2021

BIODIVERSITY AND BIOGEOGRAPHY OF PROTIST COMMUNITIES IN COASTAL MARINE ECOSYSTEMS ACROSS INDONESIA

Erin Borbee
University of Rhode Island, eborbee@uri.edu

Recommended Citation
Borbee, Erin, "BIODIVERSITY AND BIOGEOGRAPHY OF PROTIST COMMUNITIES IN COASTAL MARINE ECOSYSTEMS ACROSS INDONESIA" (2021). *Open Access Dissertations.* Paper 1278.
https://digitalcommons.uri.edu/oa_diss/1278

BIODIVERSITY AND BIOGEOGRAPHY OF PROTIST COMMUNITIES IN COASTAL MARINE ECOSYSTEMS ACROSS INDONESIA

BY

ERIN BORBEE

A DISSERTATION SUBMITTED IN PARTIAL FUFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2021

DOCTOR OF PHILOSOPHY DISSERTATION

OF

ERIN BORBEE

APPROVED:

Dissertation Committee:

Major Professor: Christopher Lane

Jonathan Puritz

Austin Humphries

Brenton DeBoef
DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

2021

ABSTRACT

Despite a long history of biodiversity and biogeography research in Indonesia we know very little about protist communities across this region. What we do know about protist communities across Indonesia is largely based on visual surveys, which have been proven to vastly underestimate protist diversity. The combination of immense biodiversity, oceanographic significance, and human activity across this region make it an interesting setting for understanding the relative effects of abiotic and biotic drivers of protist community structure. The work presented in this dissertation uses metabarcoding to characterize protist community composition and underwater visual census (UVC) data to characterize fish and benthic communities across Indonesia. Throughout each chapter the UVC data as well as socio-environmental variables like human population and distance to market are used to understand drivers of protist community structure on both broad and local geographic scales.

Chapters two and three focus on understanding abiotic and biotic factors driving broad-scale biogeographic trends in protist communities across Indonesia. Our four sampling regions span from Pacific to Indian Ocean across a gradient of fishing pressure and fish biomass. Despite the shift in biomass at upper levels of the food web, protist communities appeared minimally impacted by fishing pressure. Instead, protist communities showed a sharp community shift between the two regions with lowest fishing pressure in the east. This community shift appears to be driven by surface currents, specifically the Indonesian Throughflow and the Halmahera Eddy. However, due to sampling timelines, seasonal community shifts cannot be ruled out either.

Chapter three further explores how fisheries management impacts protist communities on a local scale. Similar to fishing pressure, fisheries management strategies impact fish biomass levels across sites within sampling regions. The highest fish biomass occurred in sites where fishing was prohibited and lowest fish biomass occurred in sites where fishing was unrestricted. Just as with fishing pressure, the shifts in biomass at the upper levels of the food web appeared to have minimal impact on protist communities at the base of the food web.

Chapter four narrows the focus to Lombok, Indonesia and explores how disturbances and biotic interactions shape protist communities on a local scale. Lombok was the most heavily fished of our four sampled regions. Natural disturbances and destructive fishing practices have resulted in high coral rubble at many sites. These disturbances play a role in structuring protist communities on a local scale across the island. Rubble fields were characterized by increased relative abundance of small heterotrophic protists like ciliates and cercozoans, and also by increase relative abundance of diatoms. While ciliate and cercozoan success is typically characteristic of increased bacterial growth, diatom success is typically a result of increased nutrient levels. In this case, the nutrients likely originate from sewage effluent across the island.

This work is the first to characterize protist communities across Indonesia using metabarcoding data. These data highlight the importance of abiotic factors like surface currents in structuring protist communities on a broad geographic scale, while also highlighting the lack of anthropogenic impact on structuring protist communities. Additionally, this work explores the roles natural disturbances and destructive fishing practices have on protist communities on a local scale. The findings further expand our knowledge on drivers of protist biogeography across the globe, and provide insight on how these data can inform management and policy decisions in the future.

ACKNOWLEDGEMENTS

I have never been good at sentimentality, but there are a lot of people that I need to thank for all of their help over the past four years, so here goes nothing. First and foremost, I need to thank my advisor Christopher Lane for all of his support and guidance during my time at URI. Likewise, I need to thank my committee members Austin Humphries and Jon Puritz for their feedback and support throughout this process. I also want to thank both Chris and Austin for helping me navigate this project and an international collaboration for the first time in my research career. While the path of the collaboration and outcomes of this project were not always clear, you always had our back and I appreciate all you've done to make this work possible.

This work would not have been possible without our collaborators in Indonesia. I want to thank the students, in particular, for their help in the lab and making me feel like family during my trips over there. Inna, I cannot thank you enough for all the help you gave me in the lab and constantly looking out for me when I was traveling by myself. Ester, you are a wizard in the lab. We would never have been able to finishing processing samples without your help. Iqbal and Abby, thank you for helping us in the field and lab, but especially for being our translators at the immigration office. I still don't know what magic you worked to get our permits processed in such a short amount of time, but I am forever grateful to you for that. Budi, thank you for all your work in the field and your willingness to jump into lab work head on so that we could get all of our samples done in time. There are so many more people I could mention, but to Selia, Arina, Titi, Lita, Dije, Begin, Ubun, and whoever else I’m missing, thank you. All of your friendship and help during long days in the lab and field were greatly appreciated and I hope our paths cross again someday.

Finally, I need to thank the CBLS 260 crew and a few other students at URI that have been a part of this project and more generally have just been great friends during my time here. To all the current and former Lane Lab and Jenkins Lab members that have been around these past four years, thank you for the tolerating my random science, data analysis, and occasional sports tangents in the office. Thanks for lots of laughs and even more baked goods. I need to thank Elaine Shen, Kristina Terpis, and Chris Paight who were a part of this project in various capacities in both the field and lab, and Paul Carvalho who led the field season in Wakatobi himself generating an entire region of data used in this dissertation. To Evelyn Spencer, Katie Nickles, and Liz Hunter, thanks for always being down to dive even when we don’t have anything to collect. To Laura Holland, thanks for being the only one who ever knew what I was talking about when it came to most sporting events. And finally, to Jillian Freese and Alexa Sterling, thanks for peer pressuring my first-year, anti-social self into joining SWMS. Being a part of that community has been a really wonderful part of my grad school experience. We’ve all gone down lots of data analysis and science rabbit-holes and I don’t think I could possibly count how many times we’ve all said the phrase “nothing means anything” to one another. So, thank you for your support and most importantly your friendship these past four years. I would not be here without you.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

CHAPTER 2

Supplemental Tables

CHAPTER 3

Supplemental Tables

CHAPTER 4

LIST OF FIGURES

FIGURE PAGE

CHAPTER 1

CHAPTER 2

Supplemental Figures

CHAPTER 3

CHAPTER 4

CHAPTER 1: INTRODUCTION

Origins of biogeography

Indonesia has a long history of biodiversity and biogeography research dating back to the mid 1800s and Wallace's Line. Wallace's Line was first described by Alfred Russel Wallace in 1859 and was later named by Thomas Huxley in 1868 (Huxley, 1868). The line aimed to denote a boundary dividing islands in the region into those dominated by Asian fauna in the west and Australian fauna in the east. Over the next few decades the line has been redrawn by multiple scientists including Huxley (1868), Lydekker (1896), and Weber (1902) to best approximate the boundary between these two distinct faunae (Mayr, 1944; Simpson, 1977) (Figure 1). These lines, however, do not represent a hard break between Asian and Australian faunae, but rather a line of faunal balance with islands in the west comprised of greater than 50% Asian fauna and islands in the east comprised of greater than 50% Australian fauna (Mayr, 1944).

The theory behind these lines originates from the geographic history of the Indo-Pacific region, and more specifically from the dramatic changes in sea level across these regions that united various islands at different points in time. As recent as the Pleistocene (~17,000 years ago), the sea level in the Indo-Pacific was estimated to be 120m lower than its present day level (Voris, 2000). This sea level change united islands on the Sunda (Asian) shelf and Sahul (Australian) shelf allowing for exchange of terrestrial faunae across islands that are now isolated from one another (Figure 2). Fluctuations in sea level and their subsequent effects on speciation and structuring of communities across the Indo-Pacific, led to pivotal research on speciation, population structures and the evolutionary mechanisms behind these including genetic drift and gene flow between populations (Mayr, 1954a). One particularly notable theory that emerged from evolutionary research in this region was the Equilibrium Theory of Insular Zoogeography, which helped explain patterns of adaptive radiation and gene flow between populations across islands in the Indo-Pacific (MacArthur & Wilson, 1963). However, these studies were often limited to terrestrial systems, and by the mid 20th century biodiversity and biogeography research on marine systems in the Indo-Pacific had taken off driving scientists to ask the question of whether the mechanisms for speciation on land were applicable in marine systems.

The Coral Triangle and origins of marine biodiversity

The Coral Triangle, which sits at the center of the Indo-Pacific, is widely considered the epicenter of marine biodiversity with diversity decreasing both latitudinally and longitudinally outward from this region (Allen, 2008; Bowen, Rocha, Toonen, & Karl, 2013; Hoeksema, 2007; Veron et al., 2009) (Figure 3). Over the course of the 20th century a number of different hypotheses emerged in attempt to explain the vast diversity in this region. The first of these hypotheses is the Center of Origin hypothesis which posits that the Coral Triangle is the origin of speciation and therefore has the highest diversity (Briggs, 1999a, 1999b, 2003). On the other hand, the Center of Accumulation hypothesis suggests that speciation occurs on the periphery of this region and as species ranges expand outwards, diversity accumulates in the Coral Triangle (Ladd, 1960). Finally, the Center of Overlap hypothesis suggests that the Coral Triangle is not the center of speciation, but rather that the high diversity in this region is due to the overlap between distinct faunae from the Pacific and Indian Oceans coming together in this region (Woodland, 1983).

Support for each of these hypotheses has been found in studies on population structure of various marine metazoans across the Indo-Pacific, but to date there is no consensus on any one hypothesis (Barber, Cheng, Erdmann, Tenggardjaja, & Ambariyanto, 2011; Bowen et al., 2013; Crandall et al., 2019; Gaither et al., 2011; Gaither & Rocha, 2013; Tornabene, Valdez, Erdmann, & Pezold, 2015). Instead, there is growing support that a combination of the existing hypotheses best explains the immense diversity observed in this region (Barber, 2009; Barber et al., 2011). These hypotheses have also faced many criticisms throughout time as well. One criticism of the Center of Origin hypothesis was that it lacked a convincing mechanism for creating such diversity within the Coral Triangle and must therefore rely on sympatric speciation to create such novel diversity (Briggs, 1999c; Jokiel & Martinelli, 1992). At that point in time, sympatric speciation was not thought to be common amongst metazoans and the primary mode of speciation was thought to have been allopatric (Barraclough, 1998; Bush, 1975; Futuyma & Mayer, 1980). However, marine environments where there is clear evidence of geographic structure among marine metazoans despite physical barriers to gene flow being seemingly absent have helped reshape our understanding of speciation and more specifically expanded our understanding of sympatric speciation (Mayr, 1954b).

Biogeography of marine metazoans across the Indo-Pacific

In terrestrial ecosystems geographic barriers that lead to speciation differ across groups of organisms depending on their life history and their dispersal ability.

Differences in effectiveness of geographic barriers are evident when looking at terrestrial faunae patterns across islands in the Indo-Pacific. While Wallace's line aimed to divide islands dominated by Australian fauna and Asian fauna, Pelseneer recognized in 1904 that the lines would not represent a hard boundary for some animals like birds which have the ability to disperse among islands on either side of the line despite being separated by a body of water (Mayr, 1944). In marine environments, many animals rely on a planktonic larval phase for dispersal away from their source population, which in turn plays an important role in population structure for those species (Bahonak, 1999; Cowen & Sponaugle, 2009; Gilg & Hilbish, 2003). Variation in reproductive output and larval duration leads to variation in effectiveness of different geographic barriers among species (Cowen & Sponaugle, 2009; Treml et al., 2012). Methods for evaluating dispersal potential and geographic structure of different organisms include both indirect and direct methods of tracking larvae as well as phylogeographic studies on adult populations of a species (Avise, 2009; Kool, Moilanen, & Treml, 2013; Treml, Roberts, Halpin, Possingham, & Riginos, 2015).

Phylogeographic studies have played a particularly important role in elucidating geographic structure in populations of marine organisms across the Indo-Pacific. Comparison of phylogeographic structure across the region has revealed patterns of concordance among certain species, while others lack phylogeographic structure altogether (Carpenter et al., 2011). While data from some fish species supported east-west divisions concordant with Wallace's line (Lourie & Vincent, 2004), data from some invertebrate species suggested the potential existence of a marine equivalent of Wallace's Line dividing populations into north and south through the Java Sea (Barber, Palumbi, Erdmann, & Moosa, 2000). The studies revealing concordant phylogeographic patterns across invertebrates specifically highlight the potential role of surface currents in shaping population structure across the region (Barber et al., 2011; Crandall et al., 2008; DeBoer et al., 2008). However, while surface currents appear to play an important role in shaping population structure on broad geographic scales, environmental selection and biotic interactions appear to play more important roles shaping populations locally (Crandall et al., 2008, 2019).

Larval dispersal has long been evaluated in terms of dispersal potential by currents, however potential dispersal is often not realized dispersal for many species. Despite having the ability to disperse over great distances (>100km), many species will choose to settle closer to the source population (<40km) (Cowen & Sponaugle, 2009; G P Jones, Milicich, Emslie, & Lunow, 1999; Geoffrey P. Jones, Planes, & Thorrold, 2005; Swearer et al., 2002; Thorrold et al., 2002; Williamson et al., 2016). These dispersal patterns can be explained by environmental conditions like temperature (Teske et al., 2008; Thompson et al., 2018; Woolsey, Byrne, & Baird, 2013), but they can also be explained by biotic interactions among species. One example of this is the population structure of certain sea stars matching the geographic distributions of gastropod species that engage in a commensal relationships with those sea stars (Crandall et al., 2008). In addition to symbiotic relationships, top-down pressures including fishing and predator-prey interactions also play important roles in shaping population structure for metazoans across the region.

Nearly 60% of Indonesia’s population, 1.7 million of which are coral reef fishers, live in coastal areas (Siry, 2007; Teh, Teh, & Sumaila, 2013). However, fishing is not evenly distributed across the region. Instead, fishing is highest in the west where human population is highest, and lowest on remote reefs in the east. This gradient in fishing pressure is inversely related with metazoan biodiversity and fish biomass across Indonesia (Campbell et al., 2020). The shifts in diversity and biomass on reefs has had clear impacts on the size and composition of fish populations across Indonesia on local scales (Carvalho et al., 2021). Fish biomass was significantly lower at intensively fished sites, and mean length was significantly smaller in catch at intensively fished sites (Pet-Soede, Van Densen, Pet, & Machiels, 2001). In attempt to mitigate the effects of fishing on these communities, management schemes and marine protected areas (MPAs) have been established to help conserve biodiversity and promote sustainable fishing practices. These protections have in turn impacted communities resulting in higher biomass and biodiversity of metazoans in protected areas (Campbell et al., 2020; Campbell, Edgar, Stuart-Smith, Soler, & Bates, 2018; Campbell, Mukminin, Kartawijaya, Huchery, & Cinner, 2014). Despite the wealth of knowledge we have on metazoan communities across the Indo-Pacific, we lack data on microbial communities across this region and how the same abiotic and biotic factors that shape metazoan communities shape microbial communities at the base of the food web.

Marine protist biodiversity and biogeography

Protists exhibit immense morphological and ecological diversity and play important roles in oceanic processes including in biogeochemical cycling (Caron, Countway, Jones, Kim, & Schnetzer, 2012). Autotrophic protist groups contribute significantly to net primary production (NPP) across the globe, with diatoms alone contributing to nearly 40% of marine NPP and 20% of global NPP (Brzezinski, Villareal, & Lipschultz, 1998; Field, Behrenfeld, Randerson, & Falkowski, 1998). Heterotrophic groups like ciliates, radiolarians, and foraminiferans play important roles in both pelagic and benthic food webs as consumers of bacteria and other protists aiding in carbon cycling in their ecosystems (Flues, Bass, & Bonkowski, 2017; Glücksman, Bell, Griffiths, & Bass, 2010; Gonzalez, Sherr, & Sherr, 1990; Hall, Barrett, & James, 1993; Lipps & Valentine, 1970; Suzuki & Not, 2015). These free-living protists exhibit a latitudinal diversity gradient across the globe, with highest diversity occurring in the mid to low latitudes where NPP is greatest (Chust, Irigoien, Chave, & Harris, 2013; Sunagawa et al., 2015). In addition to free-living protists, there are also protists that engage in a full spectrum of symbioses. One of the most notable examples of these symbioses that holds particular importance for coral reef ecosystems, is the obligate relationship between tropical corals and their dinoflagellate symbionts (Clerissi et al., 2018). These photosynthetic symbionts provide corals with energy required for survival and thus help create a foundation for these incredibly biodiverse ecosystems to thrive. And yet, what we currently know about protist biodiversity is only scratching the surface.

Development of molecular tools and high-throughput sequencing has enabled us to uncover vast diversity across protists that was previously overlooked (Mahé et al., 2017; Moreira & López-García, 2002; Savin, Martin, LeGresley, Giewat, & Rooney-Varga, 2004; Zimmermann, Glöckner, Jahn, Enke, & Gemeinholzer, 2015). The ability of sequencing to distinguish between cryptic species and capture rare species missed in visual surveys has also helped elucidate biogeographic structure in protist communities across the globe (De Luca, Piredda, Sarno, & Kooistra, 2021; De Vargas, Norris, Zaninetti, Gibb, & Pawlowski, 1999; Šlapeta, López-García, & Moreira, 2006). Prior to the use of sequencing, many species of marine protists were assumed to be cosmopolitan and the primary factor limiting their geographic distribution was dispersal limitation by ocean currents (Mann & Droop, 1996). The belief that dispersal limitation was the primary driver of geographic structure in protist communities was directly in line with Hubbell's neutral model of biodiversity (Rosindell, Hubbell, & Etienne, 2011). However, the use of sequencing data to evaluate these communities has revealed that many of these assumed cosmopolitan species are in fact multiple cryptic species with distinct geographic ranges (Casteleyn et al., 2010; Kooistra et al., 2008). Furthermore, the geographic structure observed in many of these species appeared to be linked to both dispersal limitation and environmental conditions like temperature. These findings support the Bass-Becking hypothesis, which suggests environmental selection is the primary driver of community structure by stating, "everything is everywhere, but the environment selects" (Cermeño, de Vargas, Abrantes, & Falkowski, 2010; Cermeño & Falkowski, 2009).

Our understanding of the tradeoff between dispersal limitation and environmental selection and their role in shaping geographic structure in protist communities is constantly evolving. Expeditions including Tara Oceans and Malaspina have greatly expanded our knowledge of these communities on a global scale using both high-throughput sequencing and visual methods (Duarte, 2015; Pesant et al., 2015). Studies emerging from these expeditions have provided support for both the role of dispersal limitation and environmental selection, and acknowledged that these communities are likely structured by a combination of the two (Sunagawa et al., 2015). While dispersal limitation has been supported by studies showing correlations between biogeographic structure of protist communities and ocean circulation patterns (Richter et al., 2020), environmental selection has continued to be supported by studies on the latitudinal diversity gradient and the inability of certain species to disperse through colder latitudes around continents despite connection via currents (Chust et al., 2013; Malviya et al., 2016). Studies from these expeditions also highlight the important role grazers play in shaping protist community structure from the top-down. In particular, grazers appear to play significant roles in shaping protist community size-structure and composition on a local scale (Moffett & Landry, 2019; Sommeria-Klein, Watteaux, Iudicone, Bowler, & Morlon, 2020). Despite these expeditions greatly expanding our understanding of the structure and function of protist communities on a global scale, we still lack data on these communities in the Indo-Pacific (Figure 4).

