Title: Investigating marine biodiversity patterns and drivers at the global epicenter using environmental DNA (eDNA) metabarcoding

Christopher E. Bird, Yajuan Lin

Abstract

Marine ecosystems provide important socio-economic and ecological services both at local and global scales, and regions with high marine biodiversity are often designated as focal areas for management and conservation. The marine ecosystems with the greatest biodiversity, those in the tropical Indo-Pacific region which include the Coral Triangle and the centers of reef and shore fish diversity, are also much less studied than other more depauperate regions, such as the temperate Atlantic and Pacific regions. The center of marine biodiversity is predicted to be in the Indo-Malay-Philippines Archipelago by evolutionary models based on the centers of overlap, origin, and survival hypotheses, and data on both shore and reef fish species range overlap predicts that the center of diversity is in the Central Visayas (CV) region of the Philippines. Observational surveys of fishes, however, found the CV to have the lowest species richness. Further, across the Philippines, fishes commercially exploited for food and the aquarium trade exhibited the lowest species richness. This highlights the potentially ephemeral nature of biodiversity patterns, the impact that anthropogenic activities can have on biodiversity in relatively short periods of time, and the importance of observational data in assessing biodiversity patterns. Further, it highlights the need for more rigorous study of the factors affecting marine diversity – commercially exploited fish might exhibit lower richness because there are fewer species that are commercially fished. More rigorous study is necessary to associate biodiversity patterns with their drivers.

To advance our knowledge about patterns of biodiversity in the hottest hotspot of marine biodiversity, we propose to collaborate with four Philippine universities to conduct a pilot study to rapidly assess the biodiversity of not only fishes, but also other eukaryotic metazoa such as invertebrates, eukaryotic zooplankton, photosynthesizers, and microbes in a latitudinal transect through the Philippines using use environmental DNA (eDNA) metabarcoding. Sampling will include the “center of fish diversity” (Central Visayas, Verde Island Passage) as well as locations outside of this region (West Philippine Sea, Celebes Sea). We plan to leverage existing data on (1) geographical location, (2) oceanographic variables, (3) primary productivity, (4) human population density, and (5) fishing effort to test for statistically significant relationships with observed biodiversity and community composition in the broad taxonomic groupings listed above. We will also test for concordance in biodiversity among the taxonomic groups which will allow more and better comparisons with commercially exploited taxa.

Overall, this pilot study will help to shed light on both the biogeography of marine biodiversity and its drivers in the center of marine biodiversity. It can also help to inform spatial conservation priorities by providing more comprehensive information on marine biodiversity that is not limited to a narrowly-defined taxonomic group. Furthermore, this research will be done alongside local collaborators, thus improving scientific partnerships within the region, and bridging the technological gap between high-income and developing countries, in concert with the UN Ocean Decade solutions for global ocean sustainability.

We plan to leverage the results of this study in a new proposal to the National Science Foundation (NSF) Biological Oceanography Program (PD 23-1650). The pilot will strengthen the proposal by helping us to demonstrate proof of concept with preliminary data, iron out methodological details, strengthen partnerships, and hone the hypotheses.

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*POSITION TITLE Associate Professor

*PRIMARY ORGANIZATION & LOCATION Texas A&M - Corpus Christi, Corpus Christi, TX, USA

***PROFESSIONAL PREPARATION - (see PAPPG Chapter II.D.2.h.i.a.3)**

PREVIOUS ORGANIZATION(S) & LOCATION(S)	DEGREE (if applicable)	RECEIPT DATE* (MM/YYYY)	FIELD OF STUDY
University of Hawai'i, Manoa, HI, USA	PhD	06/2006	Botany: Ecol, Evol, Cons, Biol
University of Connecticut, Storrs, CT, USA	BS	12/1997	Liberal Arts & Science: Ecolo & Evol Biol, Magna Cum Laude

Note - For Fellowship applicants only, please include the start date of the Fellowship.

***APPOINTMENTS AND POSITIONS - (see PAPPG Chapter II.D.2.h.i.a.4)**

Start Date - End Date	Appointment or Position Title, Organization, and Location
2018 - present	Associate Professor, Dept of Life Sci, Texas A&M University, Corpus Christi, TX, USA
2015 - present	Director, Genomics Core Laboratory, Texas A&M University, Corpus Christi, TX, USA
2012 - 2018	Assistant Professor, Dept of Life Sci, Texas A&M University, Corpus Christi, TX, USA
2012 - 2018	Affiliate Researcher, Hawai'i Institute of Marine Biology, U of Hawai'i, Kaneohe, HI, USA
2007 - 2012	Post-doc Research Fellow, Hawai'i Inst. of Marine Biology, U of Hawai'i, Kaneohe, HI, USA

