marine organisms in water samples, amplifying that eDNA with PCR, sequencing the eDNA samples to depths of 10,000-100,000 reads per sample, bioinformatically classifying the reads into amplicon sequence variants (ASV), and assigning the ASV to specific taxa using public reference databases, such as the Barcode of Life Database<sup>19,20</sup>. eDNA has many advantages over observational biodiversity assessment methods, e.g., it is less labor intensive, less invasive, and more cost effective and it is at the forefront in marine conservation efforts<sup>20-22</sup>. It has been widely used for biomonitoring marine systems to inform management<sup>22-26</sup>. Meanwhile, there are only three eDNA research on Philippine marine systems that were published, two of which focused on marine microbial communities<sup>27,28</sup>, and one on fishes<sup>29</sup>, and there remains a huge gap in the literature in terms of taxonomic and spatial coverage.

We propose to test for patterns in, and drivers of, marine biodiversity of fishes, invertebrates, eukaryotic plankton, and microbes among the different marine biogeographic regions in the Philippines using eDNA metabarcoding. First, we will test for differences in biodiversity indices (e.g., Chao1, Shannon, Simpson)<sup>30,31</sup> and taxonomic composition in relation to three biogeographic regions, 12 locations within each region, and taxonomic group. Second, we will test for a relationship between biodiversity and measured environmental variables that potentially shape diversity, focusing on the factors mentioned above. In performing these tests, we can address whether (1) marine biodiversity in VR is lower than the other regions, as was previously documented in observational surveys of economically important fishes<sup>7</sup>; (2) if different marine biogeographic regions have different community compositions and if variation in biodiversity is greater within or among regions; and (3) if observed patterns of biodiversity are strongly correlated with the environmental factors mentioned, and if those relationships vary by taxonomic group. The methodology for this research proposal is detailed in Appendix I and Appendix Figure 1.

### *Impact/Explanation of Innovative Nature of the Project*

The impact and innovative nature of the project is twofold. First, it can inform spatial management and conservation priorities by identifying regions of high biodiversity. eDNA metabarcoding enables simultaneous surveys of diversity patterns across different taxonomic groups, thus the inferences will be transformational. ***It will be the first time that this will be done in the epicenter of marine biodiversity.*** Second, it fosters international scientific collaboration, advancing environmental equity especially in the emerging field of eDNA metabarcoding. Currently, the eDNA approach is still primarily confined to high-income countries, and one way for developing countries to have access to this technology is through cohesive international collaboration with developed countries to encourage technical exchange, develop capacity, and share facilities and resources<sup>32</sup>. This initiative aligns seamlessly with the goals of the UN Ocean Decade, which aims to restore ocean health and gather ocean stakeholders worldwide. The partnerships formed from this seed grant will be pivotal for future research projects, not just with Filipino collaborators, but on a global scale.

### Expected Career Impact

Securing funding from the National Science Foundation, and other federal sources, is a critical factor in earning Tenure and Promotion for Assistant Professor Dr. Lin, and Associate Professor Dr. Bird. It will also help support a Ph.D. student, Kevin Labrador, conduct research for his dissertation. This seed project will significantly enhance the research capacity of an early-career faculty member, Dr. Lin, in domains of eDNA and marine biodiversity. Furthermore, it will foster in-depth collaboration with Dr. Bird's lab and expand international collaborations, laying the foundation for future federal grant applications such as NSF CAREER. The work of Dr. Bird in the Philippines has led to career development opportunities for himself and his students such as invited presentations, membership in the Philippines Fishes Genomics Working Group, a collaborative proposal to NSF DEB, and consistently being selected to represent TAMUS LSAMP since 2017 at the Louis Stokes Alliance for Minority Participation – NSF International Center of Excellence meetings, which was instrumental in securing funding from the NSF IRES program. This seed grant will develop the framework for our eDNA research in the Philippines, which can be leveraged in a full proposal to the National Science Foundation (NSF) Biological Oceanography Program where international collaboration is highly encouraged. In this project, we will foster and expand existing collaborations with Silliman University and University of the Philippines (UP) – Mindanao as well as establish collaborations with the UP-Marine Science Institute, Batangas State University, and the Philippines National Fisheries Research and Development Institute. Collaborations with Filipino institutions are mandatory to secure the collection permits required to complete the proposed work. Demonstrating that TAMU-CC can lead such an international effort will be an important consideration by NSF program officers and reviewers in making funding decisions.

### Timeline of Deliverables

[illegible]

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