Marine protist biodiversity and biogeography in the Indo-Pacific

Research on marine protists across Indonesia has primarily centered around toxic and bloom-forming diatom and dinoflagellate species in coastal environments (Hasani, Adiwilaga, & Pratiwi, 2013; Likumahua et al., 2020; Nasution, Dian Takarina, & Thoha, 2021; Rahmadyani, Widiarti, & Hendrayanti, 2017; Sidabutar, Thoha, et al., 2016). These studies have provided insight on how environmental factors including salinity, temperature, water quality, and more shape protist communities. Water quality is particularly important in regards to harmful algal blooms (HABs) along Indonesia’s coastlines. Increased nutrient input, runoff, and pollution levels resulting from increased human activity along the coasts all corresponded to increased abundance of HAB-causing protist species (Hasani et al., 2013; Likumahua et al., 2020; Nasution et al., 2021; Rahmadyani et al., 2017; Y. Suteja & Purwiyanto, 2018; Syakti, Idris, Koenawan, Asyhar, & Apriadi, 2019). The weather patterns across this region also play an important role in driving nutrient levels and, as a result, protist community dynamics. The southwest monsoon brings heavy rains to Southeast Asia which results in increased runoff in coastal ecosystems and decreased velocity of the Indonesian Throughflow, the current carrying water from the Pacific Ocean through Indonesia to the Indian Ocean (Gordon & Fine, 1996; Gordon, Susanto, & Vranes, 2003; Lee, Fournier, Gordon, & Sprintall, 2019). The changes in salinity, nutrient levels, and flow through the region during the southwest monsoon provides ideal conditions for many HAB species to thrive (Mahmudi, Lusiana, Herawati, & Serihollo, 2020; Tang, Rachman, Fitria, Thoha, & Chen, 2018).

These studies, however, are limited in geographic scope and rely on visual methods for characterization of these communities. The majority of research on marine protists in Indonesia, especially in relation to HABs, takes place on islands in the western part of the country where human population and activity is highest. Within western Indonesia the majority of these studies have taken place around the island of Java further restricting their geographic range (Hasani et al., 2013; Nasution et al., 2021; Sidabutar, Bengen, Wouthuyzen, & Partono, 2016; Y. Suteja & Purwiyanto, 2018; Yulianto Suteja et al., 2021; Syakti et al., 2019; Tang et al., 2018; Thoha et al., 2015). Studying HABs in areas of high human population and activity is useful for understanding local drivers of protist community structure, but they don’t allow for investigation of what factors drive broad-scale shifts in community structure over the transition from the Pacific to the Indian Ocean. Furthermore, these studies fail to identify the vast majority of diversity in these communities. The visual based methods used in these studies identify anywhere from 20-40 species of protists species. All of the species identified belong to either diatoms or dinoflagellates, which excludes a wide array of other planktonic and benthic protists that also play important roles in the ecosystem. The pore size on the plankton nets used in these studies also often target the microplankton (20-200µm) biassing the data against smaller species. Meanwhile, metabarcoding data from other regions of the globe specifically highlight that the diversity in the nano and picoplankton have been vastly underestimated by these kinds of visual methods (Le Bescot et al., 2016; López-García, Rodriguez-Valera, Pedrós-Alló, & Moreira, 2001).

While sequencing has not been utilized to characterize free-living protist communities across Indonesia, it has been utilized to evaluate geographic structure in protist symbionts. Dinoflagellates belonging to the class Symbiodiniaceae are common symbionts in marine invertebrates, and most notably are the obligate photosynthetic symbiont in tropical corals (Baker, 2003). Sequencing efforts have shown that temperature appears to play a role in structuring symbioses between these dinoflagellates and their invertebrate hosts across Indonesia. Where temperature is warmer, the symbionts tend to belong to thermally tolerant clades as opposed to those found at other sites, potentially giving their host a competitive advantage (DeBoer et al., 2012; Roriris, Agung, Astuty, & Mulyani, 2017). While these studies demonstrate the usefulness of sequencing data in understanding geographic distribution and structure of protists, they are still limited in geographic scope just as the studies discussed above were.

Future directions for protist research in the Indo-Pacific

High-throughput sequencing and global sampling efforts have greatly advanced our understanding of protist community structure across the world's oceans in recent years. However, difficult permitting processes and limited resources for molecular lab work have restricted our ability to characterize these communities across the Indo-Pacific (Barber et al., 2014). The data that are available on protist communities in the Indo-Pacific are limited in geographic scope and are primarily from visual surveys which are known to vastly underestimate protist biodiversity. Despite shortfalls of prior studies on protist communities across this region, the immense biodiversity and unique oceanographic features make it an ideal setting for studying both abiotic and biotic drivers of protist community structure.

Oceanographically, the Indo-Pacific is unlike any other region in the world. As the only low-latitude choke-point in ocean circulation, this region would allow us to better understand the role of dispersal limitation in shaping protist community structure independent of broad-scale latitudinal shifts driven primarily by environmental changes. Seasonal changes in current velocity and water chemistry as a result of monsoons, further allow us to investigate the relative roles of environmental selection and dispersal limitation on shaping community structure. Just as the unique circulation and weather patterns in the Indo-Pacific allow us to investigate drivers of protist community structure from the bottom-up, the immense biodiversity in this region and variation in fishing pressure allow us to investigate drivers of protist community structure from the top-down. The gradient in biodiversity and fishing pressure enables us to investigate how shifts in biomass and diversity at different trophic levels affect protists at the base of the food web. Furthermore, the variation in fisheries management strategies will allow us to understand how certain protections that aim to protect metazoan species impact microbial communities.

Protists are an essential part of marine ecosystems, but have long been overlooked in the Indo-Pacific. Understanding both abiotic and biotic drivers of protist community structure across this region will greatly expand our understanding of protist communities globally. In addition to increasing knowledge of protist community dynamics on a global scale these data could also be informative for conservation purposes. Understanding the environmental factors that shape protist communities and overall connectivity among different levels of the food web can provide important insight on how fisheries management strategies and MPAs across the region affect microbial organisms at the base of the food web to help inform future policy decisions and management design.

REFERENCES

Allen, G. R. (2008). Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*, 541–556. https://doi.org/10.1002/aqc

Avise, J. C. (2009). Phylogeography: Retrospect and prospect. *Journal of Biogeography*, *36*(1), 3–15. https://doi.org/10.1111/j.1365-2699.2008.02032.x

Bahonak, A. J. (1999). Dispersal , Gene Flow , and Population Structure. *The Quarterly Review of Biology*, *74*(1), 21–45.

Baker, A. C. (2003). Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 661–689. https://doi.org/10.1146/annurev.ecolsys.34.011802.132417

Barber, P. H. (2009). The challenge of understanding the Coral Triangle biodiversity hotspot. *Journal of Biogeography*, *36*(10), 1845–1846. https://doi.org/10.1111/j.1365-2699.2009.02198.x

Barber, P. H., Ablan-Lagman, M. C. A., Berlinck, R. G., Cahyani, D., Crandall, E. D., Ravago-Gotanco, R., … Willette, D. A. (2014). Advancing biodiversity research in developing countries: the need for changing paradigms. *Bulletin of Marine Science*, *90*(1), 187–210. https://doi.org/10.5343/bms.2012.1108

Barber, P. H., Cheng, S. H., Erdmann, M. V, Tenggardjaja, K., & Ambariyanto. (2011). Evolution and conservation of marine biodiversity in the Coral Triangle:

insights from stomatopod Crustacea. *CrustIssues19*, *12*(17), 1–32.

Barber, P. H., Palumbi, S. R., Erdmann, M. V., & Moosa, M. K. (2000). A marine Wallace's line? *Nature Brief Communications*, *406*, 692–693.

Barraclough, T. G. (1998). Revealing the factors that promote speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *353*(1366), 241–249. https://doi.org/10.1098/rstb.1998.0206

Bowen, B. W., Rocha, L. A., Toonen, R. J., & Karl, S. A. (2013). The origins of tropical marine biodiversity. *Trends in Ecology and Evolution*, *28*(6), 359–366. https://doi.org/10.1016/j.tree.2013.01.018

Briggs, J. C. (1999a). Coincident Biogeographic Patterns: Indo-West Pacific Ocean. *Evolution*, *53*(2), 326. https://doi.org/10.2307/2640770

Briggs, J. C. (1999b). Extinction and replacement in the Indo-West Pacific Ocean. *Journal of Biogeography*, *26*(4), 777–783. https://doi.org/10.1046/j.1365-2699.1999.00322.x

Briggs, J. C. (1999c). Modes of speciation: Marine Indo-West Pacific. *Bulletin of Marine Science*, *65*(3), 645–656.

Briggs, J. C. (2003). Marine centres of origin as evolutionary engines. *Journal of Biogeography*, *30*, 1–18. Retrieved from http://dx.doi.org/10.1046/j.1365-2699.2003.00810.x

Brzezinski, M. A., Villareal, T. A., & Lipschultz, F. (1998). Silica production and the contribution of diatoms to new and primary production in the central North

Pacific. *Marine Ecology Progress Series*, *167*, 89–104. https://doi.org/10.3354/meps167089

Bush, G. L. (1975). Modes of Animal Speciation. *Annual Review of Ecology and Systematics*, *6*(1), 339–364. https://doi.org/10.1146/annurev.es.06.110175.002011

Campbell, S. J., Darling, E. S., Pardede, S., Ahmadia, G., Mangubhai, S., Amkieltiela, … Maire, E. (2020). Fishing restrictions and remoteness deliver conservation outcomes for Indonesia's coral reef fisheries. *Conservation Letters*, *13*(2), 1–9. https://doi.org/10.1111/conl.12698

Campbell, S. J., Edgar, G. J., Stuart-Smith, R. D., Soler, G., & Bates, A. E. (2018). Fishing-gear restrictions and biomass gains for coral reef fishes in marine protected areas. *Conservation Biology*, *32*(2), 401–410. https://doi.org/10.1111/cobi.12996

Campbell, S. J., Mukminin, A., Kartawijaya, T., Huchery, C., & Cinner, J. E. (2014). Changes in a coral reef fishery along a gradient of fishing pressure in an Indonesian marine protected area. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(1), 92–103. https://doi.org/10.1002/aqc.2359

Caron, D. A., Countway, P. D., Jones, A. C., Kim, D. Y., & Schnetzer, A. (2012). Marine protistan diversity. *Annual Review of Marine Science*, *4*, 467–493. https://doi.org/10.1146/annurev-marine-120709-142802

Carpenter, K. E., Barber, P. H., Crandall, E. D., Ablan-Lagman, M. C. A., Ambariyanto, Mahardika, G. N., … Toha, A. H. A. (2011). Comparative phylogeography of the coral triangle and implications for marine management. *Journal of Marine Biology*, *2011*, 1–14. https://doi.org/10.1155/2011/396982

Carvalho, P. G., Setiawan, F., Fahlevy, K., Subhan, B., Madduppa, H., Zhu, G., & Humphries, A. T. (2021). Fishing and habitat condition differentially affect size spectra slopes of coral reef fishes. *Ecological Applications*, *0*(0), 1–14. https://doi.org/10.1002/eap.2345

Casteleyn, G., Leliaert, F., Backeljau, T., Debeer, A. E., Kotaki, Y., Rhodes, L., … Vyverman, W. (2010). Limits to gene flow in a cosmopolitan marine planktonic diatom. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(29), 12952–12957. https://doi.org/10.1073/pnas.1001380107

Cermeño, P., de Vargas, C., Abrantes, F., & Falkowski, P. G. (2010). Phytoplankton biogeography and community stability in the ocean. *PLoS ONE*, *5*(4). https://doi.org/10.1371/journal.pone.0010037

Cermeño, P., & Falkowski, P. G. (2009). Controls on Diatom Biogeography in the Ocean. *Science*, *325*(September), 1539–1542.

Chust, G., Irigoien, X., Chave, J., & Harris, R. P. (2013). Latitudinal phytoplankton distribution and the neutral theory of biodiversity. *Global Ecology and Biogeography*, *22*(5), 531–543. https://doi.org/10.1111/geb.12016

Clerissi, C., Brunet, S., Vidal-Dupiol, J., Adjeroud, M., Lepage, P., Guillou, L., … Toulza, E. (2018). Protists within corals: The hidden diversity. *Frontiers in Microbiology*, *9*(AUG), 1–13. https://doi.org/10.3389/fmicb.2018.02043

Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, *1*, 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

Crandall, E. D., Jones, M. E., Muñoz, M. M., Akinronbi, B., Erdmann, M. V., & Barber, P. H. (2008). Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle. *Molecular Ecology*, *17*(24), 5276–5290. https://doi.org/10.1111/j.1365-294X.2008.03995.x

Crandall, E. D., Riginos, C., Bird, C. E., Liggins, L., Treml, E., Beger, M., … Gaither, M. R. (2019). The molecular biogeography of the Indo-Pacific: Testing hypotheses with multispecies genetic patterns. *Global Ecology and Biogeography*, *28*(7), 943–960. https://doi.org/10.1111/geb.12905

De Luca, D., Piredda, R., Sarno, D., & Kooistra, W. H. C. F. (2021). Resolving cryptic species complexes in marine protists: phylogenetic haplotype networks meet global DNA metabarcoding datasets. *ISME Journal*, 16–18. https://doi.org/10.1038/s41396-021-00895-0

De Vargas, C., Norris, R., Zaninetti, L., Gibb, S. W., & Pawlowski, J. (1999). Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(6), 2864–2868. https://doi.org/10.1073/pnas.96.6.2864

DeBoer, T. S., Baker, A. C., Erdmann, M. V., Ambariyanto, Jones, P. R., & Barber, P. H. (2012). Patterns of Symbiodinium distribution in three giant clam species across the biodiverse Bird's Head region of Indonesia. *Marine Ecology Progress Series*, *444*(Baker 2003), 117–132. https://doi.org/10.3354/meps09413

DeBoer, T. S., Subia, M. D., Ambariyanto, Erdmann, M. V., Kovitvongsa, K., & Barber, P. H. (2008). Phylogeography and limited genetic connectivity in the endangered boring giant clam across the coral triangle. *Conservation Biology*, *22*(5), 1255–1266. https://doi.org/10.1111/j.1523-1739.2008.00983.x

Duarte, C. M. (2015). Seafaring in the 21st century: The Malaspina 2010 circumnavigation expedition. *Limnology and Oceanography Bulletin*, *24*(1), 11–14. https://doi.org/10.1002/lob.10008

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, *281*(5374), 237–240. https://doi.org/10.1126/science.281.5374.237

Flues, S., Bass, D., & Bonkowski, M. (2017). Grazing of leaf-associated Cercomonads (Protists: Rhizaria: Cercozoa) structures bacterial community composition and function. *Environmental Microbiology*, *19*(8), 3297–3309. https://doi.org/10.1111/1462-2920.13824

Futuyma, D. J., & Mayer, G. C. (1980). Non-Allopatric Speciation in Animals. *Systematic Zoology*, *29*(3), 254. https://doi.org/10.2307/2412661

Gaither, M. R., Bowen, B. W., Bordenave, T. R., Rocha, L. A., Newman, S. J., Gomez, J. A., … Craig, M. T. (2011). Phylogeography of the reef fish Cephalopholis argus (Epinephelidae) indicates Pleistocene isolation across the indo-pacific barrier with contemporary overlap in the coral triangle. *BMC*

Evolutionary Biology, *11*(1). https://doi.org/10.1186/1471-2148-11-189

Gaither, M. R., & Rocha, L. A. (2013). Origins of species richness in the Indo-Malay-Philippine biodiversity hotspot: Evidence for the centre of overlap hypothesis. *Journal of Biogeography*, *40*, 1638–1648. https://doi.org/10.1111/jbi.12126

Gilg, M. R., & Hilbish, T. J. (2003). The geography of marine larval dispersal: Coupling genetics with fine-scale physical oceanography. *Ecology*, *84*(11), 2989–2998. https://doi.org/10.1890/02-0498

Glücksman, E., Bell, T., Griffiths, R. I., & Bass, D. (2010). Closely related protist strains have different grazing impacts on natural bacterial communities. *Environmental Microbiology*, *12*(12), 3105–3113. https://doi.org/10.1111/j.1462-2920.2010.02283.x

Gonzalez, J. M., Sherr, E. B., & Sherr, B. F. (1990). Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Applied and Environmental Microbiology*, *56*(3), 583–589. https://doi.org/10.1128/aem.56.3.583-589.1990

Gordon, A. L., & Fine, R. A. (1996). Pathways of water between the Pacific and Indian oceans in the Indonesian seas. *Letters to Nature*, *379*(6561), 146–149. https://doi.org/10.1038/379146a0

Gordon, A. L., Susanto, R. D., & Vranes, K. (2003). Cool Indonesian throughflow as a consequence of restricted surface layer flow. *Letters to Nature*, *425*(6960), 821–824. https://doi.org/10.1038/nature02013

Hall, J. A., Barrett, D. P., & James, M. R. (1993). The importance of phytoflagellate, heterotrophic flagellate and ciliate grazing on bacteria and picophytoplankton sized prey in a coastal marine environment. *Journal of Plankton Research*, *15*(9), 1075–1086. https://doi.org/10.1093/plankt/15.9.1075

Hasani, Q., Adiwilaga, E. M., & Pratiwi, N. T. M. (2013). The Relationship between the Harmful Algal Blooms (HABs) Phenomenon with Nutrients at Shrimp Farms and Fish Cage Culture Sites in Pesawaran District Lampung Bay. *MAKARA of Science Series*, *16*(3), 183–191. https://doi.org/10.7454/mss.v16i3.1480

Hoeksema, B. W. (2007). Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity : Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity : The Coral Triangle. In W. Renema (Ed.), *Biogeography, Time, and Place: Distributions, Barriers, and Islands* (pp. 117–178). https://doi.org/10.1007/978-1-4020-6374-9

Huxley, T. H. (1868). On the classification and distribution of Alectromorphae and Heteromorphae. *Proceedings of the Zoological Society of London*, *36*(1), 294–319.

Jokiel, P., & Martinelli, F. J. (1992). The Vortex Model of Coral Reef Biogeography. *Journal of Biogeography*, *19*(4), 449–458.

Jones, G P, Milicich, M. J., Emslie, M. J., & Lunow, C. (1999). Self-recruitment in a coral reef fish population. *Environmental Protection*, *402*(December), 802–804.

Jones, Geoffrey P., Planes, S., & Thorrold, S. R. (2005). Coral reef fish larvae settle close to home. *Current Biology*, *15*(14), 1314–1318.

https://doi.org/10.1016/j.cub.2005.06.061

Kooistra, W. H. C. F., Sarno, D., Balzano, S., Gu, H., Andersen, R. A., & Zingone, A. (2008). Global Diversity and Biogeography of Skeletonema Species (Bacillariophyta). *Protist*, *159*(2), 177–193. https://doi.org/10.1016/j.protis.2007.09.004

Kool, J. T., Moilanen, A., & Treml, E. A. (2013). Population connectivity: Recent advances and new perspectives. *Landscape Ecology*, *28*(2), 165–185. https://doi.org/10.1007/s10980-012-9819-z

Ladd, H. S. (1960). Origin of the Pacific island molluscan fauna. *American Journal of Science*, Vol. 258-A, pp. 137–150.

Le Bescot, N., Mahé, F., Audic, S., Dimier, C., Garet, M. J., Poulain, J., … Siano, R. (2016). Global patterns of pelagic dinoflagellate diversity across protist size classes unveiled by metabarcoding. *Environmental Microbiology*, *18*(2), 609–626. https://doi.org/10.1111/1462-2920.13039

Lee, T., Fournier, S., Gordon, A. L., & Sprintall, J. (2019). Maritime Continent water cycle regulates low-latitude chokepoint of global ocean circulation. *Nature Communications*, *10*(1), 1–13. https://doi.org/10.1038/s41467-019-10109-z

Likumahua, S., de Boer, M. K., Krock, B., Hehakaya, S., Imu, L., Müller, A., … Buma, A. G. J. (2020). Variability of dinoflagellates and their associated toxins in relation with environmental drivers in Ambon Bay, eastern Indonesia. *Marine Pollution Bulletin*, *150*(November 2019), 110778. https://doi.org/10.1016/j.marpolbul.2019.110778

Lipps, J. H., & Valentine, J. W. (1970). the Role of Foraminifera in the Trophic Structure of Marine Communities. *Lethaia*, *3*(3), 279–286. https://doi.org/10.1111/j.1502-3931.1970.tb01271.x

López-García, P., Rodriguez-Valera, F., Pedrós-Alló, C., & Moreira, D. (2001). Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Letters to Nature*, *409*(February), 603–607.