***PRODUCTS - (see PAPPG Chapter II.D.2.h.i.a.5) Products Most Closely Related to the Proposed Project**

Rue, C.R., Selwyn, J.D., Cockett, P.M., Gillis, B., Gurski, L., Jose, P., Kutil, B.L., Magnuson, S.F., de Mesa, L.Á.L., Overath, R.D., Smee, D.L., Bird, C. E. (2021) Genetic diversity across the mitochondrial genome of eastern oysters (Crassostrea virginica) in the northern Gulf of Mexico. PeerJ, 9, e12205. https://doi.org/10.7717/peerj.12205

Thia, J.A., McGuigan, K., Liggins, L., Figueira, W.F., Bird, C.E., Mather, A., Evans, J.L. and Riginos, C. (2021). Genetic and phenotypic variation exhibit both predictable and stochastic patterns across an intertidal fish metapopulation. Molecular Ecology. https://doi.org/10.1111/mec.15829

Biesack, E. E., Dang, B. T., Ackiss, A. S., Bird, C. E., Chheng, P., Phounvisouk, L., ... & Carpenter, K. E. (2020). Evidence for population genetic structure in two exploited Mekong River fishes across a natural riverine barrier. Journal of Fish Biology, 97(3), 696-707. https://doi.org/10.1111/jfb.14424

Hamilton A, Selwyn JD, Hamner RM, Johnson HK, Brown T, Springer SK, Bird CE. (2020) Biogeography of shell morphology in over-exploited shellfish reveals adaptive tradeoffs on human-inhabited islands and incipient selectively driven lineage bifurcation. Journal of Biogeography, 47(7), 1494-1509. https://doi.org/10.1111/jbi.13845

Ackiss AS, Bird CE, Dang BT, Biesack EE, Phen C, Phounvisouk L, Stockwell BL, Vu QHD, Uy S, Carpenter KE. (2019) Cryptic lineages and a population dammed to incipient extinction in the Mekong River catfish, Hemibagrus spilopterus (Bagridae). Journal of Heredity. https://doi.org/10.1093/jhered/esz016

Other Significant Products, Whether or Not Related to the Proposed Project (see PAPPG Chapter II.D.2.h.i.a.5)

Drymon JM, Cooper PT, Powers SP, Miller MM, Magnuson S, Krell E, & Bird C. (2019) Genetic identification of depredator species in commercial and recreational fisheries. North American Journal of Fisheries Management. https://doi.org/10.1002/nafm.10292

Crandall, ED, Riginos C, Bird C, Liggins L, Treml E, Beger M, Barber PH, Connolly SR, Cowman PF, DiBattista JD, & Edble JA. (2019) The molecular biogeography of the Indo_pacific: Testing hypotheses with multispecies genetic patterns. Global Ecology and Biogeography. https://doi.org/10.1111/geb.12905

Scherer A, Bird CE, McCutcheon M, Hu X, Smee DL. (2018) Two-tierd defene strategy may compensate for predator avoidance costs of an ecosystem engineer. Marine Biology, 165(8), 131. https://doi.org/10.1007/s00227-018-3391-2

Ackiss A, Bird CE, Akita Y, Santos MD, Tachihara K, Carpenter KE. (2018) Genetic patterns in peripheral marine populations of the fusilier fish Caesio cuning within the Kuroshio Current. Ecology and Evolution, 8(23), 11875-11886. https://doi.org/10.1002/ece3.4644

Selwyn JD, Johnson JE, Downey-Wall AM, Bynum AM, Hamner RM, Hogan JD & Bird CE. (2017) Simulations indicate that scores of lionfish (Pterois volitans) were introduced to the Atlantic Ocean. PeerJ, 5: e3996. https://doi.org/10.7717%2Fpeerj.3996

***Synergistic Activities** - (**see PAPPG Chapter II.D.2.h.(i)(a)(6)**)

Texas Coastal Bend COVID-19 Pandemic Task-force, Texas A&M University - Corpus Christi, 2020 - 2022
Lead Spokesperson, Lead Modeler
Worked with Nueces County, City of Corpus Christi, Health Department, Nueces County Medical Society, and university faculty and students to model and forecast COVID cases and hospital beds required as well as communicate the results to the general public in regularly scheduled public addresses to the 12 county Texas Coastal Bend (pop. ~600,000). This work was recognized with the 2020 Caller-Times Newsmaker of the Year Award and the Westside Buisiness Association's "Year of the Hero - Torch Bearer" award in 2022.

Symposium for Innovation, Research, and Creative Activities, Texas A&M University - Corpus Christi, 2022- 2023
co-Founder, co-Chair
1 day conference where ~300 students from all colleges have presented their work in a professional conference atmosphere and receive constructive criticism.