Lourie, S. A., & Vincent, A. C. J. (2004). A marine fish follows Wallace's Line: the phylogeography of the three-spot seahorse (Hippocampus trimaculatus, Syngnathidae, Teleostei) in Southeast Asia. *Journal of Biogeography*, *31*, 1975–1985.

MacArthur, R. H., & Wilson, E. O. (1963). An equilibrium theory of insular zoogeography. *International Journal of Organic Evolution*, *17*(4), 373–387.

Mahé, F., Vargas, C. De, Bass, D., Czech, L., Stamatakis, A., Lara, E., … Dunthorn, M. (2017). Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nature Publishing Group*, *1*(March), 1–8. https://doi.org/10.1038/s41559-017-0091

Mahmudi, M., Lusiana, E. D., Herawati, E. Y., & Serihollo, L. G. (2020). Environmental factors and seasonal effect on the potential harmful algae presence at Ambon Bay, Indonesia. *Biodiversitas*, *21*(7), 3101–3107. https://doi.org/10.13057/biodiv/d210730

Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., … Bowler, C. (2016). Insights into global diatom distribution and diversity in the world's ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(11), E1516–E1525. https://doi.org/10.1073/pnas.1509523113

Mann, D. G., & Droop, S. J. M. (1996). Biodiversity, biogeography and conservation of diatoms. *Hydrobiologia*, *336*(1–3), 19–32. https://doi.org/10.1007/BF00010816

Mayr, E. (1944). Wallace's Line in the Light of Recent Zoogeographic Studies. *The Quarterly Review of Biology*, *19*(1), 1–14.

Mayr, E. (1954a). Change of Genetic Environment and Evolution. In *Evolution as a Process* (pp. 157–180).

Mayr, E. (1954b). Geographic speciation of tropical echinoids. *Evolution*, *8*(1), 1–18.

Moffett, J. W., & Landry, M. R. (2019). Grazing control and iron limitation of primary production in the Arabian Sea: Implications for anticipated shifts in Southwest Monsoon intensity. *Deep-Sea Research Part II: Topical Studies in Oceanography*, (November), 104687. https://doi.org/10.1016/j.dsr2.2019.104687

Moreira, D., & López-García, P. (2002). The molecular ecology of microbial eukaryotes unveils a hidden world. *Trends in Microbiology*, *10*(1), 31–38. Retrieved from http://dx.doi.org/10.1186/s13059-016-0960-x%0Ahttp://dx.doi.org/10.1038/s41587-019-0191-2%0Ahttps://doi.org/10.1016/j.tree.2019.08.008%0Ahttp://dx.doi.org/10.1016/j.protis.2010.01.003%0Ahttp://dx.doi.org/10.1038/ismej.2012.36%0Ahttp://dx.doi.org/10.1038/s

Nasution, A. K., Dian Takarina, N., & Thoha, H. (2021). The presence and abundance of harmful dinoflagellate algae related to water quality in Jakarta Bay, Indonesia. *Biodiversitas Journal of Biological Diversity*, *22*(5), 2909–2917. https://doi.org/10.13057/biodiv/d220556

Pesant, S., Not, F., Picheral, M., Kandels-Lewis, S., Le Bescot, N., Gorsky, G., … Searson, S. (2015). Open science resources for the discovery and analysis of Tara Oceans data. *Scientific Data*, *2*(Lmd), 1–16. https://doi.org/10.1038/sdata.2015.23

Pet-Soede, C., Van Densen, W. L. T., Pet, J. S., & Machiels, M. A. M. (2001). Impact of Indonesian coral reef fisheries on fish community structure and the resultant catch composition. *Fisheries Research*, *51*(1), 35–51. https://doi.org/10.1016/S0165-7836(00)00236-8

Rahmadyani, R. F., Widiarti, R., & Hendrayanti, D. (2017). Distribution analysis on cyst abundance of HAB causing dinoflagellates at green mussel (Perna viridis) culture area in Kamal Muara , North Jakarta. *Proceeding – ICM-MBT*, (November), 014–021. Retrieved from http://pksplipb.or.id/; email: journal@pksplipb.or.id

Richter, D., Watteaux, R., Vannier, T., Leconte, J., Reygondeau, G., Maillet, N., … Frémont, P. (2020). Genomic evidence for global ocean plankton biogeography shaped by large-scale current systems. *HAL Archives-Ouvertes*.

Roriris, E. T., Agung, M. U. K., Astuty, S., & Mulyani, Y. (2017). Molecular Identification of Thermally-Tolerant Symbiotic Dinoflagellates from Hard Coral

(Scleractinia) in Biawak Island, Indonesia. *Microbiology Indonesia*, *11*(4), 123–128. https://doi.org/10.5454/mi.11.4.3

Rosindell, J., Hubbell, S. P., & Etienne, R. S. (2011). The Unified Neutral Theory of Biodiversity and Biogeography at Age Ten. *Trends in Ecology and Evolution*, *26*(7), 340–348. https://doi.org/10.1016/j.tree.2011.03.024

Savin, M. C., Martin, J. L., LeGresley, M., Giewat, M., & Rooney-Varga, J. (2004). Plankton diversity in the bay of fundy as measured by morphological and molecular methods. *Microbial Ecology*, *48*(1), 51–65. https://doi.org/10.1007/s00248-003-1033-8

Sidabutar, T., Bengen, D. G., Wouthuyzen, S., & Partono, T. (2016). The abundance of phytoplankton and its relationship to the N/P ratio in Jakarta Bay, Indonesia. *Biodiversitas*, *17*(2), 673–678. https://doi.org/10.13057/biodiv/d170241

Sidabutar, T., Thoha, H., Bayu, M. D., Rachman, A., Sianturi, O. R., Fitriya, N., … Masseret, E. (2016). Occurrence of Pyrodinium bahamense blooms related to cyst accumulation in the bottom sediments in the bays at Ambon, Lampung and Jakarta, Indonesia. *Harmful Algae News*, (52), 8–9. Retrieved from www.ioc-unesco.org/hab

Simpson, G. G. (1977). Too Many Lines ; The Limits of the Oriental and Australian Zoogeographic Regions. *Proceedings of the American Philisophical Society*, *121*(2), 107–120.

Siry, H. Y. (2007). Making Decentralized Coastal Zone Management Work for the Southeast Asian Region: Comparative Perspectives. In *Oceans and Law of Sea*.

Šlapeta, J., López-García, P., & Moreira, D. (2006). Global dispersal and ancient cryptic species in the smallest marine eukaryotes. *Molecular Biology and Evolution*, *23*(1), 23–29. https://doi.org/10.1093/molbev/msj001

Sommeria-Klein, G., Watteaux, R., Iudicone, D., Bowler, C., & Morlon, H. (2020). Global drivers of eukaryotic plankton biogeography in the sunlit ocean. *BioRxiv*. Retrieved from https://www.biorxiv.org/content/10.1101/2020.09.08.287524v1.abstract

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., … Lepoivre, C. (2015). *Structure and function of the global ocean microbiome*. *348*(6237), 1–10.

Suteja, Y., & Purwiyanto, A. I. S. (2018). Nitrate and phosphate from rivers as mitigation of eutrophication in Benoa bay, Bali-Indonesia. *IOP Conference Series: Earth and Environmental Science*, *162*(1). https://doi.org/10.1088/1755-1315/162/1/012021

Suteja, Yulianto, Dirgayusa, I. G. N. P., Afdal, Cordova, M. R., Rachman, A., Rintaka, W. E., … Purwiyanto, A. I. S. (2021). Identification of potentially harmful microalgal species and eutrophication status update in Benoa Bay, Bali, Indonesia. *Ocean & Coastal Management*, *210*(April), 105698. https://doi.org/10.1016/j.ocecoaman.2021.105698

Suzuki, N., & Not, F. (2015). Biology and Ecology of Radiolaria. In *Marine Protists: Diversity and Dynamics* (pp. 179–222). https://doi.org/10.1007/978-4-431-55130-0

Swearer, S. E., Shima, J. S., Hellberg, M. E., Thorrold, S. R., Jones, G. P., Robertson, D. R., … Warner, R. R. (2002). Evidence of self-recruitment in demersal marine populations. *Bulletin of Marine Science*, *70*(1 SUPPL.), 251–271.

Syakti, A. D., Idris, F., Koenawan, C. J., Asyhar, R., & Apriadi, T. (2019). Biological pollution potential in the water of Bintan-Riau Islands Province, Indonesia: First appearance of harmful algal bloom species. *Egyptian Journal of Aquatic Research*, *45*(2), 117–122. https://doi.org/10.1016/j.ejar.2019.04.002

Tang, S., Rachman, A., Fitria, N., Thoha, H., & Chen, B. (2018). Phytoplankton changes during SE monsoonal period in the Lembeh Strait of North Sulawesi, Indonesia, from 2012 to 2015. *Acta Oceanologica Sinica*, *37*(12), 9–17. https://doi.org/10.1007/s13131-018-1283-4

Teh, L. S. L., Teh, L. C. L., & Sumaila, U. R. (2013). A Global Estimate of the Number of Coral Reef Fishers. *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0065397

Teske, P. R., Papadopoulos, I., Newman, B. K., Dworschak, P. C., McQuaid, C. D., & Barker, N. P. (2008). Oceanic dispersal barriers, adaptation and larval retention: An interdisciplinary assessment of potential factors maintaining a phylogeographic break between sister lineages of an African prawn. *BMC Evolutionary Biology*, *8*(1), 1–14. https://doi.org/10.1186/1471-2148-8-341

Thoha, H., Rachman, A., Sidabutar, T., Fitriya, N., Bayu, M. D., Iwataki, M., … Masseret, E. (2015). First record of the harmful dinoflagellate Cochlodinium polykrikoides in Lampung Bay, Indonesia. *Harmful Algae News*, (50), 14–15.

Retrieved from www.ioc-unesco.org/hab

Thompson, D. M., Kleypas, J., Castruccio, F., Curchitser, E. N., Pinsky, M. L., Jönsson, B., & Watson, J. R. (2018). Variability in oceanographic barriers to coral larval dispersal: Do currents shape biodiversity? *Progress in Oceanography*, *165*(February), 110–122. https://doi.org/10.1016/j.pocean.2018.05.007

Thorrold, S. R., Jones, G. P., Hellberg, M. E., Burton, R. S., Swearer, S. E., Neigel, J. E., … Warner, R. R. (2002). Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bulletin of Marine Science*, *70*(1 SUPPL.), 291–308.

Tornabene, L., Valdez, S., Erdmann, M., & Pezold, F. (2015). Support for a "Center of Origin" in the Coral Triangle: Cryptic diversity, recent speciation, and local endemism in a diverse lineage of reef fishes (Gobiidae: Eviota). *Molecular Phylogenetics and Evolution*, *82*(PA), 200–210. https://doi.org/10.1016/j.ympev.2014.09.012

Treml, E. A., Roberts, J., Halpin, P. N., Possingham, H. P., & Riginos, C. (2015). The emergent geography of biophysical dispersal barriers across the Indo-West Pacific. *Diversity and Distributions*, *21*(4), 465–476. https://doi.org/10.1111/ddi.12307

Treml, E. A., Roberts, J. J., Chao, Y., Halpin, P. N., Possingham, H. P., & Riginos, C. (2012). Reproductive output and duration of the pelagic larval stage determine seascape-wide connectivity of marine populations. *Integrative and Comparative*

Biology, *52*(4), 525–537. https://doi.org/10.1093/icb/ics101

Veron, J. E. N., Turak, E., Stafford-Smith, M., Stuart, K., Devantier, L. M., Green, A. L., & Peterson, N. (2009). Delineating the Coral Triangle. *Galaxea, Journal of Coral Reef Studies*, *11*(2), 91–100. https://doi.org/10.3755/galaxea.11.91

Voris, H. K. (2000). Maps of Pleistocene sea levels in Southeast Asia : shorelines , river systems and time durations. *Journal of Biogeography*, *27*, 1153–1167.

Williamson, D. H., Harrison, H. B., Almany, G. R., Berumen, M. L., Bode, M., Bonin, M. C., … Jones, G. P. (2016). Large-scale, multidirectional larval connectivity among coral reef fish populations in the Great Barrier Reef Marine Park. *Molecular Ecology*, *25*(24), 6039–6054. https://doi.org/10.1111/mec.13908

Woodland, D. J. (1983). Zoogeography of the Siganidae (Pisces): An interpretation of distribution and richness patterns. *Bulletin of Marine Science*, *33*(3), 713–717.

Woolsey, E. S., Byrne, M., & Baird, A. H. (2013). The effects of temperature on embryonic development and larval survival in two scleractinian corals. *Marine Ecology Progress Series*, *493*, 179–184. https://doi.org/10.3354/meps10499

Zimmermann, J., Glöckner, G., Jahn, R., Enke, N., & Gemeinholzer, B. (2015). Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. *Molecular Ecology Resources*, *15*(3), 526–542. https://doi.org/10.1111/1755-0998.123

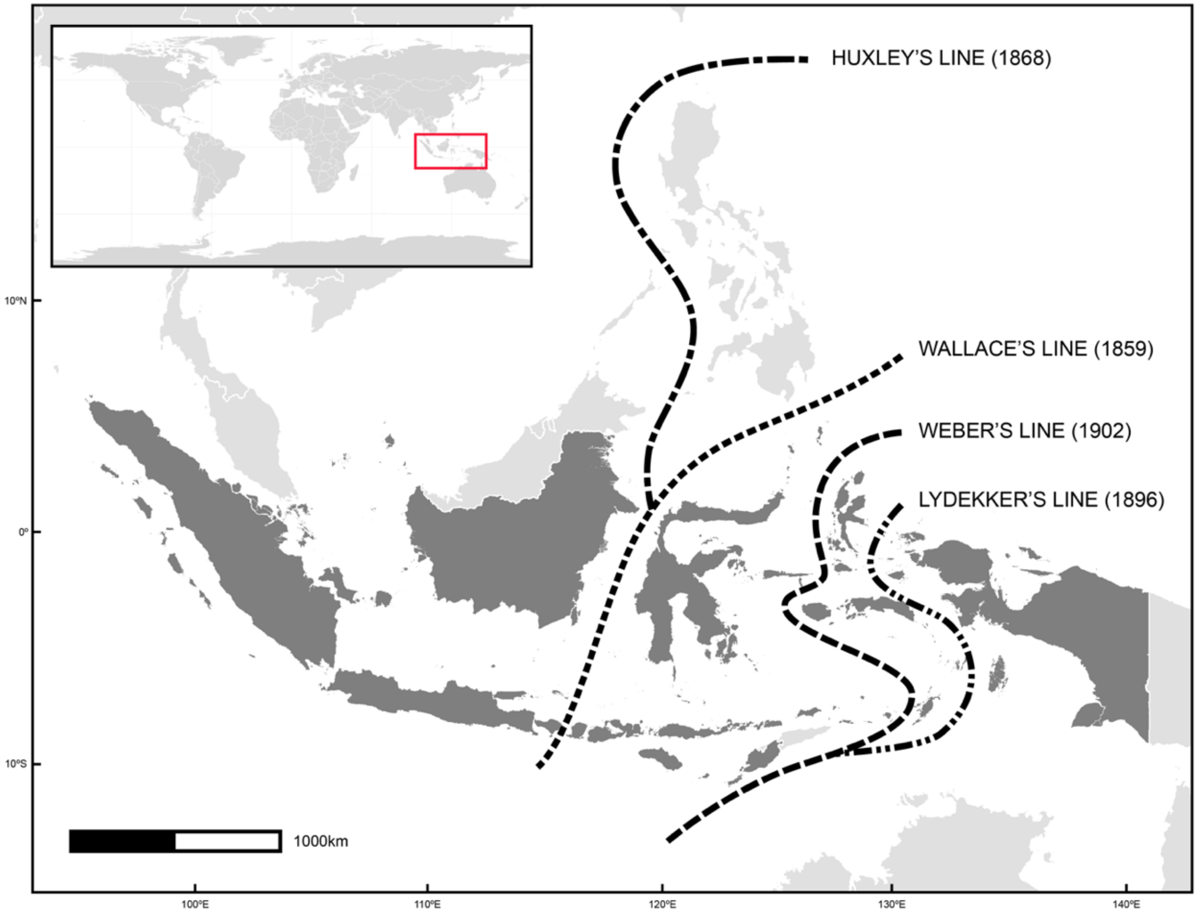


Figure 1. Map of the Indo-Pacific (Indonesia highlighted in dark grey) with major biogeographic lines used to denote the division between islands in dominated by Asian fauna in the west and Australian fauna in the east.

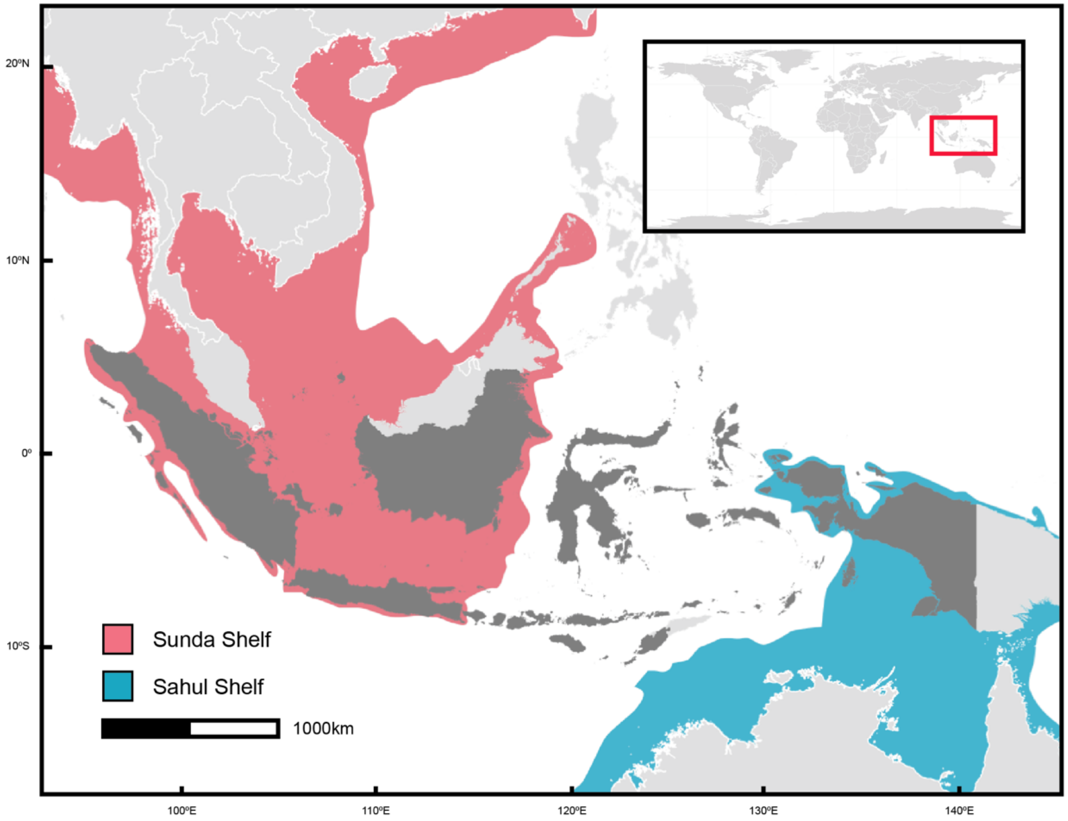


Figure 2. Map of the Indo-Pacific (Indonesia highlighted in dark grey) with contours outlining the Sunda (red) and Sahul (blue) shelves. The contours represent the portion of the shelves that were exposed during the Pleistocene when sea levels were as much as 120m below present-day levels.

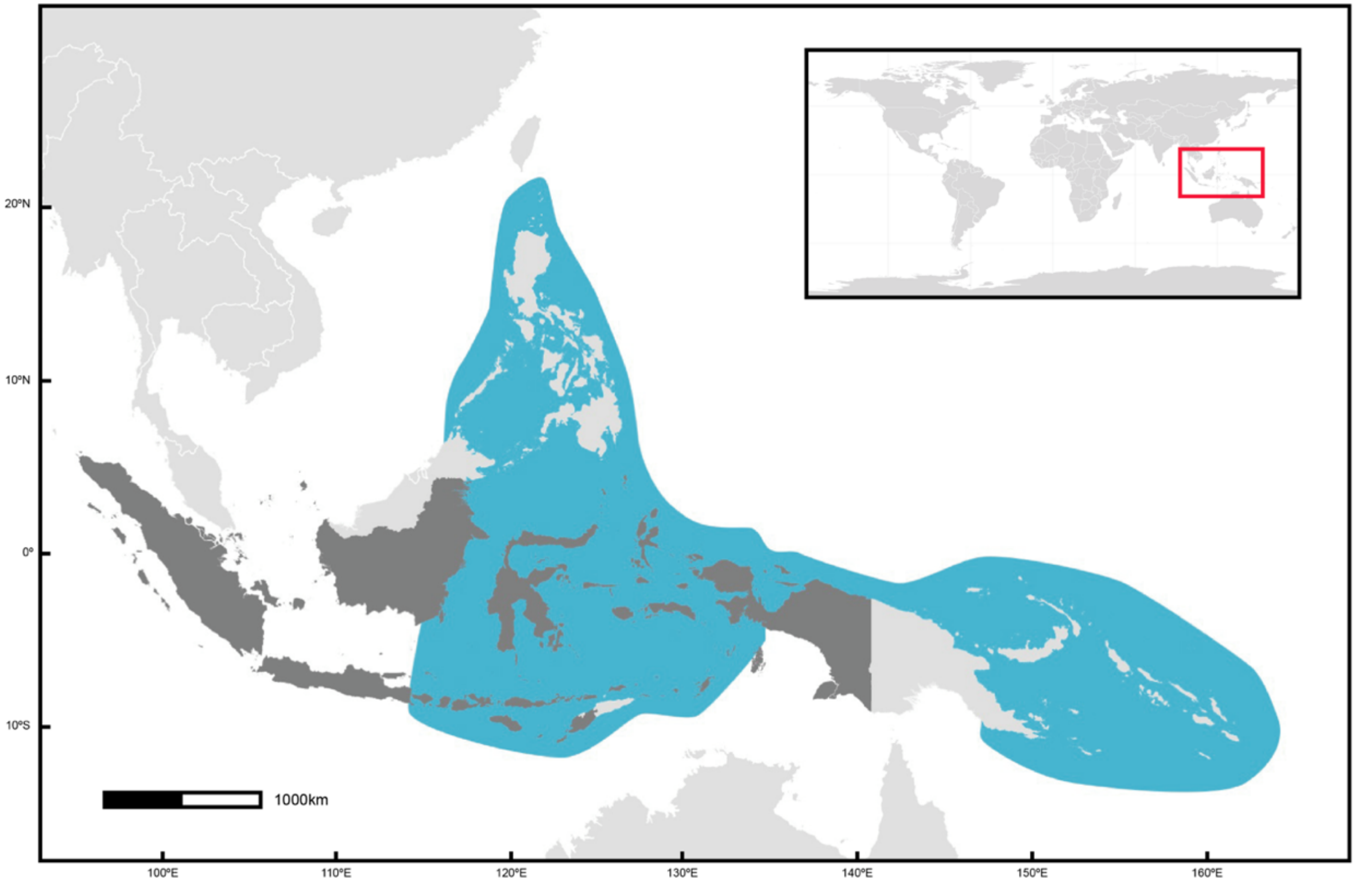


Figure 3. Map of the Indo-Pacific (Indonesia highlighted in dark grey) depicting the Coral Triangle biodiversity hotspot (blue).

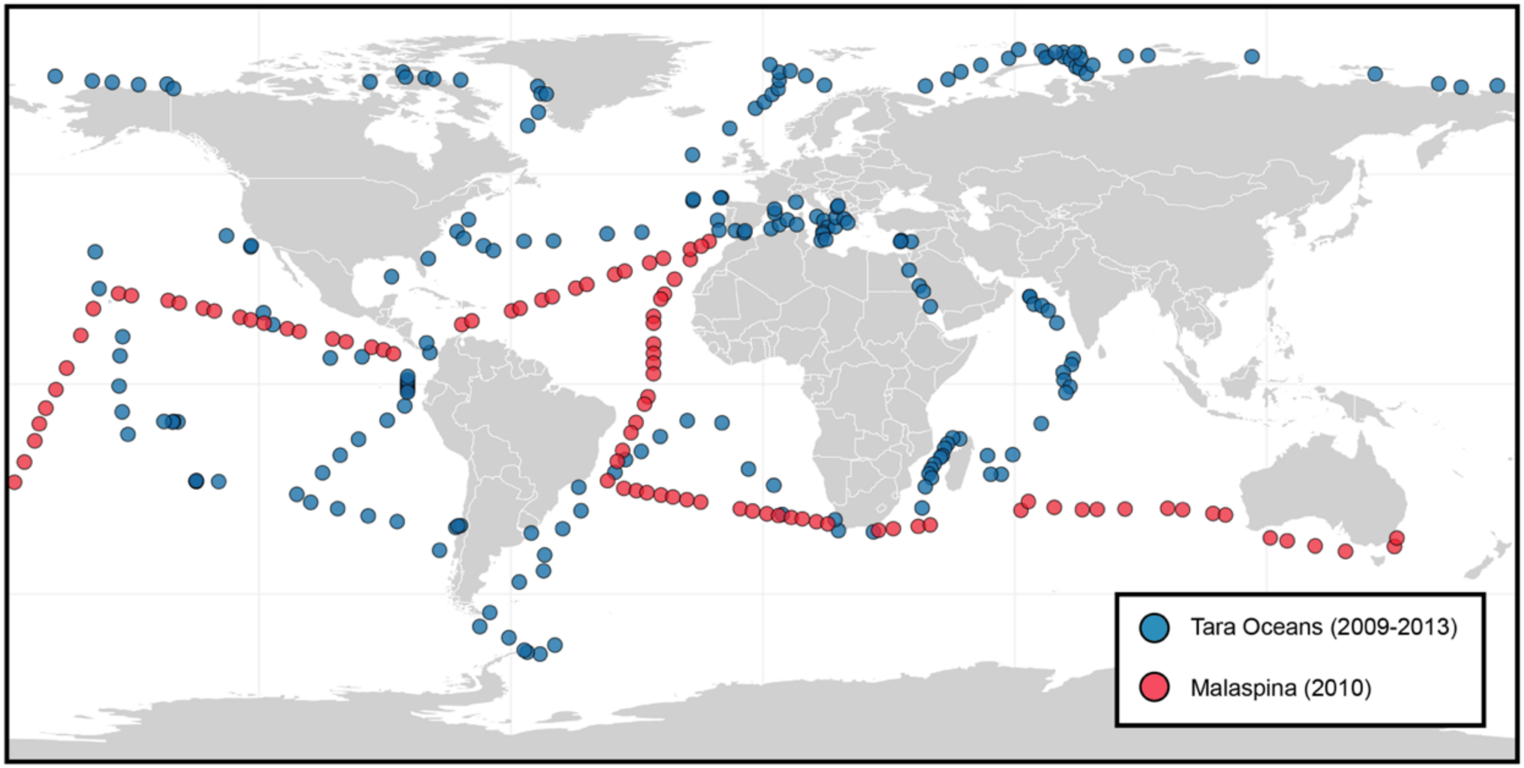


Figure 4. World map depicting Tara Oceans and Malaspina collection sites.

CHAPTER 2:

ABIOTIC DRIVERS OF PROTIST COMMUNITY STRUCTURE IN COASTAL MARINE ECOSYSTEMS ACROSS INDONESIA

ABSTRACT

Biogeographic structure in protist communities across the world's oceans is shaped by a combination of dispersal potential and environmental selection. High-throughput sequencing and global sampling efforts have helped better resolve composition and functions of these communities in the world's oceans using both molecular and visual methods. Despite such extensive sampling, data on marine protist communities across the Indo-Pacific is largely limited to visual surveys which vastly underestimate the diversity in protists. Our study uses metabarcoding to characterize protist communities in four sampling regions across Indonesia: Lombok, Wakatobi, Misool, and Waigeo. We show that each region has distinct community composition but that one region, Waigeo, has far less overlap with the other three. In Waigeo, diatom diversity increases, while dinoflagellate diversity decreases. The spatial structure and the shifts in diversity across taxa suggests that the broad-scale geographic structure observed in these communities is potentially driven by the surface current patterns across the Indo-Pacific as a result of: (1) a choke point in circulation at the Indonesian Throughflow leading to low diatom diversity in Lombok, Wakatobi, and Misool; (2) an increase in nutrient availability at the edge of the Halmahera Eddy in Waigeo leading to an increase in diatom diversity and relative abundance; or (3) seasonal variations in protist communities in line with shifts in velocity of the Indonesian Throughflow. Overall, our data highlight the importance of abiotic factors in shaping protist communities on a broad geographic scale over biotic top-down pressures such as grazing from higher trophic levels.

INTRODUCTION

Protists exhibit immense morphological, ecological, and taxonomic diversity (Caron, Countway, Jones, Kim, & Schnetzer, 2012). Ubiquitous in the world's oceans, protists make up an important fraction of the base of the food web playing essential roles in primary production, biogeochemical cycling, and as partners in a full spectrum of symbioses (Clerissi et al., 2018; Decelle, Colin, & Foster, 2015; Falkowski, Barber, & Smetacek, 1998; Field, Behrenfeld, Randerson, & Falkowski, 1998; He, Liu, Karuppiah, Ren, & Li, 2014). Our knowledge of the diversity and distribution of these groups in the world's oceans and the drivers behind their community structure has increased significantly over the years, but is still incomplete.

Much of the debate surrounding protist community structure in the world's oceans centers around the tradeoff between dispersal potential and environmental selection (Martiny et al., 2006). The connectivity of ocean basins by systems of currents combined with the small body and large population sizes of microbial species could potentially allow for these organisms to disperse globally (Cermeño & Falkowski, 2009; Lundholm & Moestrup, 2006). However, observations of protist diversity patterns across the globe have revealed biogeographic structure along a latitudinal gradient, with highest diversities in mid to low latitudes, driven by a number of abiotic factors including temperature, salinity, light availability, and more (Chust, Irigoien, Chave, & Harris, 2013; Ibarbalz et al., 2019).

The development of high throughput sequencing technologies has unveiled greater diversity in protist groups across various environments than previously thought and has shed light on the limitations of visual surveys (López-García, Rodriguez-

Valera, Pedrós-Alló, & Moreira, 2001; Mahé et al., 2017; Moreira & López-García, 2002; Savin, Martin, LeGresley, Giewat, & Rooney-Varga, 2004; Zimmermann, Glöckner, Jahn, Enke, & Gemeinholzer, 2015). Not only is it difficult to distinguish between cryptic species morphologically, but visual surveys also vastly underestimate the true diversity in many protist groups due to small and low abundant species that are often overlooked in visual methods (Colomban De Vargas, Norris, Zaninetti, Gibb, & Pawlowski, 1999; Le Bescot et al., 2016; Moon-Van Der Staay, De Wachter, & Vaulot, 2001; Šlapeta, López-García, & Moreira, 2006). In fact, molecular data has revealed that species once assumed to be cosmopolitan were actually cryptic species with differing geographies (Amato et al., 2007; Kooistra et al., 2008). As a result, molecular methods have allowed us to better resolve biogeographic structure in these communities across the globe.

Insights from data collected on the Tara Oceans expedition has revealed that biogeographic structure observed in these communities could likely be a result of a combination of dispersal limitation and environmental selection (Cermeño, de Vargas, Abrantes, & Falkowski, 2010; Sunagawa et al., 2015). However, despite various studies and expeditions like Tara Oceans and Malaspina who have sampled much of the globe, our knowledge on biodiversity and structure of plankton communities in the Indo-Pacific is largely limited to visual surveys in few parts of the region (Barber et al., 2014; Mahmudi, Lusiana, Herawati, & Serihollo, 2020; Sidabutar, Bengen, Wouthuyzen, & Partono, 2016; Tang, Rachman, Fitria, Thoha, & Chen, 2018; Thoha & Rachman, 2018; Ujianti, Anggoro, Bambang, Purwanti, & Androva, 2019).

The Indo-Pacific poses a particular interest not only because it is home to some of the most biodiverse ecosystems in the world, but it is also an oceanographically unique region. This region has incredibly biodiverse coral reefs that serve as an important food and economic resource for the countries in this region, and protists play an essential role at the base of the food web in the health of these ecosystems (G. R. Allen, 2008; Asaad, Lundquist, Erdmann, & Costello, 2018; Tittensor et al., 2010). From an oceanographic perspective, the Pacific and Indian Ocean basins meet in Indonesian waters and it is the only low latitude choke-point in ocean circulation (Lee, Fournier, Gordon, & Sprintall, 2019; Sprintall & Revelard, 2014).

Our study is the first to characterize protist communities extensively across Indonesia using metabarcoding methods. With sampling regions spanning from the Pacific to the Indian Ocean, we aim to begin to understand the diversity and distribution of different protist groups across this region and the potential drivers behind these patterns. Our data show that on a broad geographic scale, protist communities appear structured largely by abiotic factors such as surface currents across the region as opposed to top-down biotic pressures such as grazing from upper trophic levels.

METHODS

Sample collection and preservation

Sampling for this study took place from January 2018 through May 2019 and covered four sampling regions across Indonesia: Lombok (18 sites), Wakatobi (12 sites), Misool (21 sites), and Waigeo (19 sites) (Figure 1). At each site, a 4L water sample was collected at 5m depth and a surface sediment sample was collected at 10m depth by SCUBA.

Following collection, each sample was filtered over 12µm followed by 0.4µm polycarbonate filters (SterliTech) using a peristaltic pump. All 4L of water was filtered and sediment samples were filtered until the filters clogged (typically 1-2L). Filters were then cut in half and each half was place in a separate 2mL cryovial with 1mL of DNA RNA shield (Zymo Research). Samples were kept at room temperature, and when possible, at 4°C, until transported back to the lab where they were stored at 4°C.

DNA extraction, PCR, and sequencing

The Zymo Biomics DNA Miniprep Kit (Zymo Research) was used to extract DNA from the filters following manufacturer's protocol. The V9 hypervariable region of 18S rDNA was then amplified with polymerase chain reaction (PCR) using the 1398F (5' – TTGTACACACCGCCC – 3') and 1510R (5' – CCTTCYGCAGGTTCACCTAC – 3') primers (Amaral-Zettler, McCliment, Ducklow, & Huse, 2009). These primers were the same ones used by the Tara Oceans group and have been successful and relatively unbiased in PCR amplification across eukaryotic lineages (Columban de Vargas et al., 2015). PCR reactions were set up using Bioline MyTaq Red Mix (Meridian Bioscience) following the volume and concentration recommendations from the manufacturer for both primers and DNA template. The PCR began with a 3min denaturation step at 94°C, followed by 35 cycles of 94°C for 45sec, 48°C for 30sec, and 72°C for 30sec, and finishing with a final elongation step of 72°C for 5min. Successful amplification and amplicon size was confirmed using gel electrophoresis. Library prep was done by the University of Rhode Island Genomics and Sequencing Center and the amplicons were then sequenced on the Illumina MiSeq on a 2x150 run using the MiSeq Reagent V2 Kit.

In addition to our samples from the field, we also prepared a mock community using DNA samples from cultures we had available in the lab as a control on the sequencing run. The DNA used spanned different Stramenopile lineages including *Fragilariopsis cylindrus* (Bacillariophyta), *Apedinella radians* (Dictyochophyceae), *Phaeothamnion confervicola* (Phaeothamniophyceae), *Chrysosaccus* sp. (Chrysophyceae), and *Tribonema minus* (Xanthophyceae). DNA from each culture was added in equal concentrations to a single tube, and the resulting DNA sample was amplified using the same primers and PCR methods described above. The amplicons were then sequenced on the Illumina MiSeq on the same run as the environmental samples.

Bioinformatics

Forward and reverse reads were initially quality assessed in FastQC (Andrews, 2010). Primer sequences were trimmed from the paired reads using Cutadapt (v1.9.1) (Martin, 2011). After trimming, the reads were imported into QIIME2 (v2020.6) and were filtered, denoised, merged, and chimera checked using the DADA2 plugin in QIIME2 (Bolyen et al., 2019; Callahan et al., 2013). Truncation length for denoising was chosen to minimize the number of low-quality bases at the end of the reads while maximizing the amount of overlap between the forward and reverse reads to optimize merging.

The amplicon sequence variants (ASVs) resulting from denoising were then clustered at 97% similarity into operational taxonomic units (OTUs) using the VSEARCH plugin in QIIME2 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) and were then taxonomically assigned using a Naïve-Bayes classifier in QIIME2 with the Protist Ribosomal Reference Database (PR^2) (Guillou et al., 2013). Once classified, any ASV that had a classification of lower than 95% confidence at any taxonomic level was filtered out to remove low quality and low abundance ASVs from the dataset to help reduce noise in later analyses.

Spatial autocorrelation

Spatial autocorrelation was tested for between community dissimilarity (β-diversity, Bray-Curtis dissimilarity) and sampling site locations using a Mantel test with 9999 permutations. Autocorrelations were run on each subset of data separately (0.4μm water samples; 12μm water samples; 0.4μm sediment samples; and 12μm sediment samples) for samples from all regions and then for samples from each individual region. Running autocorrelations across all samples, followed by samples from each individual region allowed us to identify spatial structure within our data on both large and small spatial scales.

Alpha and Beta diversity

Alpha and Beta diversity metrics were calculated in R using the *vegan* and *phyloseq* packages (McMurdie & Holmes, 2013; Oksanen et al., 2020). For Alpha diversity, operational taxonomic unit (OTU) richness was calculated for each sample and compared across a number of metadata variables available (i.e. region, filter-size, fisheries management zone, etc.). Richness was used over other alpha diversity metrics such as Shannon diversity to avoid potential bias from 18S copy number variation across different taxonomic groups. Kruskal-Wallis and Wilcoxon tests were used to determine the significance of differences across different groups. For Beta diversity, a Bray-Curtis dissimilarity matrix was constructed to compare community composition differences across samples. The comparisons of community composition across different metadata groups (i.e. region, sample type, etc.) were done using analysis of similarity (ANOSIM) and permutational analysis of variance (PERMANOVA). Both ANOSIM and PERMANOVA were used in order to compare the outputs and select the most appropriate statistic given the sampling design and dispersion in our specific dataset. Beta diversity was then visualized using principal coordinates analysis (PCoA).

Co-occurrence networks

Co-occurrence networks were constructed using the WGCNA package in R for each individual sampling region (Langfelder & Horvath, 2008). The resulting networks organized groups of co-occurring ASVs into modules of closely co-occurring sequences, which then could be compared among sampling regions. Modules shared across regions were then detected among different pairings and groups of sampling regions to identify overlap in communities among the regions. Edge and node counts were used as a measure to show the amount of co-occurrence among regions.