Society for the Advancement of Chicanos/Hispanics & Native Americans in Science (SACNAS), Texas A&M University – Corpus Christi, 2015-present
Chapter Faculty Advisor
Provide guidance to TAMU-CC's SACNAS chapter. Assist in organizing STEM outreach efforts and professional development activities.

'Opihi Partnership, State of Hawai'i, 2008-present
Founding Partner
Collaborative interagency effort to enable sustainable management of near-shore fisheries, and restore crashed fisheries. This work has resulted in two community managed marine areas in Maui and the Kīpahulu CBSFA.

Louis Stokes Alliance for Minority Participation - NSF International Center of Excellence, 2017-present
Meeting Attendee & Participant
Representing Philippines PIRE, Philippines IRES, and TAMUS LSAMP, I have attended, contributed, and presented results at LSAMP-NICE meetings focused on facilitating international research for US students from underrepresented groups in STEM.

***Certification**:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§287, 1001, 1031 and 31 U.S.C. §§3729-3733 and 3802.

Signature (Please type out full name): Christopher E Bird Date: 08/14/2023

NSF BIOGRAPHICAL SKETCH

Provide the following information for the Senior personnel.
Follow this format for each person. **DO NOT EXCEED 3 PAGES.**

IDENTIFYING INFORMATION:

NAME: Lin, Yajuan

NSF ID: 000803646@nsf.gov
ORCID: 0000-0002-9057-9321

POSITION TITLE: Assistant Professor

ORGANIZATION AND LOCATION: Texas A&M University Corpus Christi, Corpus Christi, TX, USA

Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE (if applicable)	DATE RECEIVED	FIELD OF STUDY
Institut Universitaire Européen de la Mer (IUEM), Brest, Plouzané, France	Postdoctoral Fellow	2016 - 2018	Biogeochemistry
Duke University, Durham, NC, USA	Postdoctoral Fellow	2013 - 2015	Biogeochemistry
Duke University, Durham, NC, USA	PHD	05/2013	Marine Science and Conservation
Peking University, Beijing, Beijing, China	BS	06/2006	Geology/Biology (double major)

Appointments and Positions

2022 - present Assistant Professor, Texas A&M University Corpus Christi, Department of Life Sciences, Corpus Christi, TX, USA

2020 - 2022 Assistant Professor, Duke Kunshan University, Division of Natural and Applied Sciences, Kunshan, Not Applicable, N/A, China

2018 - 2020 Research Scientist, Duke University, Nicholas School of the Environment, Durham, NC, USA

Products

Products Most Closely Related to the Proposed Project

1. Wang S, Lin Y, Gifford S, Eveleth R, Cassar N. Linking patterns of net community production and marine microbial community structure in the western North Atlantic. The ISME Journal. 2018 June 22; 12(11):2582-2595. Available from: https://www.nature.com/articles/s41396-018-0163-4 DOI: 10.1038/s41396-018-0163-4
2. Lin Y, Moreno C, Marchetti A, Ducklow H, Schofield O, Delage E, Meredith M, Li Z, Eveillard D, Chaffron S, Cassar N. Decline in plankton diversity and carbon flux with reduced sea ice extent along the Western Antarctic Peninsula. Nature Communications. 2021 August 16; 12(1):-

. Available from: https://www.nature.com/articles/s41467-021-25235-w DOI: 10.1038/s41467-021-25235-w

3. Lin Y, Cassar N, Marchetti A, Moreno C, Ducklow H, Li Z. Specific eukaryotic plankton are good predictors of net community production in the Western Antarctic Peninsula. Scientific Reports. 2017 November 01; 7(1):-. Available from: https://www.nature.com/articles/s41598-017-14109-1 DOI: 10.1038/s41598-017-14109-1
4. Lin Y, Gifford S, Ducklow H, Schofield O, Cassar N. Towards Quantitative Microbiome Community Profiling Using Internal Standards. Applied and Environmental Microbiology. 2019 March; 85(5):-. Available from: https://journals.asm.org/doi/10.1128/AEM.02634-18 DOI: 10.1128/AEM.02634-18
5. Gorsky G, Bourdin G, Lombard F, Pedrotti M, Audrain S, Bin N, Boss E, Bowler C, Cassar N, Caudan L, Chabot G, Cohen N, Cron D, De Vargas C, Dolan J, Douville E, Elineau A, Flores J, Ghiglione J, Haëntjens N, Hertau M, John S, Kelly R, Koren I, Lin Y, Marie D, Moulin C, Moucherie Y, Pesant S, Picheral M, Poulain J, Pujo-Pay M, Reverdin G, Romac S, Sullivan M, Trainic M, Tressol M, Troublé R, Vardi A, Voolstra C, Wincker P, Agostini S, Banaigs B, Boissin E, Forcioli D, Furla P, Galand P, Gilson E, Reynaud S, Sunagawa S, Thomas O, Thurber R, Zoccola D, Planes S, Allemand D, Karsenti E. Expanding Tara Oceans Protocols for Underway, Ecosystemic Sampling of the Ocean-Atmosphere Interface During Tara Pacific Expedition (2016–2018). Frontiers in Marine Science. 2019 December 11; 6:-. Available from: https://www.frontiersin.org/article/10.3389/fmars.2019.00750/full DOI: 10.3389/fmars.2019.00750