RESULTS

Sequencing and filtering results

The V9 sequencing encompassed 293 samples and greater than 18 million total reads with an average of 63,668 reads per sample. After quality filtering, denoising, and merging we retained on average 84% of our reads. Our additional filtering following taxonomic assignment eliminated 16% of the remaining reads. That 16% was largely undefined, low abundance ASVs. The remaining sequences consisted of 48,656 ASVs which classified to 1,387 taxa. The final filtering step which isolated the sequences classifying to Stramenopiles, Alveolates, and Rhizaria, eliminated 65% of the remaining data, resulted in 12,085 ASVs that classified to 839 taxa. The Stramenopiles, Alveolates, and Rhizaria were chosen specifically because they encompass the most abundant and diverse lineages of microbial eukaryotes across the world's oceans and have been the dominant protist groups in metabarcoding surveys across the globe (Columban de Vargas et al., 2015). The 65% of reads that were eliminated in that final filtering step belonged primarily to undefined eukaryotes (28%) and metazoans (24%).

Mock community

After taxonomic assignment of the sequences in our mock community, 95% of reads were assigned to Stramenopiles. Most of the remaining 5% of reads were classified as undefined Eukaryotes (4.23%), while the remaining fraction of a percent came from various Opisthokonts (0.56%) and other protist groups (0.24%).

Within the Stramenopiles, we saw all of the groups that were added to the mock community in varying proportions. The majority of reads came back classified to Bacillariophyta (86.66%), followed distantly by Dictyochophyceae (3.62%), Chrysophyceae (2.38%), Phaeothamnion (2.05%), and Xanthophyceae (0.26%) (supplemental fig). Within each of these groups, species identification was as expected with exception of the Xanthophyte, which classified to the correct genus but a different species than what we expected (*Tribonema ulotrichoides*). The misidentification of the Xanthophyte is likely a result of either misidentification of the culture or poor representation of Xanthophyte sequences in the reference database. The difference in the proportions of reads across taxonomic groups, particularly with the Bacillariophyta, despite starting with approximately equal concentrations of DNA, is likely a result of variation in 18S copy number across different taxonomic groups or primer bias (Medinger et al., 2010; Zhu, Massana, Not, Marie, & Vaulot, 2005).

Spatial autocorrelation

The full dataset shows strong spatial autocorrelation across each filter size and sample type (water vs. sediment) (Mantel, $p < 0.05$), with the exception of the 12µm water samples (Mantel, $p = 0.377$) (Supplemental Table 1). This spatial autocorrelation indicates that community composition of samples was more similar to community composition of samples within the same region than samples from other regions.

However, on a smaller geographic scale, there is minimal spatial autocorrelation among sites within each sampling region. In Lombok, spatial autocorrelation is only apparent in water samples, and in Misool it is only apparent in

12μm water samples (Mantel, $p = 0.0001$) and 0.4μm sediment samples (Mantel, $p = 0.0004$). In Lombok, the spatial autocorrelation among water samples is likely a result of sampling design with three distinct groupings of six sites around the island. In Wakatobi and Waigeo, spatial autocorrelation is not apparent in any samples (Mantel, $p < 0.05$) (Supplemental Table 1). The lack of spatial autocorrelation within individual sampling regions, allowed us to proceed using regional groupings of sites as categorical designations for alpha and beta diversity statistics.

Community composition

Taxonomic assignment of ASVs revealed that samples across all regions were largely dominated by diatoms and dinoflagellates making up 22% and 42% of reads respectively across all samples, followed distantly by the Ciliates (7%), Radiolarians (7%), and Apicomplexans (4%). While diatoms consistently accounted for 22% of reads in both water and sediment samples, dinoflagellates made up 47% of reads in water samples in only 37% of reads in sediment samples. In addition to differences between water and sediment samples, there are also differences in percent composition of diatoms and dinoflagellates between the different filter size fractions. In the 12μm size fraction, diatoms made up 35% of reads in water samples and 31% of reads in sediment samples, while only making up 9% and 13% of reads in the 0.4μm size fraction. Dinoflagellates on the other hand made up higher proportions of the 0.4μm size fraction samples. In the 0.4μm water samples, dinoflagellates made up 52% of reads, and in sediment samples, they made up 41%, both of which are about 8% higher than their composition in 12μm size fraction samples.

The community composition in samples also shifts noticeably across the different sampling regions (Figure 2). Diatoms consistently showed the highest relative abundance of reads in Waigeo (37%) compared to their relative abundance in Lombok (23%), Wakatobi (18%), and Misool (15%). Dinoflagellate reads on the other hand were much more consistent in relative abundance across sampling regions (Lombok = 37%; Wakatobi = 40%; Misool = 52%; Waigeo = 40%).

Alpha diversity by region

Overall OTU richness of protist communities was significantly lower in Waigeo than in the other three sampling regions (Wilcoxon, $p < 0.05$) (Supplemental Table 2). This trend was consistent in both water and sediment samples of the 0.4µm and 12µm size fractions. Dinoflagellate OTU richness was significantly lower in Waigeo when compared to the other regions (Wilcoxon, $p < 0.05$), with the exception of Wakatobi in the 0.4µm size fraction water samples. However, diatom OTU richness showed a different trend entirely (Figure 3). In the 0.4µm samples for both sediment and water, no sampling region significantly differed from one another. In the 12µm size fraction samples, the diatom OTU richness was significantly higher in Waigeo when compared to the other three regions (Wilcoxon, $p < 0.05$). The one exception to the trend appeared in the 12µm size fraction water samples, where Waigeo samples had significantly higher diatom OTU richness than Lombok and Misool samples, but were not significantly different from Wakatobi samples (Wilcoxon, $p < 0.05$) (Supplemental Table 2).

Beta diversity by region

Principal Coordinate Analysis (PCoA) ordinations, constructed using Bray-Curtis distances, showed that samples clustered most closely with other samples from the same region (Figure 4). The ANOSIM and PERMANOVA results slightly differed from one another. PERMANOVA results showed that all sampling regions were significantly different from one another (PERMANOVA, $p < 0.05$), while ANOSIM showed that most regions were significantly different from one another with the exception of a few. The few exceptions where regions were not distinct from one another using ANOSIM, were Waigeo and Wakatobi in the 0.4µm water samples (ANOSIM, $p = 0.074$), Waigeo and Wakatobi in the 0.4µm sediment samples (ANOSIM, $p = 0.419$), and Waigeo and Lombok in the 12µm water samples (ANOSIM, $p = 0.131$) (Supplemental Table 3). The difference between the two statistics is likely a result of unbalanced sampling design (Wakatobi has about half as many sites as the other regions) (Anderson & Walsh, 2013). Because ANOSIM was more conservative in detecting differences in community structure across regions with varying dispersion and sample sizes, that statistic was used moving forward.

Co-occurrence networks

The co-occurrence networks revealed that of all the sampling regions, Wakatobi consistently had the highest number of edges, and therefore, the highest amount of co-occurrence across all of its sites (Figure 5). Lombok and Misool had the next highest number of edges followed by Waigeo, which had the least. This suggests that Waigeo had the most variation in community composition from site to site, while Wakatobi had the most uniformity from site to site.

Identification of consensus modules shared among sampling regions revealed that Waigeo stood apart from the others. Consensus modules of taxa were identified between Lombok and Wakatobi, Lombok and Misool, Wakatobi and Misool, and among those three regions together. However, not a single consensus module was detected between any region and Waigeo (Figure 5). This suggests that Waigeo has a more distinct community composition compared to the other sampling regions.

DISCUSSION

Spatial structure in protist communities and the importance of dispersal limitation vs. environmental selection

The primary drivers of protist community structure across the globe are dispersal limitation and environmental selection by abiotic factors like temperature and nutrient levels (Martiny et al., 2006; Sunagawa et al., 2015). These factors are especially important in shaping biogeographic structure in protist communities on large geographic scales along latitudinal gradients (Chust et al., 2013; Ibarbalz et al., 2019). While ocean currents theoretically allow microbial organisms to disperse globally (Cermeño & Falkowski, 2009; Lundholm & Moestrup, 2006), temperature and nutrient concentrations restrict the geographic ranges of protists creating the spatial structure we observe in these communities across the world's oceans (Chust et al., 2013; Ibarbalz et al., 2019; Kafouris et al., 2019). The selection by temperature along latitudinal gradients appears to be particularly important in transitions between ocean basins as noted from samples collected from the transition from the Indian Ocean to the Atlantic, and the Atlantic Ocean to the Pacific (Chust et al., 2013; Malviya et al., 2016). However, unlike the transitions between the Indian and Atlantic oceans and Atlantic and Pacific oceans, the transition from the Pacific to the Indian Ocean does not span a wide latitudinal range. Instead, water passes east to west, from the Pacific through the Indonesian archipelago to the Indian Ocean.

The spatial autocorrelation among the full dataset indicates that on a large geographic scale protist community structure could be linked to connectivity and transport time between regions. However, the lack of spatial autocorrelation among samples within individual sampling regions, suggests that the variables that govern local shifts in protist community structure differ from those that shape protist communities on a broad geographic scale. While shifts in protist communities across transitions between ocean basins in other parts of the world appear primarily driven by temperature changes along a latitudinal gradient (Chust et al., 2013; Muñiz et al., 2018; Schaum, Buckling, Smirnoff, Studholme, & Yvon-Durocher, 2018), those temperature shifts are likely not a factor in the Indo-Pacific given its comparatively narrow latitudinal range. However, the correlation between temperature and protist community shifts was not tested. Co-occurrence data on the other hand does reveal highly distinct communities in each of the four sampling regions, the most overlap between regions occurring between neighboring regions, which reinforces that dispersal limitation and transport time between regions plays a large role in structuring these communities on a broad scale. Therefore, unlike other transitions between ocean basins, the communities we sampled across the Indo-Pacific are likely shaped first and foremost by dispersal limitation as opposed to environmental selection. Shifts in alpha and beta diversity statistics by sampling region also support this and demonstrate how surface currents across the Indo-Pacific could play an important role in structuring these communities.

Community composition shifts and the role of surface currents

Community composition differed significantly among the sampling regions suggesting broad-scale biogeographic structure across the transition from the Pacific Ocean to the Indian ocean. However, the similarity in community composition and co-occurrence of taxa among different sampling regions suggests that surface currents may play a role in structuring these communities on a large geographic scale. Co-occurrence networks constructed for each region show strong connectivity of sites within regions and minimal overlap of co-occurring modules among regions. The only modules shared among or between regions involved Lombok, Wakatobi, and Misool. Waigeo, despite its geographic proximity to other sampling regions like Misool, had minimal overlap with any other sampling region. In addition to the geographic proximity between Waigeo and Misool, these two regions also have similar levels of human population and metazoan biodiversity. The similarity in human activity and metazoan diversity between these two regions, suggests that top-down pressures like grazing from organisms at upper trophic levels are likely not playing a strong role in shaping these communities, which further supports the potential role of dispersal limitation in explaining these shifts. The lack of overlap in community composition between Misool and Waigeo further suggests the presence of a barrier or division separating the communities in these two regions. The location of this barrier appears consistent with a dispersal barrier driving population breaks in a number of marine invertebrate populations across this region and has been hypothesized to be driven by ocean currents across the region (Carpenter, Barber, Crandall, Ablan-lagman, et al., 2011).

The geographic structure in protist communities across this region was most apparent in the highest diversity groups, the diatoms, dinoflagellates, ciliates, and radiolarians, likely a result of statistical power in those groups. The lower diversity groups remained consistent in diversity and relative abundance across all sampling regions. However, some of these groups showed distinct profiles from their diversity and abundance in other environments. Specifically, Apicomplexans, which account for high relative abundance of protist sequences in neotropical soils (Mahé et al., 2017) and are the dominant parasitic group in Antarctic marine sediments (Cleary & Durbin, 2016), only accounted for on average 5% of reads per sample in our sediment data. Instead, the dominant parasitic groups in our data belonged to the dinoflagellate group Syndiniales which accounted for on average 13% of reads per sample in our sediment data.

The two most abundant groups in our samples were the diatoms and dinoflagellates. Previous metabarcoding surveys across the globe show that these two groups are some of the most diverse and abundant protist groups in the world’s oceans (Armbrust, 2009; Columban de Vargas et al., 2015; Le Bescot et al., 2016). Shifts in abundances of these groups across sampling regions show that while dinoflagellate relative abundance in the community remains relatively steady, diatom relative abundance is higher in Waigeo compared to the other three regions. Likewise, diatoms appear to be more diverse in Waigeo compared to the other three regions. This increase in diversity of diatoms in Waigeo is the opposite of what is observed in the overall protist diversity and dinoflagellate diversity, which decreases significantly in this region.

On a global scale, diatom and dinoflagellate communities appear to be driven by a number of abiotic factors. Diatoms are typically fast growers and most successful in high nutrient environments, whereas dinoflagellates have slower growth rates and are successful in low-nutrient conditions (Edwards, Thomas, Klausmeier, & Litchman, 2015; Litchman, Klausmeier, Schofield, & Falkowski, 2007). Therefore, in tropical oligotrophic environments like those across the Indo-Pacific, we would expect dinoflagellates to dominate the communities, which is the case for three of the four sampling regions. However, data on diatom abundance and diversity from other oligotrophic regions across the globe show that diversity of diatoms can still be high at sites where relative abundance is low (Malviya et al., 2016). In these regions, diatoms are able to survive through various ecological mechanisms like dormancy or symbioses with N-fixers until more favorable conditions arise. In the presence of higher nutrient levels, diatoms have a clear competitive advantage over other phytoplankton groups due to their fast growth rates and ability to take up and store nitrates (A. E. Allen, Vardi, & Bowler, 2006; Smith et al., 2019).

The differences in ecology between diatoms and dinoflagellates and their ability to respond and compete in varying environmental conditions are likely responsible for the dramatic shift in diversity we see in Waigeo. In addition to the shift between diatom and dinoflagellate diversity between Misool and Waigeo, the spatial autocorrelation among sites in the full dataset emphasize the role that connectivity and transport time between regions may play in explaining these patterns. There are a few potential explanations for how this may be happening, all of which appear to be linked to the surface currents throughout this region (Table 1):

Choke points in circulation lead to lower diatom diversity

Choke points in ocean circulation, like those at Drake Passage and Agulhas Retroflection, have been shown to correspond with choke points for diatom diversity (Malviya et al., 2016) (Figure 6). The Indonesian Throughflow (ITF), which carries water from the Pacific through the Indonesian archipelago to the Indian Ocean, is the only low-latitude chokepoint in ocean circulation. The ITF flows through the archipelago by three of our four sampling regions (Figure 6). The sampling region that sits just beyond the ITF is Waigeo. The three sampling regions it does flow through are the three regions with significantly lower diatom diversity (Figure 6).

Fronts of eddies as diatom diversity hotspots

In biogeographic studies of invertebrate populations across Indonesia, the ITF has been hypothesized to aid in dispersal of planktonic larvae, and thus connecting populations over long distances (Carpenter, Barber, Crandall, Ablan-Lagman, et al., 2011; Nuryanto & Kochzius, 2009). But the ITF is not the only current at play in this region. The Halmahera Eddy (HE) sits just north of the Bird's Head Peninsula of Papua and east of Halmahera, which places it just northeast of Waigeo (Figure 6). The HE circulates water from the New Guinea Coastal Current (NGCC) and sends it out to the Pacific via the Equatorial Countercurrent (ECC). The HE has been hypothesized to act as a potential barrier to dispersal for planktonic larvae preventing transport across the Molucca Sea (Barber, Erdmann, & Palumbi, 2006; Carpenter, Barber, Crandall, Ablan-Lagman, et al., 2011; DeBoer et al., 2008). From an oceanographic perspective though there could be other factors at play driving these observed shifts in community structure.

Dinoflagellates and diatoms compete with one another for resources in their environment and are best equipped for competing in different conditions. As mentioned above, dinoflagellates are more successful in low nutrient conditions, whereas diatoms are more successful competitors in higher nutrient conditions. Eddies have been shown to create environmental gradients on a small geographic scale that can influence protist community structure (Tréguer et al., 2018). The centers of eddies are known to be nutrient poor and therefore a dead-zone for diatom diversity. The fronts of eddies however are nutrient-rich and therefore hotspots for diatom diversity (Clayton, Nagai, & Follows, 2014; Peterson, Crawford, & Harrison, 2011; Romero, Fischer, Karstensen, & Cermeño, 2016). Waigeo sits just beyond the edge of the HE which as a result, may be supplying nutrients to the waters around Waigeo allowing diatoms to be more successful in this region. However, more sampling and nutrient data would be required to investigate this further.

Seasonality in currents across Indonesia

One other factor that may be driving these differences is seasonal shifts in community structure. Protists communities in many other places around the globe have predictable seasonal cycles, often driven by environmental changes throughout the year like temperature, light availability, and precipitation (Benedetti et al., 2019; Draredja et al., 2019; Gasinaite et al., 2005; Muñiz et al., 2018). In the Indo-Pacific, temperature remains fairly consistent in our sampling regions over the course of the year, but monsoons may play a role in shaping community structure. The northwest monsoon results in increased waterfall and run off into the ITF (Lee et al., 2019). During this period (Jan-Feb) the ITF velocity decreases, reducing transport through the Makassar Strait and at some points, along with other currents in the regions can shift in direction (Gordon, Susanto, & Vranes, 2003; Shinoda, Han, Metzger, & Hurlburt, 2012). Lombok, Wakatobi, and Misool were all sampled in the summer months during the dry season, but Waigeo was sampled in January. During this period, the ITF would have been slower, potentially making small changes in community composition more pronounced during this time.

CONCLUSION

Overall, our data show that protist communities across Indonesia exhibit high spatial structure. The geographic structure in protist communities across the transition from the Pacific to the Indian Ocean appears to be linked to surface current patterns and connectivity among regions. Unlike transitions between ocean basins in other parts of the world where environmental selection by variables like temperature and nutrient concentrations are the primary drivers of biogeographic structure in protist communities, the transition in protist community structure between the Pacific and Indian oceans appears largely driven by dispersal limitation. In addition to the broad geographic structure observed in our data across Indonesia, we also document a lack of geographic structure in protist communities within sampling regions, emphasizing how dispersal limitation plays an important role on a large geographic scale, while environmental selection and biotic interactions are likely play a more important role in shaping protist communities on a local geographic scale.

REFERENCES

Allen, A. E., Vardi, A., & Bowler, C. (2006). An ecological and evolutionary context for integrated nitrogen metabolism and related signaling pathways in marine diatoms. *Current Opinion in Plant Biology*, *9*(3), 264–273. https://doi.org/10.1016/j.pbi.2006.03.013

Allen, G. R. (2008). Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*, 541–556. https://doi.org/10.1002/aqc

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). *A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes*. *4*(7), 1–9. https://doi.org/10.1371/journal.pone.0006372

Amato, A., Kooistra, W. H. C. F., Levialdi Ghiron, J. H., Mann, D. G., Pröschold, T., & Montresor, M. (2007). Reproductive Isolation among Sympatric Cryptic Species in Marine Diatoms. *Protist*, *158*(2), 193–207. https://doi.org/10.1016/j.protis.2006.10.001

Anderson, M. J., & Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, *83*(4), 557–574. https://doi.org/10.1890/12-2010.1

Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data*.

Armbrust, E. V. (2009). The life of diatoms in the world's oceans. *Nature*, *459*(7244), 185–192. https://doi.org/10.1038/nature08057

Asaad, I., Lundquist, C. J., Erdmann, M. V., & Costello, M. J. (2018). Delineating priority areas for marine biodiversity conservation in the Coral Triangle. *Biological Conservation*, *222*(March), 198–211. https://doi.org/10.1016/j.biocon.2018.03.037

Barber, P. H., Ablan-Lagman, M. C. A., Berlinck, R. G., Cahyani, D., Crandall, E. D., Ravago-Gotanco, R., … Willette, D. A. (2014). Advancing biodiversity research in developing countries: the need for changing paradigms. *Bulletin of Marine Science*, *90*(1), 187–210. https://doi.org/10.5343/bms.2012.1108

Barber, P. H., Erdmann, M. V., & Palumbi, S. R. (2006). Comparative phylogeography of three codistributed stomatopods: Origins and timing of regional lineage diversification in the Coral Triangle. *Evolution*, *60*(9), 1825–1839.