Other Significant Products, Whether or Not Related to the Proposed Project

1. Larkin A, Blinebry S, Howes C, Lin Y, Loftus S, Schmaus C, Zinser E, Johnson Z. Niche partitioning and biogeography of high light adapted Prochlorococcus across taxonomic ranks in the North Pacific. The ISME Journal. 2016; 10(7):1555-1567. Available from: https://www.nature.com/articles/ismej2015244 DOI: 10.1038/ismej.2015.244
2. Johnson Z, Lin Y. *Prochlorococcus* : Approved for export. Proceedings of the National Academy of Sciences. 2009 June 30; 106(26):10400-10401. Available from: https://pnas.org/doi/full/10.1073/pnas.0905187106 DOI: 10.1073/pnas.0905187106
3. Ribalet F, Swalwell J, Clayton S, Jiménez V, Sudek S, Lin Y, Johnson Z, Worden A, Armbrust E. Light-driven synchrony of *Prochlorococcus* growth and mortality in the subtropical Pacific gyre. Proceedings of the National Academy of Sciences. 2015 June 15; 112(26):8008-8012. Available from: https://pnas.org/doi/full/10.1073/pnas.1424279112 DOI: 10.1073/pnas.1424279112
4. Lin Y, Gazsi K, Lance V, Larkin A, Chandler J, Zinser E, Johnson Z. *In situ* activity of a dominant *Prochlorococcus* ecotype (eHL-II) from rRNA content and cell size. Environmental Microbiology. 2013 June; :n/a-n/a. Available from: https://onlinelibrary.wiley.com/doi/10.1111/1462-2920.12135 DOI: 10.1111/1462-2920.12135
5. Hunt D, Lin Y, Church M, Karl D, Tringe S, Izzo L, Johnson Z. Relationship between Abundance and Specific Activity of Bacterioplankton in Open Ocean Surface Waters. Applied and Environmental Microbiology. 2013 January; 79(1):177-184. Available from: https://journals.asm.org/doi/10.1128/AEM.02155-12 DOI: 10.1128/AEM.02155-12

Synergistic Activities

1. International collaboration: participated in large scale international cruises including 1) TARA Pacific (coral reef focused) and 2) Antarctic Circumnavigation Expedition, involving researchers from 22 countries.
2. Reviewer: National Science Foundation (OPP's Antarctic Sciences Section), the North Pacific Research Board (NPRB), *The ISME Journal*, *Microbiome*, *Limnology and Oceanography*, *Frontiers Microbiology*, *Aquatic Microbial Ecology*, *Scientific Reports*, *Journal of Geophysical Research*, *Deep Sea Research Part II*.
3. Undergraduate mentoring: two NSF REU students and 6 undergraduate students for senior thesis.
4. Science communication: TARA Pacific Expedition "meet a scientist on board" to allow students and teachers to explore the scientific instruments used aboard the schooner TARA, in Lorient, France (2016) and Boston, USA (2018).
5. K-12 education: lecture on 'Phytoplankton and the Ocean Carbon Cycle' for Miss Katie Doyle's Ocean's Class program, with asynchronous session sent to 20 classrooms across the US.

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

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Budget and Justification

Table 1. Proposed budget for this research proposal.

Line Item	Description		Cost
Personnel	PhD student salary, fringe benefits, and tuition for one semester	$	14,819
eDNA Collection Kit	Items that will be sent to collaborators to collect and filter seawater	$	2,932
Laboratory Supplies	Laboratory items and reagents needed to process samples, from eDNA extraction up to library preparation for sequencing	$	8,886
Contractual	Sequencing costs; shipment fees of items (e.g., collection kits, samples) to and from the Philippines	$	3,350
Total		$	29,987

The total budget requested by this project is **$29,987.** The line items and justifications are provided below and summarized in Table 1:

Personnel: $14,819

A PhD student will help to assemble the eDNA collections kits, carry out laboratory work and data analysis. We are requesting one semester (4.5 mo at 50% FTE) of support for the graduate student (4.5 mo * $2000 = $9,000). Fringe benefits for a student are 10.7% of their salary ($9000 * 0.107 = $963) and insurance is $562 per month (0.5 FTE * 4.5 mo * $562 = $1265), and tuition and fees is estimated to be $3,591 per semester.

Collection Kit: $2,932

The collection kit will be sent to the four collaborating institutions in the Philippines for the collection and filtration of seawater samples. Each kit contains the following: a 5-gallon bucket to hold the seawater prior to filtration ($23.04); 300 mL syringes to aspirate and administer the seawater to the filtration set up ($145.44); the serial filtration set-up, consisting of 80 µm Nitex Nylon Mesh Filter Sieve ($264.00), 0.45 µm Sterivex cartridges ($1,176.88), and 0.22 µm Sterivex cartridges ($1,174.44); 3 mL syringes to administer the preservative to the Sterivex cartridges ($13.99); dual male-female luer-lok caps to seal the Sterivex cartridges ($79.96); and 1 oz Whirl-Pak sterile bags to store the cartridges and prevent contamination or leaks during transport ($53.76). We will be sending 39 Sterivex cartridges per size fraction per site, summing up to 156 samples per size fraction for processing.

Laboratory Supplies: $8,886

The laboratory supplies will be used to process samples in TAMU-CC Genomics Core Laboratory. A PVC pipe cutter will be used to open the Sterivex cartridge ($25.98), and the filters will be placed and cut into smaller sections on sterile 90 mm disposable petri dishes ($214.00). eDNA from the 0.45 (n = 156) µm and 0.22 µm filters (n = 156) will be extracted using Qiagen Blood and Tissue Kit ($2,127.76) and Qiagen Plant Mini Kit ($1916.76), respectively. Metabarcoding markers will be amplified using custom oligos and PCR kits ($557.33), which will then be used to prepare the TaggiMatrix libraries for high

throughput sequencing (156 samples * 4 loci = 624 libraries @ $6.48/library = $4,044.38). Costs for the library preparation include PCR (2016 reactions including triplicates and controls: $947.01), agarose gel electrophoresis ($120.96), SPRI select ($1,233.12), ligation of iTru primers and clean-up ($383.93), and the consumables for all lab work including pipet tips, microtubes, gloves, bleach, ethanol, and sterile water ($1,359.36).

Contractual: $3,350

The contractual fees will cover the sequencing and shipping costs. The amplicon libraries will be sequenced using a NovaSeq 6000 PE 250 lane (400M read pairs); we will aim 130 M read pairs ($1,787.50). We will also ship memoranda of agreements ($328.24) and eDNA sample collection kits to the Philippines ($851.38), and eDNA samples back to TAMU-CC ($383.16).

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[1,2]. 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[3]. 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[4]. Given the global-scale declines in marine biodiversity[2,5], environmental DNA (eDNA) metabarcoding, a rapid, non-invasive biomonitoring tool that can comprehensively identify species from environmental samples[6], provides a solution to implement rapid spatial monitoring and management responses to the biodiversity crisis[3].

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[7–10], 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[5,9,11]. Identifying the threats brought about by direct and indirect anthropogenic activities, such as climate change, is critical to inform management, conservation, and restoration efforts[3]. However, the highest concentration of global marine biodiversity is located within the Indo-Pacific region in the Indo-Malay-Philippines Archipelago[4,8,12], which is comprised mostly of resource-poor tropical countries that are most vulnerable to biodiversity threats[3]. 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[8,12]. Spatial variation in biodiversity, geomorphology, and oceanography across the Philippine archipelago

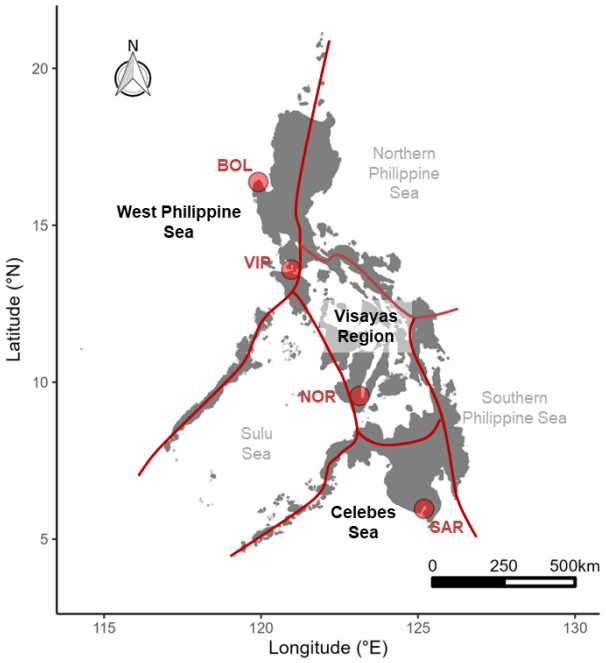

Figure 1. Sampling locations in the Philippines. The different marine biogeographic regions are delineated by the red line. The selected sites (red points) and biogeographic regions (bold text) are (1) BOL = Bolinao, West Philippine Sea; (2) VIP = Verde Island Passage, transition between West Philippine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