Benedetti, F., Jalabert, L., Sourisseau, M., Beker, B., Cailliau, C., Desnos, C., … Pouline, P. (2019). The seasonal and inter-annual fluctuations of plankton abundance and community structure in a North Atlantic Marine Protected Area. *Frontiers in Marine Science*, *6*(APR), 1–16. https://doi.org/10.3389/fmars.2019.00214

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … 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

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2013). DADA2: High resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869.DADA2

Caron, D. A., Countway, P. D., Jones, A. C., Kim, D. Y., & Schnetzer, A. (2012). Marine protistan diversity. *Annual Review of Marine Science*, *4*, 467–493. https://doi.org/10.1146/annurev-marine-120709-142802

Carpenter, K. E., Barber, P. H., Crandall, E. D., Ablan-Lagman, M. C. A., Ambariyanto, Mahardika, G. N., … Toha, A. H. A. (2011). Comparative phylogeography of the coral triangle and implications for marine management. *Journal of Marine Biology*, *2011*, 1–14. https://doi.org/10.1155/2011/396982

Carpenter, K. E., Barber, P. H., Crandall, E. D., Ablan-lagman, M. C. A., Mahardika, G. N., Manjaji-matsumoto, B. M., … Toha, A. H. A. (2011). Comparative Phylogeography of the Coral Triangle and Implications for Marine Management. *Journal of Marine Biology*, *2011*. https://doi.org/10.1155/2011/396982

Cermeño, P., de Vargas, C., Abrantes, F., & Falkowski, P. G. (2010). Phytoplankton biogeography and community stability in the ocean. *PLoS ONE*, *5*(4). https://doi.org/10.1371/journal.pone.0010037

Cermeño, P., & Falkowski, P. G. (2009). Controls on Diatom Biogeography in the Ocean. *Science*, *325*(September), 1539–1542.

Chust, G., Irigoien, X., Chave, J., & Harris, R. P. (2013). Latitudinal phytoplankton distribution and the neutral theory of biodiversity. *Global Ecology and Biogeography*, *22*(5), 531–543. https://doi.org/10.1111/geb.12016

Clayton, S., Nagai, T., & Follows, M. J. (2014). Fine scale phytoplankton community structure across the Kuroshio Front. *Journal of Plankton Research*, *36*(4), 1017–1030. https://doi.org/10.1093/plankt/fbu020

Cleary, A. C., & Durbin, E. G. (2016). Unexpected prevalence of parasite 18S rDNA sequences in winter among Antarctic marine protists. *Journal of Plankton Research*, *38*(3), 401–417. https://doi.org/10.1093/plankt/fbw005

Clerissi, C., Brunet, S., Vidal-Dupiol, J., Adjeroud, M., Lepage, P., Guillou, L., … Toulza, E. (2018). Protists within corals: The hidden diversity. *Frontiers in Microbiology*, *9*(AUG), 1–13. https://doi.org/10.3389/fmicb.2018.02043

De Vargas, Colomban, Norris, R., Zaninetti, L., Gibb, S. W., & Pawlowski, J. (1999). Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(6), 2864–2868. https://doi.org/10.1073/pnas.96.6.2864

de Vargas, Columban, Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., … Karsenti, E. (2015). Eukaryotic plankton diversity in the sunlit ocean. *Science*, *348*(6237), 1261605-1/11. https://doi.org/10.1007/s13398-014-0173-7.2

DeBoer, T. S., Subia, M. D., Ambariyanto, Erdmann, M. V., Kovitvongsa, K., & Barber, P. H. (2008). Phylogeography and limited genetic connectivity in the endangered boring giant clam across the coral triangle. *Conservation Biology*, *22*(5), 1255–1266. https://doi.org/10.1111/j.1523-1739.2008.00983.x

Decelle, J., Colin, S., & Foster, R. A. (2015). Photosymbiosis in Marine Planktonic Protists. In *Marine Protists: Diversity and Dynamics* (pp. 465–500). https://doi.org/10.1007/978-4-431-55130-0

Draredja, M. A., Frihi, H., Boualleg, C., Gofart, A., Abadie, E., & Laabir, M. (2019). Seasonal variations of phytoplankton community in relation to environmental factors in a protected meso-oligotrophic southern Mediterranean marine ecosystem (Mellah lagoon, Algeria) with an emphasis of HAB species. *Environmental Monitoring and Assessment*, *191*(10). https://doi.org/10.1007/s10661-019-7708-5

Edwards, K. F., Thomas, M. K., Klausmeier, C. A., & Litchman, E. (2015). Light and growth in marine phytoplankton: Allometric, taxonomic, and environmental variation. *Limnology and Oceanography*, *60*(2), 540–552. https://doi.org/10.1002/lno.10033

Falkowski, P. G., Barber, R. T., & Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science*, *281*(5374), 200–206. https://doi.org/10.1126/science.281.5374.200

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, *281*(5374), 237–240. https://doi.org/10.1126/science.281.5374.237

Gasinaite, Z. R., Cardoso, A. C., Heiskanen, A. S., Henriksen, P., Kauppila, P., Olenina, I., … Wasmund, N. (2005). Seasonality of coastal phytoplankton in the Baltic Sea: Influence of salinity and eutrophication. *Estuarine, Coastal and Shelf Science*, *65*(1–2), 239–252. https://doi.org/10.1016/j.ecss.2005.05.018

Gordon, A. L., & Fine, R. A. (1996). Pathways of water between the Pacific and Indian oceans in the Indonesian seas. *Letters to Nature*, *379*(6561), 146–149. https://doi.org/10.1038/379146a0

Gordon, A. L., Susanto, R. D., & Vranes, K. (2003). Cool Indonesian throughflow as a consequence of restricted surface layer flow. *Letters to Nature*, *425*(6960), 821–824. https://doi.org/10.1038/nature02013

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., … Christen, R. (2013). The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, *41*(D1), 597–604. https://doi.org/10.1093/nar/gks1160

He, L., Liu, F., Karuppiah, V., Ren, Y., & Li, Z. (2014). Comparisons of the Fungal and Protistan Communities among Different Marine Sponge Holobionts by Pyrosequencing. *Microbial Ecology*, *67*(4), 951–961. https://doi.org/10.1007/s00248-014-0393-6

Ibarbalz, F. M., Henry, N., Brandão, M. C., Martini, S., Busseni, G., Byrne, H., … Zinger, L. (2019). Global Trends in Marine Plankton Diversity across Kingdoms of Life. *Cell*, *179*(5), 1084–1097. https://doi.org/10.1016/j.cell.2019.10.008

Kafouris, S., Smeti, E., Spatharis, S., Tsirtsis, G., Economou-Amilli, A., & Danielidis, D. B. (2019). Nitrogen as the main driver of benthic diatom composition and diversity in oligotrophic coastal systems. *Science of the Total Environment*, *694*, 1–9. https://doi.org/10.1016/j.scitotenv.2019.133773

Kooistra, W. H. C. F., Sarno, D., Balzano, S., Gu, H., Andersen, R. A., & Zingone, A. (2008). Global Diversity and Biogeography of Skeletonema Species (Bacillariophyta). *Protist*, *159*(2), 177–193. https://doi.org/10.1016/j.protis.2007.09.004

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*. https://doi.org/10.1186/1471-2105-9-559

Le Bescot, N., Mahé, F., Audic, S., Dimier, C., Garet, M. J., Poulain, J., … Siano, R. (2016). Global patterns of pelagic dinoflagellate diversity across protist size classes unveiled by metabarcoding. *Environmental Microbiology*, *18*(2), 609–626. https://doi.org/10.1111/1462-2920.13039

Lee, T., Fournier, S., Gordon, A. L., & Sprintall, J. (2019). Maritime Continent water cycle regulates low-latitude chokepoint of global ocean circulation. *Nature Communications*, *10*(1), 1–13. https://doi.org/10.1038/s41467-019-10109-z

Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. *Ecology Letters*, *10*(12), 1170–1181. https://doi.org/10.1111/j.1461-0248.2007.01117.x

López-García, P., Rodriguez-Valera, F., Pedrós-Alló, C., & Moreira, D. (2001). Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Letters* *to Nature*, *409*(February), 603–607.

Lundholm, N., & Moestrup, Ø. (2006). The Biogeography of Harmful Algae. In *Ecology of Harmful Algae* (Vol. 189, pp. 23–35). https://doi.org/10.1007/978-3-540-32210-8_3

Mahé, F., Vargas, C. De, Bass, D., Czech, L., Stamatakis, A., Lara, E., … Dunthorn, M. (2017). Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nature Publishing Group*, *1*(March), 1–8. https://doi.org/10.1038/s41559-017-0091

Mahmudi, M., Lusiana, E. D., Herawati, E. Y., & Serihollo, L. G. (2020). Environmental factors and seasonal effect on the potential harmful algae presence at Ambon Bay, Indonesia. *Biodiversitas*, *21*(7), 3101–3107. https://doi.org/10.13057/biodiv/d210730

Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., … Bowler, C. (2016). Insights into global diatom distribution and diversity in the world's ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(11), E1516–E1525. https://doi.org/10.1073/pnas.1509523113

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 5–7.

Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., … Staley, J. T. (2006). Microbial biogeography: Putting microorganisms on the map. *Nature Reviews Microbiology*, *4*(2), 102–112. https://doi.org/10.1038/nrmicro1341

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwälder, B., Schlötterer, C., & Boenigk, J. (2010). Diversity in a hidden world: Potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Molecular Ecology*, *19*(SUPPL. 1), 32–40. https://doi.org/10.1111/j.1365-294X.2009.04478.x

Moon-Van Der Staay, S. Y., De Wachter, R., & Vaulot, D. (2001). Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. *Nature*, *409*(6820), 607–610. https://doi.org/10.1038/35054541

Moreira, D., & López-García, P. (2002). The molecular ecology of microbial eukaryotes unveils a hidden world. *Trends in Microbiology*, *10*(1), 31–38. Retrieved from http://dx.doi.org/10.1186/s13059-016-0960-x%0Ahttp://dx.doi.org/10.1038/s41587-019-0191-2%0Ahttps://doi.org/10.1016/j.tree.2019.08.008%0Ahttp://dx.doi.org/10.1016/j.protis.2010.01.003%0Ahttp://dx.doi.org/10.1038/ismej.2012.36%0Ahttp://dx.doi.org/10.1038/s

Muñiz, O., Rodríguez, J. G., Revilla, M., Laza-Martínez, A., Seoane, S., & Franco, J. (2018). Seasonal variations of phytoplankton community in relation to environmental factors in an oligotrophic area of the European Atlantic coast (southeastern Bay of Biscay). *Regional Studies in Marine Science*, *17*, 59–72.

https://doi.org/10.1016/j.rsma.2017.11.011

Nuryanto, A., & Kochzius, M. (2009). Highly restricted gene flow and deep evolutionary lineages in the giant clam Tridacna maxima. *Coral Reefs*, *28*(3), 607–619. https://doi.org/10.1007/s00338-009-0483-y

Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., … Szoecs, E. (2020). *Package ' vegan ': Community ecology package*.

Peterson, T. D., Crawford, D. W., & Harrison, P. J. (2011). Evolution of the phytoplankton assemblage in a long-lived mesoscale eddy in the eastern Gulf of Alaska. *Marine Ecology Progress Series*, *424*, 53–73. https://doi.org/10.3354/meps08943

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *2016*(10), 1–22. https://doi.org/10.7717/peerj.2584

Romero, O. E., Fischer, G., Karstensen, J., & Cermeño, P. (2016). Eddies as trigger for diatom productivity in the open-ocean Northeast Atlantic. *Progress in Oceanography*, *147*, 38–48. https://doi.org/10.1016/j.pocean.2016.07.011

Savin, M. C., Martin, J. L., LeGresley, M., Giewat, M., & Rooney-Varga, J. (2004). Plankton diversity in the bay of fundy as measured by morphological and molecular methods. *Microbial Ecology*, *48*(1), 51–65. https://doi.org/10.1007/s00248-003-1033-8

Schaum, C. E., Buckling, A., Smirnoff, N., Studholme, D. J., & Yvon-Durocher, G.

(2018). Environmental fluctuations accelerate molecular evolution of thermal tolerance in a marine diatom. *Nature Communications*, *9*(1), 1–14. https://doi.org/10.1038/s41467-018-03906-5

Shinoda, T., Han, W., Metzger, E. J., & Hurlburt, H. E. (2012). Seasonal Variation of the Indonesian Throughflow in Makassar Strait. *Journal of Physical Oceanography*, *42*(7), 1099–1123. https://doi.org/10.1175/jpo-d-11-0120.1

Sidabutar, T., Bengen, D. G., Wouthuyzen, S., & Partono, T. (2016). The abundance of phytoplankton and its relationship to the N/P ratio in Jakarta Bay, Indonesia. *Biodiversitas*, *17*(2), 673–678. https://doi.org/10.13057/biodiv/d170241

Šlapeta, J., López-García, P., & Moreira, D. (2006). Global dispersal and ancient cryptic species in the smallest marine eukaryotes. *Molecular Biology and Evolution*, *23*(1), 23–29. https://doi.org/10.1093/molbev/msj001

Smith, S. R., Dupont, C. L., McCarthy, J. K., Broddrick, J. T., Oborník, M., Horák, A., … Allen, A. E. (2019). Evolution and regulation of nitrogen flux through compartmentalized metabolic networks in a marine diatom. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-12407-y

Sprintall, J., & Revelard, A. (2014). The Indonesian Throughflow response to Indo-Pacific climate variability. *Journal of Geophysical Research: Oceans*, *119*, 8410–8421. https://doi.org/10.1002/2013JC009533.Received

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., … Lepoivre, C. (2015). *Structure and function of the global ocean microbiome*. *348*(6237), 1–10.

Tang, S., Rachman, A., Fitria, N., Thoha, H., & Chen, B. (2018). Phytoplankton changes during SE monsoonal period in the Lembeh Strait of North Sulawesi, Indonesia, from 2012 to 2015. *Acta Oceanologica Sinica*, *37*(12), 9–17. https://doi.org/10.1007/s13131-018-1283-4

Thoha, H., & Rachman, A. (2018). Marine Phytoplankton in Port and Ship's Ballast Water at Tanjung Priok Harbour, North Jakarta, Indonesia. *ASEAN Journal on Science & Technology for Development*, *35*(1–2), 71–77.

Tittensor, D. P., Mora, C., Jetz, W., Lotze, H. K., Ricard, D., Berghe, E. Vanden, & Worm, B. (2010). Global patterns and predictors of marine biodiversity across taxa. *Nature Letters*, *466*(7310), 1098–1101. https://doi.org/10.1038/nature09329

Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., … Pondaven, P. (2018). Influence of diatom diversity on the ocean biological carbon pump. *Nature Geoscience*, *11*(1), 27–37. https://doi.org/10.1038/s41561-017-0028-x

Ujianti, R. M. D., Anggoro, S., Bambang, A. N., Purwanti, F., & Androva, A. (2019). Environmental Study on Phytoplankton in Garang Watershed, Central Java, Indonesia and Its Water Quality. *IOP Conference Series: Earth and Environmental Science*, *246*(1). https://doi.org/10.1088/1755-1315/246/1/012070

Zhu, F., Massana, R., Not, F., Marie, D., & Vaulot, D. (2005). Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiology Ecology*, *52*(1), 79–92. https://doi.org/10.1016/j.femsec.2004.10.006

Zimmermann, J., Glöckner, G., Jahn, R., Enke, N., & Gemeinholzer, B. (2015). Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. *Molecular Ecology Resources*, *15*(3), 526–542. https://doi.org/10.1111/1755-0998.12336

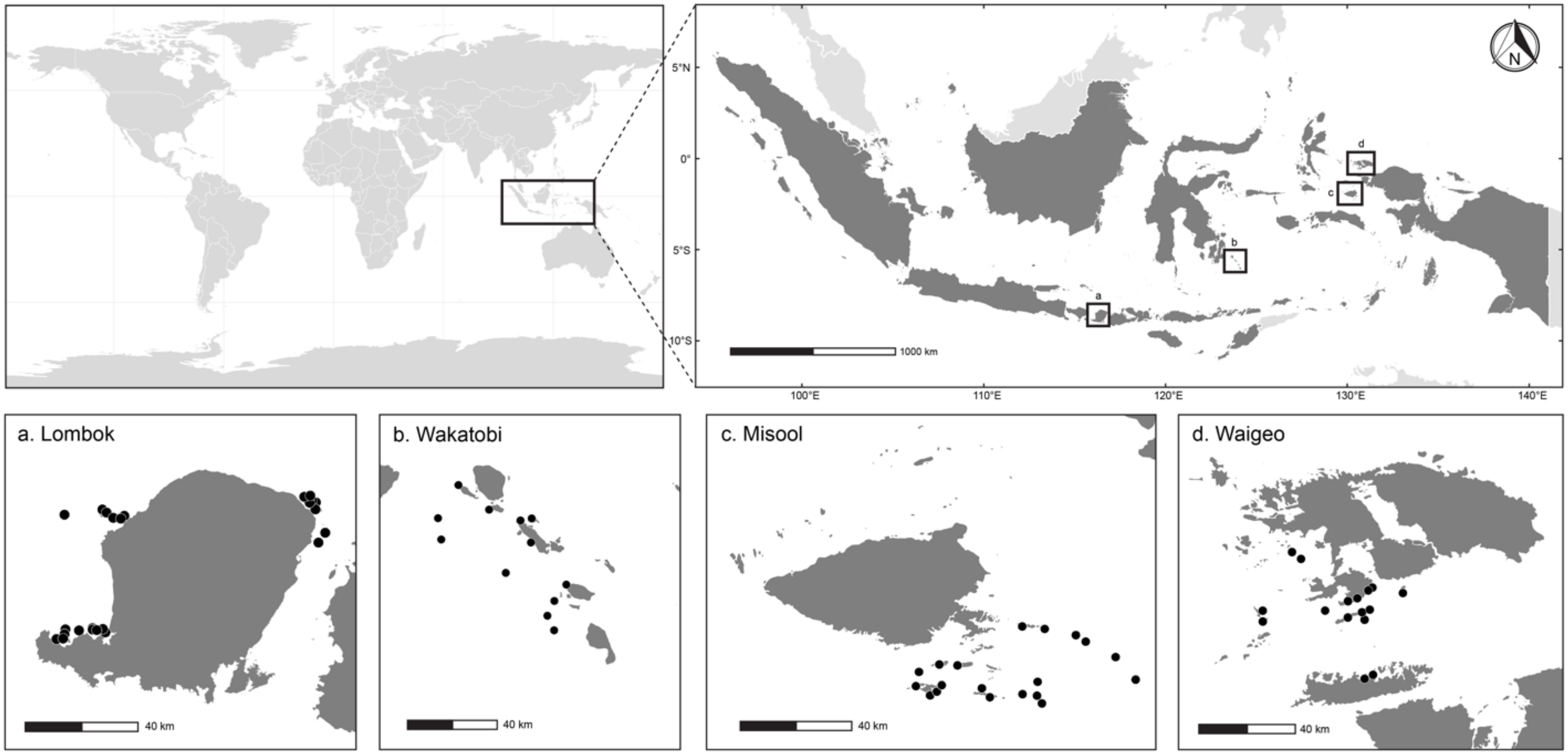


Figure 1. Maps of Indonesia and individual collection regions Lombok (a), Wakatobi (b), Misool (c), and Waigeo (d) with points indicating the collection sites within each sampling region.