characterize its six marine biogeographic regions (Figure 1)[7,13,14], which provide the geographical framework upon which management and conservation of marine resources are implemented[15,16]. 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)[8], underwater fish visual census done from 1991 - 2008 suggested otherwise[7]. The biodiversity loss in VR underscores the need to refocus management and conservation efforts on areas where diversity has likely declined[7,9,10]. 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[3]. 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[7,17] 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)[11,18], 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[19,20]. 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[20–22]. It has been used widely used for biomonitoring marine systems to inform management[22–26]. Meanwhile, there are only three eDNA research on Philippine marine systems that were published, two of which focused on marine microbial communities[27,28], and one on fishes[29], 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)[30,31] 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[7]; (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[32]. 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 gobal 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

Deliverable	23-Aug	23-Sep	23-Oct	23-Nov	23-Dec	24-Jan	24-Feb	24-Mar	24-Apr	24-May	24-Jun	24-Jul	24-Aug
Invite collaborators from the Philippines													
Formalize partnerships with scientific agreements													
Coordinate sampling with collaborators													
Prepare sampling kits													
Fieldwork and sampling													
Receive filtered seawater samples from collaborators													
Build scripts, aggregate correlates for modelling biodiversity patterns													
Laboratory Work													
Sequencing													
Analysis													
Manuscript													

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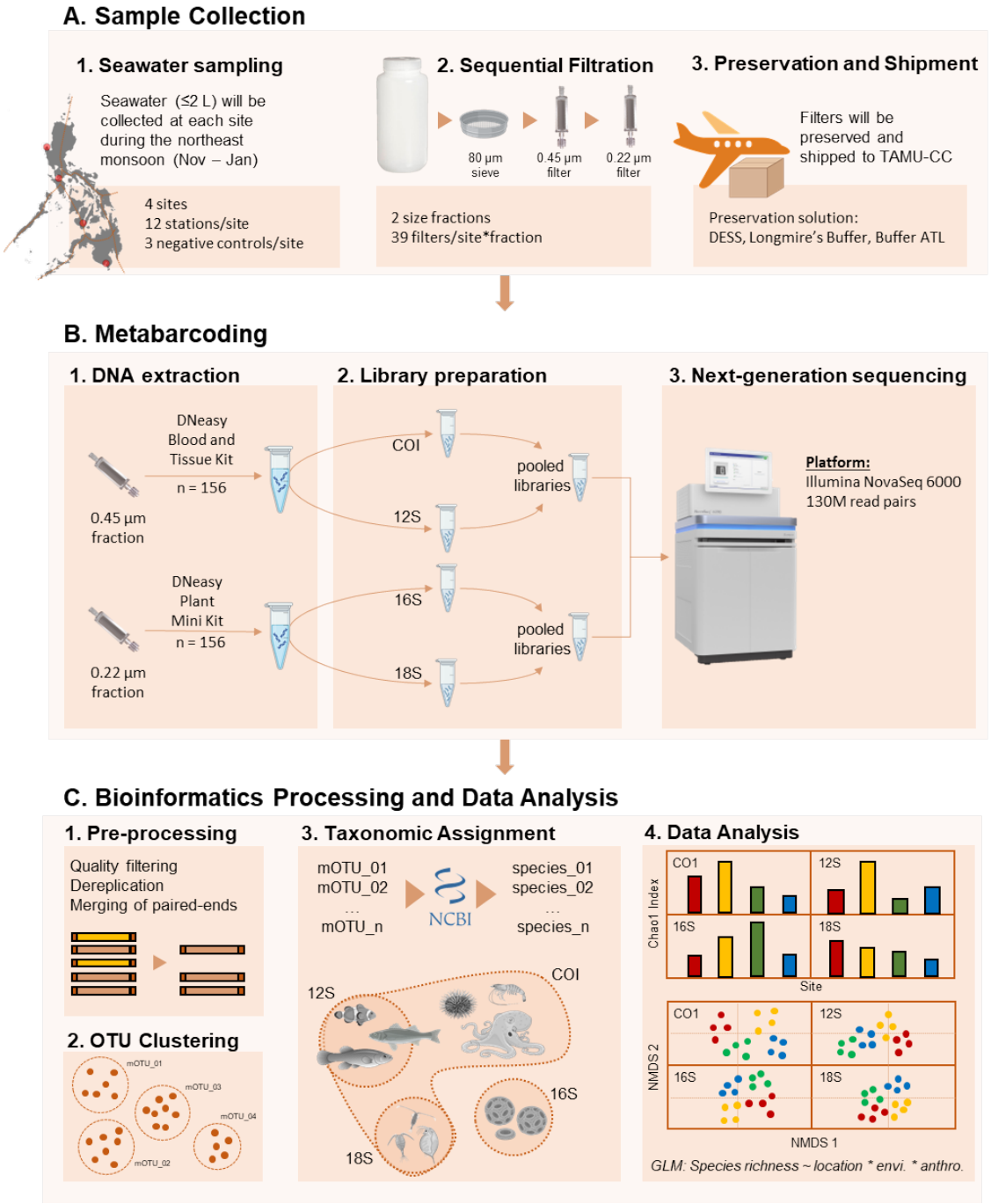
Appendix I. Materials and Methods



Appendix Figure 1. Overall schematics for the materials and methods. (A) Collected seawater samples will be sequentially filtered, preserved, and then shipped to Texas A&M University – Corpus Christi Genomics Core Laboratory (TAMU-CC GCL) for processing. (B) eDNA will be extracted and used as templates to amplify the target metabarcoding markers. The amplicon libraries will be pooled and then sequenced. (C) Pre-processed reads will be clustered based on a similarity threshold to obtain the molecular operational taxonomic units (mOTUs); these will be identified using a curated database, with each amplicon library targeting a specific taxonomic group. The read counts for each mOTU will then be used to test the hypotheses on patterns and drivers of marine biodiversity.

Invitation to Collaborate

Filipino researchers across four academic institutions were invited to collaborate on this project, each covering a sampling location: (1) University of the Philippines Diliman – The Marine Science Institute in Bolinao, Pangasinan; (2) University of the Philippines Mindanao in Sarangani Bay, (3) Batangas State University in the Verde Island Passage, and (4) Silliman University in Negros Oriental. In addition, the national agency tasked to conduct research and development for fisheries, the Department of Agriculture – National Fisheries Research and Development Institute (DA – NFRDI), was also invited to strengthen the partnership with the Philippine government. The research partnerships will be formalized with a Memorandum of Agreement for Educational and Scientific Cooperation which will stipulate the roles and responsibilities of each party, as well as the guidelines for data sharing and scientific output coming from this research grant.

Seawater Collection, Serial Filtration, and Preservation

There will be four sites in this study, each covering a different marine biogeographic region (Figure 1): (1) Bolino, Pangasinan in the West Philippine Sea (WPS), (2) Negros Oriental in the Visayas Region (VR), (3) and Sarangani in the Celebes Sea (CS); meanwhile, (4) the Verde Island Passage is a strait that connects WPS and VR. A total of 12 triplicate seawater samples (≤ 2 L; n = 36) will be collected from each site, and a negative field control (2 L sterile distilled water; n = 3) will be included to assess contamination[33,34]. To minimize seasonal variation, collection will only be done from November – January, which coincides with the northeast monsoon season. The water samples (n = 39/site) will be pre-filtered using 80 µm sieve into bleach-sterilized water containers to remove large particles (e.g., tissue debris, larvae), then serially filtered through 0.45 µm and 0.22 µm Sterivex cartridges using a sterile 300 mL syringe[34–36]. Filtration paraphernalia (e.g., silicone tubing, syringes) will be rinsed with 10% bleach and sterile distilled water before each use. Salt saturated DMSO buffer with EDTA (DESS), Longmire's Buffer, or Buffer ATL will be introduced to the cartridges for preservation[21,37]; the cartridges will then be sealed using Luer Lok cap, stored on ice, and then transported to a laboratory where it will be stored at -20 °C. The cartridges will be shipped to Texas A&M University – Corpus Christi Genomics Core Laboratory in dry ice for DNA extraction and sequencing.

DNA Extraction, Library Preparation, and Next-Generation Sequencing

eDNA will be extracted from 0.45 µm and 0.22 µm filter units using DNeasy Blood and Tissue Kit and DNeasy Plant Mini Kit, respectively[26,38], following the open Sterivex extraction method[35]. Briefly, DESS preservative will be flushed out from the Sterivex cartridge with a syringe, and the cartridge will be cut open by a PVC pipe cutter to allow removal of the filter. The filter will be placed in a Petri dish, cut into smaller pieces, and then placed in a fresh microtube for lysis. The remaining steps will be based following the manufacturer's protocol. All steps will be done aseptically under a laminar flow hood dedicated for eDNA extraction to minimize contamination. A negative laboratory control (ultrapure water) will also be processed alongside the samples.