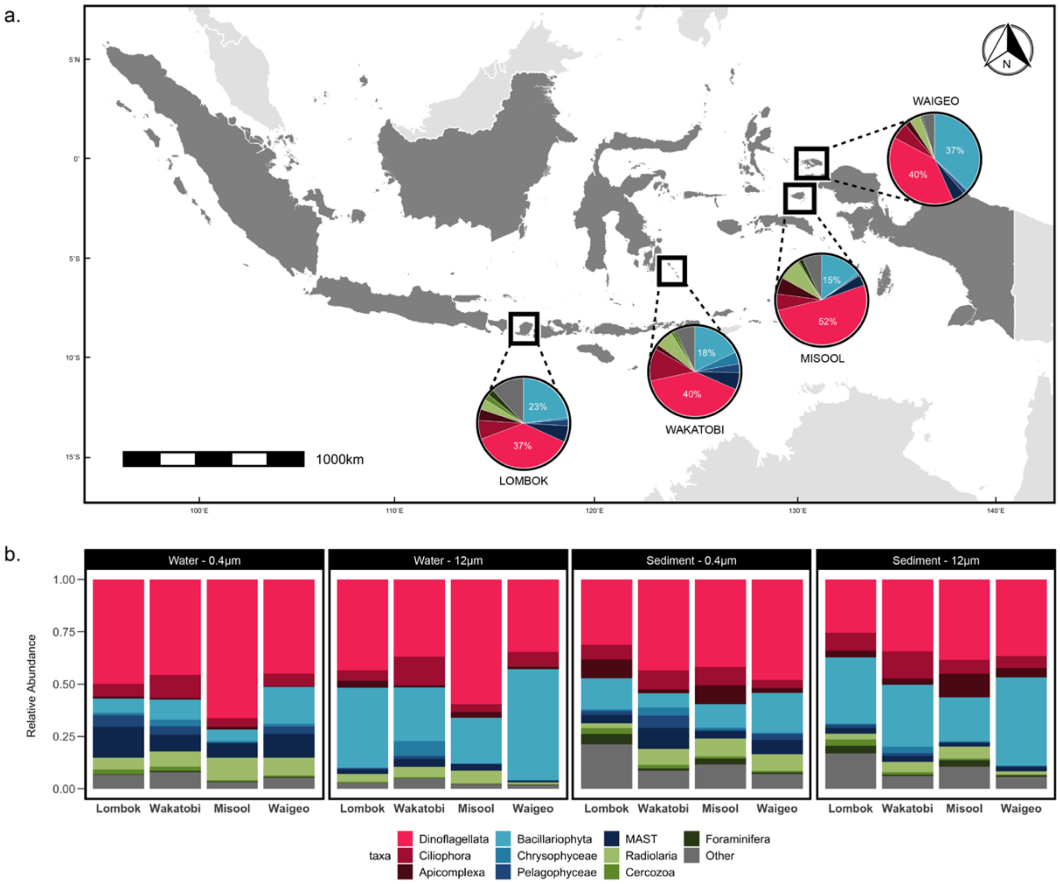


Figure 2. (a) Relative abundance of reads of major eukaryotic groups by sampling region and (b) further sorted by size fraction and sample type (water vs. sediment). Taxa contributing to less than 1% of relative abundance of the total dataset were collapsed into "Other".

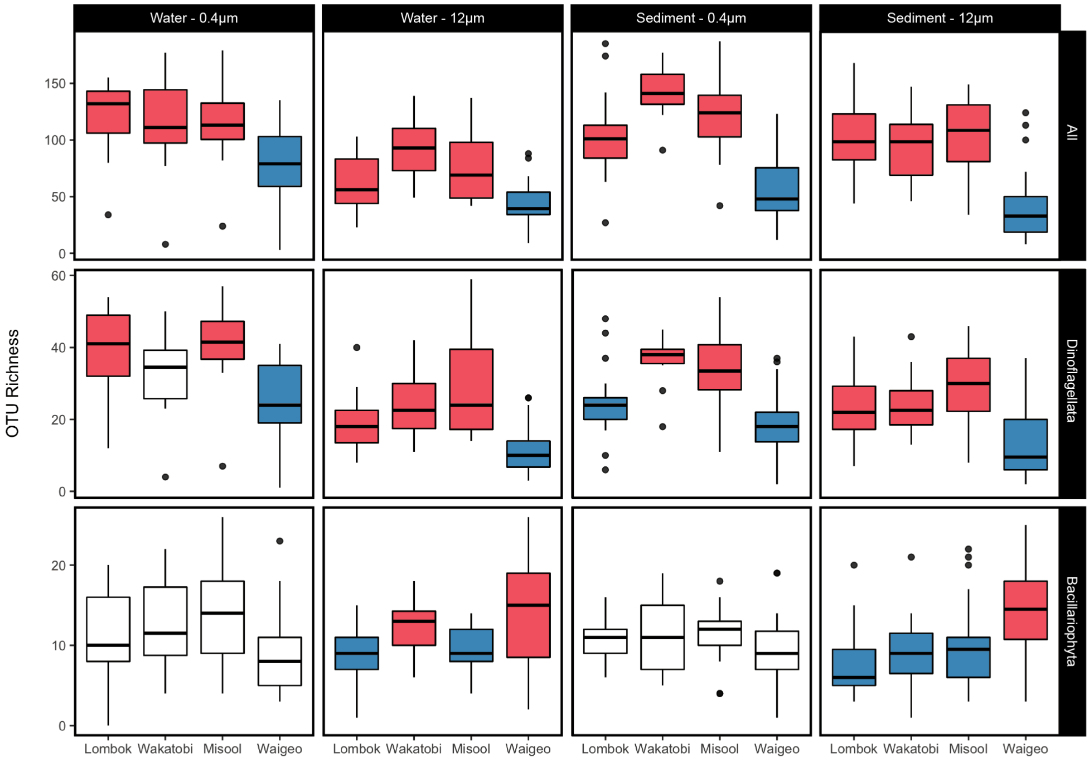


Figure 3. Protist OTU richness by region of different sample subsets and different taxonomic groups. Color indicates significant differences among regions. Red indicates significantly higher richness than other regions (Wilcoxon, $p \leq 0.05$), blue indicates significantly lower richness than other regions (Wilcoxon, $p \leq 0.05$), white indicates no significant differences among regions (Wilcoxon, $p > 0.05$).

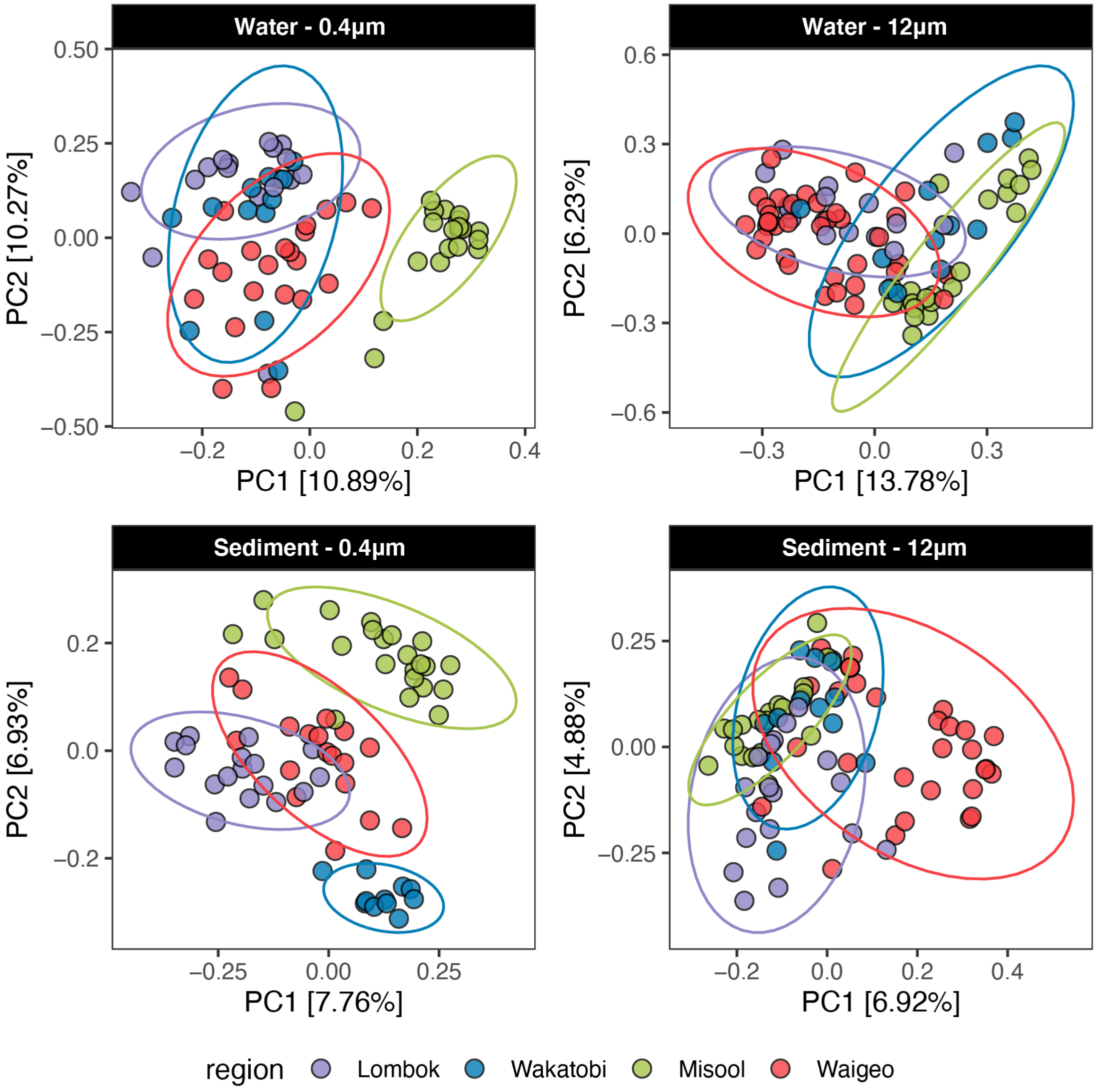


Figure 4. PCoA ordinations constructed using Bray-Curtis dissimilarity where each point represents a single sample and points are colored by sampling region. Full summary of pairwise ANOSIM statistics in Supplemental Table 3.

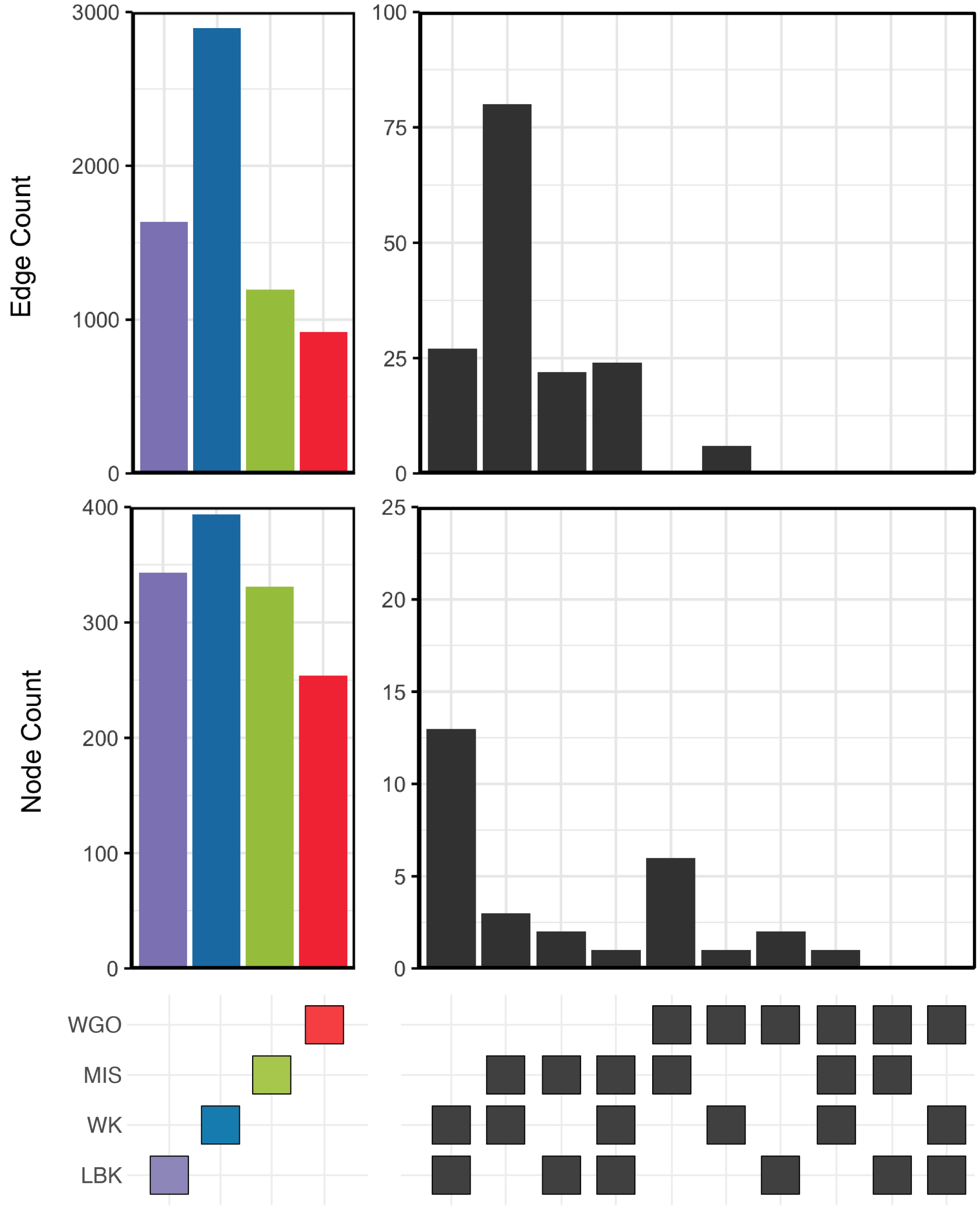


Figure 5. Edge and node counts from co-occurrence networks constructed using WGCNA. Bars with color indicate edge counts from network for that individual region, while grey bars indicated edges in networks shared across regions indicated by the squares in the bottom of the plot.

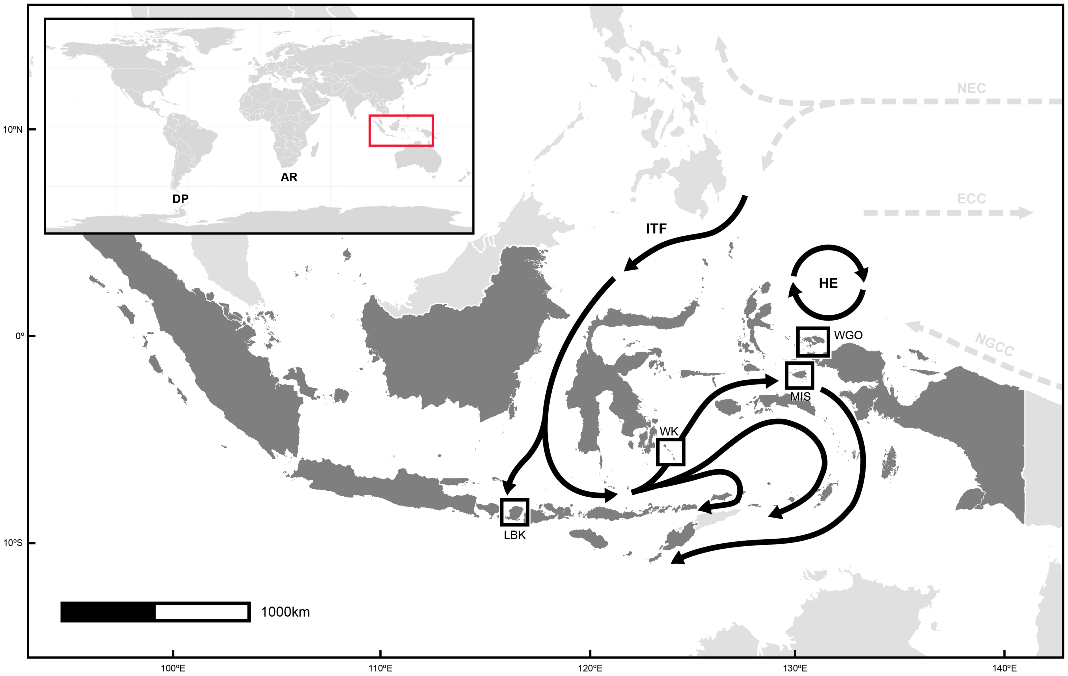
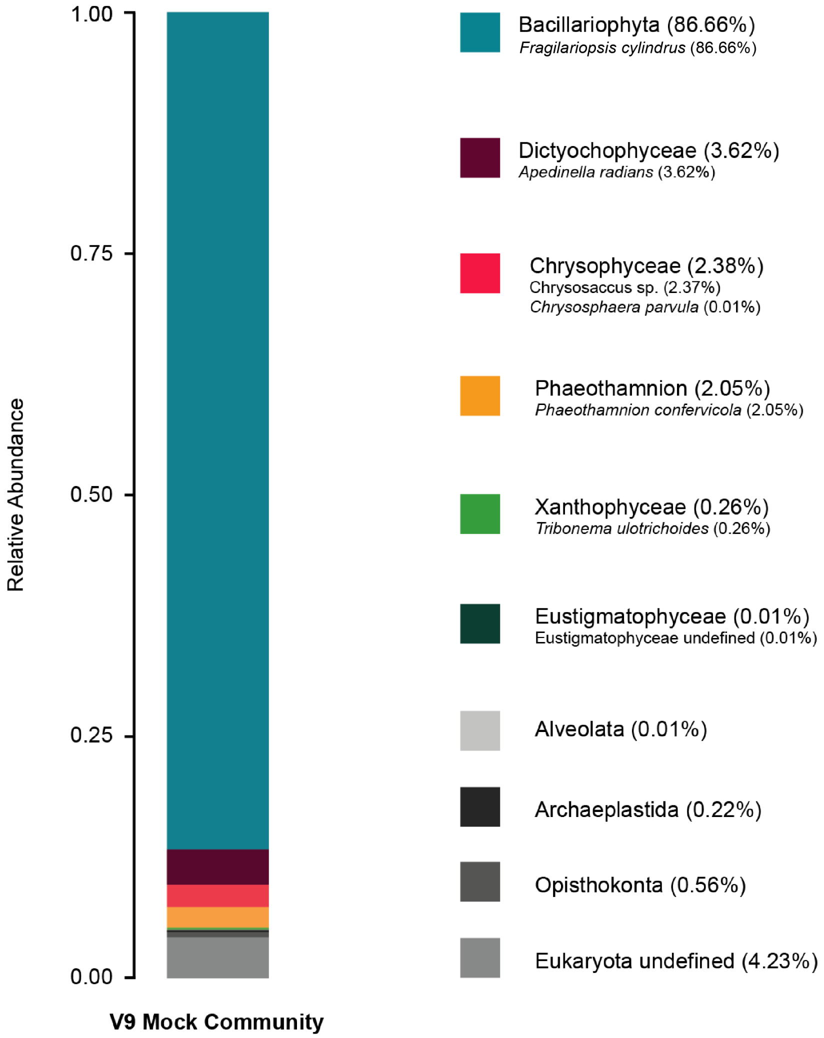