Standard metabarcoding primers will be used to amplify target regions for each taxonomic group of interest (Appendix Table I). Library preparation will follow the Adapterama II protocol to allow pooling of libraries amplified using different primers[39], and sequencing of 2 x 250 paired-end reads will be done on Illumina NovaSeq 6000 platform[40].

Bioinformatics processing and data analysis

Bioinformatics pipeline will follow published protocols[35,41,42]. The raw sequences will be pre-processed by removing low quality reads and sequence pairs that do not contain the primer sequences. Forward and reverse reads will be merged in case they overlap, or will be concatenated otherwise; duplicate reads, singletons, chimeric sequences will then be removed. Pre-processed reads will be clustered based on a % similarity threshold (97 – 99%, depending on the marker) to identify the molecular operation taxonomic units (mOTUs). Taxonomic assignment will be done by querying a representative mOTU to a curated reference database; the identification will be done up to the species if possible. A

community matrix based on the read count of each mOTU for each sampling location will be generated for downstream analyses.

Data analysis will be done on R[43] following published analytical approaches[41], with the aid of the following packages: *vegan*[44], *ape*[45], and *tidyverse*[46]. The dataset will be partitioned based on the taxonomic groups recovered from each metabarcoding primer used. We will compare the alpha biodiversity indices (e.g., Chao1, Shannon, Simpson)[31] among the different biogeographic regions using multivariate mixed modeling[47]. We will use non-metric multidimensional scaling (NMDS) to visualize the differences in community composition among biogeographic regions, test for significant differences using Permutational Analysis of Variance (PERMANOVA), and then assess the effects of the explanatory variables of interest by fitting them in the ordination. The explanatory variables are: (1) geographical location, (2) sea surface temperature, (3) chlorophyll-a concentration, (4) population density, and (5) fishing effort. Where possible, ecological and anthropogenic variables will be retrieved from online databases such as the National Aeronautics and Space Administration's Earth Observing System Data and Information System (NASA - EOSDIS; https://oceancolor.gsfc.nasa.gov) and the CountryStat database of Philippine Statistics Authority (https://openstat.psa.gov.ph/Featured/CountrySTAT-Philippines).

Appendix Table I. Primers to amplify metabarcoding markers for various marine taxa.

Taxonomic Group	Target Marker	Primer Pair	Primer Sequence (5' – 3')	Reference
Fishes	12S (170 bp)	MiFish-U-F	GTCGGTAAAACTCGTGCCAGC	Miya et al., 2015
		MiFish-U-R	CATAGTGGGGTATCTAATCCCAGTTTG	
Eukaryotic Metazoa	COI (313 bp)	mlCOIintF-XT	GGWACWRGWTGRACWITITAYCCYCC	Wangensteen et al., 2018
		jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA	
Eukaryotic Plankton	18S rRNA (270 bp)	EukF	CCAGCASCYGCGGTAATTCC	Lin et al., 2019
		EukR	ACTTTCGTTCTTGAT	
Bacteria and Archaea	16S rRNA (290 bp)	515F	GTGYCAGCMGCCGCGGTAA	Lin et al., 2019
		805R	GACTACNVGGGTATCTAAT	

Bird, Chris

From: Hernandez, Florencio
Sent: Wednesday, August 30, 2023 3:27 PM
To: Bird, Chris; Williams, Kyra
Cc: Lin, Yajuan; Labrador, Kevin
Subject: RE: TCRF Approval

Categories: Blue Category

Dear Chris,

I approve the submission of this proposal.

Cheers,

Eloy

From: Bird, Chris <Chris.Bird@tamucc.edu>
Sent: Wednesday, August 30, 2023 2:10 PM
To: Hernandez, Florencio <florencio.hernandez@tamucc.edu>; Williams, Kyra <Kyra.Williams@tamucc.edu>
Cc: Lin, Yajuan <yajuan.lin@tamucc.edu>; Labrador, Kevin <klabrador@islander.tamucc.edu>
Subject: TCRF Approval

Dear Dean Hernandez,

As part of the submission process to the R&I TCRF competition, we are requesting your approval of our application for the funds. If you approve, you can respond in the affirmative to this email and we will include that in the submission.

Per our conversation last week, we are planning to submit a proposal to the R&I TCRF competition entitled "Investigating marine biodiversity patterns and drivers at the global epicenter using environmental DNA (eDNA) metabarcoding " to collect preliminary data testing for drivers of marine eDNA biodiversity, best eDNA sample preservative, as well as establish collaborations with Philippines institutions, and secure sampling permits that can be leveraged in an NSF BioOce proposal.

Attached is a draft of the proposal for your review.

Please let us know if you have any questions or feedback.

Cheers,

Chris

Christopher E. Bird
Associate Professor, Life Sciences
Director, Genomics Core Laboratory
Texas A&M University – Corpus Christi