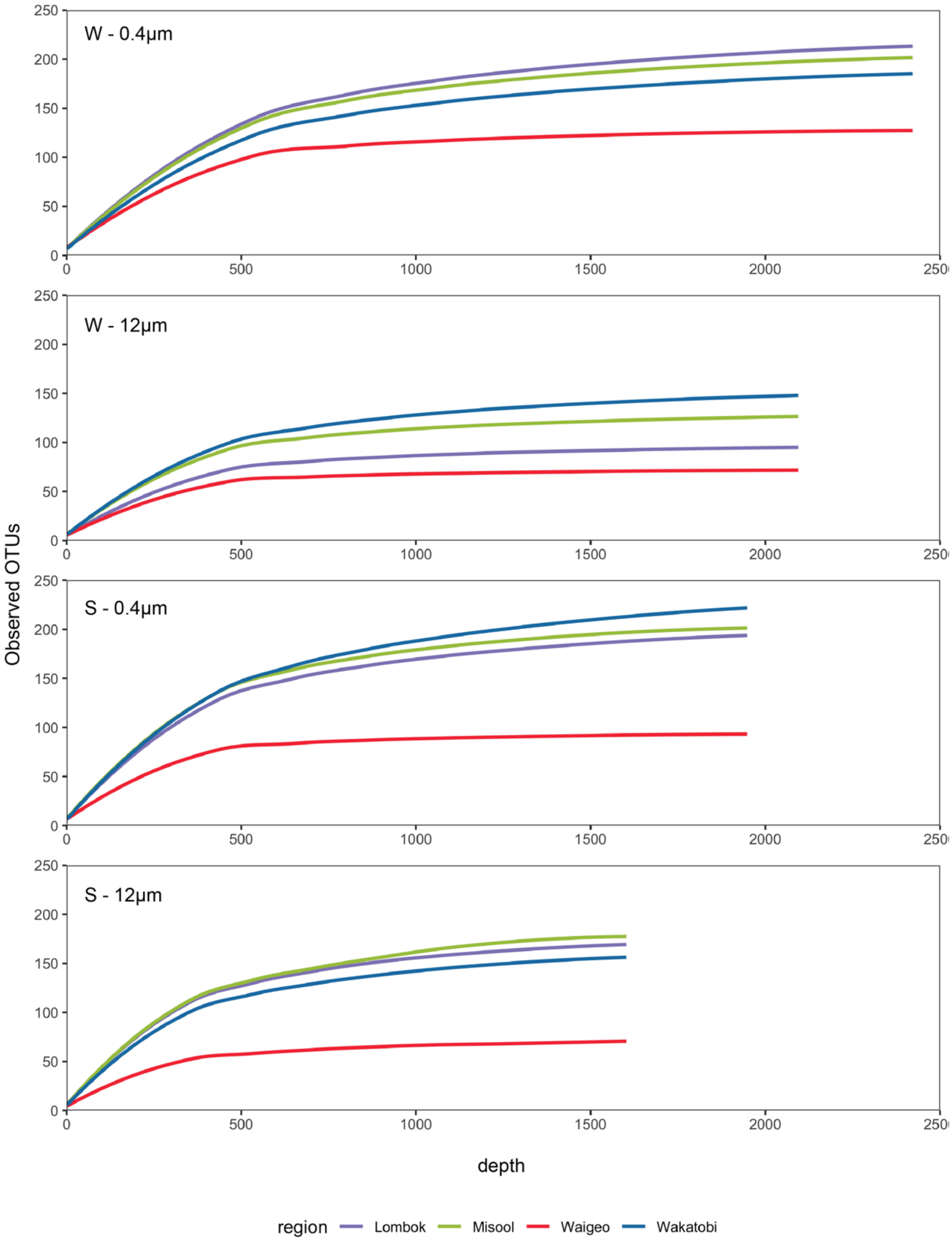
Figure 6. Map of Indonesia showing sampling regions (LBK = Lombok, WK = Wakatobi, MIS = Misool, and WGO = Waigeo) and major currents through the region including the Indonesian Throughflow (ITF), Halmahera Eddy (HE), New Guinea Coastal Current (NGCC), North Equatorial Current (NEC), and Equatorial Counter Current (ECC). Inset shows world map and location of two additional choke points in ocean circulation (AR = Agulhas Retroflection; DP = Drake Passage).

Table 1. Summary of hypotheses explaining how surface currents across Indonesia may be shaping protist community structure with supporting references.

Hypothesis	**Explanation**	**References**
ITF as chokepoint for diatom diversity in LBK, WK, and MIS	Agulhas Retroflection and Drake passage both correspond to chokepoints in diatom diversity at high latitudes. The Makassar Strait which the ITF passes through is the only low latitude chokepoint in ocean circulation and could also be creating a choke point in diatom diversity at subsequent sampling regions (LBK, WK, & MIS).	(Malviya et al., 2016) (Lee et al., 2019)
HE as hotspot for diatom diversity	The centers of eddies are known as diatom dead zones, while the fronts are diatom hotspots due to influx of nutrients at those points. Additionally, the HE has been proposed as a dispersal barrier for metazoan larvae, driving population breaks in eastern Indonesia.	(Tréguer et al., 2018) (Carpenter et al., 2011)
Seasonal changes in current patterns	Sampling occurred at different points in the year. The velocity of the ITF fluctuates with season potentially affecting connectivity and dispersal of communities. Sampling in Waigeo took place during period when surface velocity of ITF was slowest, while other regions were sampled during peak surface velocity.	(Lee et al., 2019) (Gordon & Fine, 1996) (Gordon et al., 2003)



Supplemental Figure 1. Mock community results showing the proportion of reads classifying to different eukaryotic lineages. The bars with color indicate Stramenopile lineages expected in the mock community (Bacillariophyta, Dictyochophyceae, Chrysophyceae, Phaeothamnion, Xanthophyceae), while bars with shades of grey indicate sequences from lineages not expected in the mock community (Alveolata, Archaeplastida, Opisthokonta, Eukaryota undefined).



Supplemental Figure 2. Rarefaction curves for each filter size, and sample type (water vs. sediment). Sequencing reached saturation in observed OTUs per region by about 1000 sequences.

Supplemental Table 1. Summary of Mantel statistics for spatial autocorrelation among different groupings of sites for each filter size and sample type (water vs. sediment). Asterisks (*) indicate significant p-value ($\alpha = 0.05$).

All Sites

sample type	filter size	r statistic	p-value
water	0.4μm	0.128	0.014*
	12μm	0.014	0.377
sediment	0.4μm	0.151	0.004*
	12μm	0.071	0.065*

Lombok

sample type	filter size	r statistic	p-value
water	0.4μm	0.388	0.0003*
	12μm	0.293	0.014*
sediment	0.4μm	0.115	0.934
	12μm	-0.032	0.618

Wakatobi

sample type	filter size	r statistic	p-value
water	0.4μm	-0.012	0.519
	12μm	0.089	0.222
sediment	0.4μm	-0.056	0.642
	12μm	0.048	0.374

Misool

sample type	filter size	r statistic	p-value
water	0.4μm	0.434	0.053
	12μm	0.464	0.0001*
sediment	0.4μm	0.5322	0.0004*
	12μm	0.276	0.059

Waigeo

sample type	filter size	r statistic	p-value
water	0.4μm	-0.108	0.7109
	12μm	0.092	0.181
sediment	0.4μm	0.244	0.923
	12μm	0.038	0.365

Supplemental Table 2. Summary of Wilcoxon pairwise statistics comparing richness of protist taxa among different sampling regions for each filter size and sample type (water vs. sediment). Asterisks (*) indicate significant p-value (α = 0.05).

	region 1	region 2	p-value (W-0.4)	p-value (W-12)	p-value (S-0.4)	p-value (S-12)
All	Lombok	Wakatobi	0.574	0.017*	0.0145*	0.88215
		Misool	0.494	0.183	0.0767	0.72639
		Waigeo	0.0024*	0.021*	0.0032*	0.000011*
	Wakatobi	Misool	0.8762	0.126	0.0617	0.68976
		Waigeo	0.0315*	0.000041*	0.000079*	0.00019*
	Misool	Waigeo	0.003*	0.000048*	0.000079*	0.0000083*
Dinoflagellata	Lombok	Wakatobi	0.2286	0.14883	0.0126*	0.83205
		Misool	0.6252	0.02675*	0.0505	0.1297
		Waigeo	0.0016*	0.00176*	0.0126*	0.00329*
	Wakatobi	Misool	0.0612	0.48155	0.4332	0.24048
		Waigeo	0.0915	0.00016*	0.0008*	0.00329*
	Misool	Waigeo	0.0003*	0.00000039*	0.0008*	0.00014*
Bacillariophyta	Lombok	Wakatobi	0.673	0.034*	0.92	0.446
		Misool	0.47	0.592	0.84	0.215
		Waigeo	0.224	0.034*	0.92	0.004*
	Wakatobi	Misool	0.673	0.034*	0.92	0.928
		Waigeo	0.196	0.477	0.92	0.029*
	Misool	Waigeo	0.057	0.034*	0.59	0.027*

Supplemental Table 3. Summary of pairwise PERMANOVA and ANOSIM results comparing protist community composition across sampling regions for each filter size and sample type (water vs. sediment). Asterisks (*) indicate significant p-value (α = 0.05).

Water - 0.4μm

Group 1	Group 2	Sample size	pseudo-F	p-value (PERMANOVA)	R	p-value (ANOSIM)
Lombok	Misool	37	8.2565722	0.001*	0.53754962	0.001*
	Waigeo	38	4.53058072	0.001*	0.27425074	0.001*
	Wakatobi	29	2.57929388	0.001*	0.2948942	0.001*
Misool	Waigeo	41	5.75307404	0.001*	0.33544643	0.001*
	Wakatobi	32	5.51111743	0.001*	0.51124674	0.001*
Waigeo	Wakatobi	33	2.34182463	0.001*	0.1096302	0.084

Water - 12μm

Group 1	Group 2	Sample size	pseudo-F	p-value (PERMANOVA)	R	p-value (ANOSIM)
Lombok	Misool	38	6.05498208	0.001*	0.45742683	0.001*
	Waigeo	52	3.66821531	0.001*	0.0903912	0.128
	Wakatobi	28	3.38742263	0.001*	0.38925291	0.001*
Misool	Waigeo	58	7.97192474	0.001*	0.36413496	0.001*
	Wakatobi	34	3.39737906	0.001*	0.38395317	0.001*
Waigeo	Wakatobi	48	4.75816472	0.001*	0.41514674	0.001*

Sediment – 0.4μm

Group 1	Group 2	Sample size	pseudo-F	p-value (PERMANOVA)	R	p-value (ANOSIM)
Lombok	Misool	39	4.64175302	0.001*	0.60353495	0.001*
	Waigeo	35	2.81579059	0.001*	0.36220232	0.001*
	Wakatobi	28	4.47726076	0.001*	0.24943304	0.004*
Misool	Waigeo	40	3.32141603	0.001*	0.36913405	0.001*
	Wakatobi	33	5.48450137	0.001*	0.365977	0.001*
Waigeo	Wakatobi	29	3.38415841	0.001*	0.00565754	0.415

Sediment - 12μm						
Group 1	Group 2	Sample size	pseudo-F	p-value (PERMANOVA)	R	p-value (ANOSIM)
Lombok	Misool	40	2.71120066	0.001*	0.35028672	0.001*
	Waigeo	47	3.39076538	0.001*	0.29905277	0.001*
	Wakatobi	30	2.28135805	0.001*	0.36827752	0.001*
Misool	Waigeo	51	3.70202893	0.001*	0.36574509	0.001*
	Wakatobi	34	2.41709945	0.001*	0.39856902	0.001*
Waigeo	Wakatobi	41	2.6703387	0.001*	0.23962595	0.011*

CHAPTER 3:

FISHING HAS MINIMAL IMPACT ON PROTIST COMMUNITY STRUCTURE

ABSTRACT

Indonesia is home to more coral reef fishers than any other country in the world, many of whom rely on fisheries as their only source of livelihood. Therefore, the sustainability of coral reefs and the fish they support is important for the well-being of millions of Indonesians. Marine protected areas (MPAs) have been implemented across Indonesia to conserve coral reefs and promote sustainable fisheries. While there are potential benefits to these protections, including increased abundance and diversity of fishes and corals, the effect of fishing on protist communities at the base of the food web is not well understood. In this study we use metabarcoding to characterize protist communities in both water and sediments across Indonesia in both protected and unprotected areas. Our results suggest that fishing plays a minimal role in shaping protist community structure on both broad and local geographic scales. The spatial structure in our data combined with protist alpha diversity across the region, instead suggest that protist community structure is likely driven by abiotic forces, such as surface currents. Overall, the lack of impact that fishing and MPAs have on protist communities suggests that the effects of protections at upper trophic levels are not strong enough to cascade to the base of the food web. Therefore, protist communities should not hold a strong influence for future conservation and fisheries management decisions.

INTRODUCTION

The Coral Triangle is home to some of the most biodiverse ecosystems on the planet and is widely accepted as the epicenter of marine biodiversity (Allen, 2008; Bowen, Rocha, Toonen, & Karl, 2013; Hoeksema, 2007; Veron et al., 2009). The coral reef ecosystems across this region are vital economic and food resources for the countries that occupy it (Cruz-Trinidad, Aliño, Geronimo, & Cabral, 2014; Foale et al., 2013). Indonesia is the largest country in this region and is home to nearly 17% of coral reefs in the world, across their more than 17,000 islands (Elliott, Mitchell, Wiltshire, Manan, & Wismer, 2001). Around 60% percent of Indonesia's population live in coastal areas and often rely on small-scale fisheries for their livelihood (Siry, 2007; Teh, Teh, & Sumaila, 2013). In fact, in some heavily fished areas of Indonesia, these fisheries account for almost 35% of all reported catch (Sadovy, 2005). Given the reliance on these fisheries across the country, protecting coral reef ecosystems is of vital importance from both conservation and food security perspectives (Dulvy, Freckleton, & Polunin, 2004; Halim A., 2002; Mous et al., 2005; Pet-Soede, Cesar, & Pet, 2012).

Fishing pressure across Indonesia correlates with human population along a gradient from west to east. Fishing pressure is highest on reefs in the west, where population is most dense and distance to the nearest market is relatively low, while fishing pressure is considerably lower on reefs in the east, where human population is low and distance to the nearest market is greatest. The effects of this variation in fishing pressure on coral reef ecosystems has been well documented across marine animals (Pet-Soede, Van Densen, Pet, & Machiels, 2001). In regions of low fishing pressure, reefs typically exhibit higher biodiversity and fish biomass, while heavily fished regions' reefs exhibit lower biodiversity and fish biomass (Campbell et al., 2020; Campbell, Mukminin, Kartawijaya, Huchery, & Cinner, 2014a). As reefs in the west are heavily fished and resources are depleted, there is concern that pressure on the healthy reefs in the east will begin to increase, potentially leading to exploitation of those resources as well (Mous et al., 2005). Management and marine protected area (MPA) design strategies may be useful preventative measures against overfishing, harmful fishing practices, and ultimately resource depletion on those reefs.

Fisheries management strategies and MPAs have been established across Indonesia, with the combined goals of conserving biodiversity and promoting sustainable fishing practices (Gaines, White, Carr, & Palumbi, 2010). These protections vary from no-take reserves, to areas where fishing gear is restricted, to areas that are open-access. As of 2018, the Coral Triangle had nearly 2000 MPAs that encompass over 200,000 km^2. This area, however, covers less than 4% of the marine area across this region (Asaad, Lundquist, Erdmann, & Costello, 2018). As such, protections across this region are constantly being assessed and redesigned to approach better ways to achieve both conservation and sustainable fisheries goals. Factors like size, location and connectivity to fished regions play important roles in MPA design, in attempt to optimize effectiveness of these protections (Gaines et al., 2010; Halpern, 2003; Halpern & Warner, 2003).

Whereas MPAs and fisheries management strategies promote higher diversity, abundance, and fecundity in across metazoan groups (Campbell et al., 2020; Campbell, Edgar, Stuart-Smith, Soler, & Bates, 2018a; Campbell et al., 2014a; Lester

& Halpern, 2008; Lester et al., 2009), their impact on microbial communities at the base of the food web is far less studied. Investigating how fishing pressure and various protection strategies impact microbial communities across Indonesia is essential for understanding how the effects of these management strategies and protections cascade down trophic levels in ecosystems, and can also provide insight on how these strategies may impact biogeochemical cycling and other microbial processes in reef ecosystems. Here we use metabarcoding data of protist communities to evaluate how fishing pressure and management strategies impact the base of the eukaryotic food web. Our data suggest that, while management leads to increased biomass in fish in protected areas, they have minimal impact on protist communities. The lack of connectivity between upper trophic levels and microbial eukaryotic organisms, suggests that future management and MPA design should not be strongly influenced by these communities.

METHODS

Sample collection and preservation

Sampling was conducted from January 2018 through May 2019 in four regions across Indonesia. The regions were selected to span a range of human population and fishing pressure from high in the southwest (Lombok), moderate in central Indonesia (Wakatobi), to low in the northeast region of Raja Ampat (Misool and Waigeo). Within each region, ~20 different sites were chosen (Lombok = 18 sites; Wakatobi = 12 sites; Misool = 21 sites; Waigeo = 19 sites) with a range of protection schemes that included no-take zones, gear-restricted zones, and open access zones (Figure 1). No-take zones and gear restricted zones are both categorized here as marine protected areas, while open-access zones are unprotected.

At each site, 4L of water were collected at 5m depth, and surface sediment was collected at 10m. Water and sediment samples were then filtered over consecutive 12µm and 0.4µm polycarbonate filters (SterliTech), using a peristaltic pump. Sediment samples included ~1L of sediment suspended in water and were filtered until the filters clogged – often after filtering ~2L. Filters were then cut in half and preserved in 1mL DNA RNA shield (Zymo Research), in 2mL cryovials, until transported back to the lab.

In addition to water and sediment samples, underwater visual census (UVC) data was collected for benthic and fish communities at each site. Both benthic and fish data were collected by two separate observers along three 50m transects. Observers counted and estimated fish size withing 2.5m on either side of the transect. Fish were identified as close to species level as possible and were later sorted into trophic and functional groups based on diet information and published literature available for each species (Froese & Pauly, 2021). Trophic levels included, carnivore, omnivore, benthic invertivore, corallivore, herbivore, planktivore, and detritivore. Fish biomass was later calculated based on length-weight parameters on FishBase (Froese & Pauly, 2021).

Finally, socio-environmental variables were downloaded from the Marine Socio-Environmental Covariates (MSEC) database for each of our sites (Yeager, Marchand, Gill, Baum, & McPherson, 2017). The variables downloaded from the database included human population within a 25km radius, distance to nearest market, and land area and reef area within a 15km radius of each site. These variables were then in turn used as proxy for fishing pressure at each of our sites in later analysis.

DNA extraction, PCR, and sequencing

The Zymo Biomics DNA Miniprep Kit was used to extract DNA from each filter, following the manufacturer's protocol. The V9 hypervariable region of 18S rDNA was then amplified with polymerase chain reaction (PCR) using 1398F (5' – TTGTACACACCGCCC – 3') and 1510R (5' – CCTTCYGCAGGTTCACCTAC – 3') primers (Amaral-Zettler, McCliment, Ducklow, & Huse, 2009). PCRs were set up using Bioline MyTaq Red Mix (Meridian Bioscience) following the volume and concentration recommendations from the manufacturer, for both primers and DNA template. The PCR cycle began with a 3min denaturation step at 94°C, followed by 35 cycles of 94°C for 45sec, 48°C for 30sec, and 72°C for 30sec, and finishing with a final elongation step of 72°C for 5min. Successful amplification and amplicon size were confirmed using gel electrophoresis. Library prep was done by the University of Rhode Island Genomics and Sequencing Center and the amplicons were then sequenced on the Illumina MiSeq on a 2x150bp run using the MiSeq Reagent V2 Kit.

In addition to our samples from the field, a mock community was prepared using DNA samples from cultures available in the lab, as a control on the sequencing run. The mock community spanned different Stramenopile lineages, including *Fragilariopsis cylindrus* (Bacillariophyta), *Apedinella radians* (Dictyochophyceae), *Phaeothamnion confervicola* (Phaeothamniophyceae), *Chrysosaccus* sp. (Chrysophyceae), and *Tribonema minus* (Xanthophyceae). DNA from each culture was added in equal concentrations to a single tube, and the resulting DNA sample was amplified using the same primers and PCR methods described above. The amplicons were then sequenced on the Illumina MiSeq, on the same run as the environmental samples.

Bioinformatics

Initial quality assessment of forward and reverse reads was performed in FastQC (Andrews, 2010). Primer sequences were trimmed from the paired reads using Cutadapt (v1.9.1) (Martin, 2011). After trimming, the reads were imported into QIIME2 (v2020.6) and were filtered, denoised, merged, and chimera checked, using the DADA2 plugin in QIIME2 (Bolyen et al., 2019; Callahan et al., 2013). Truncation length for denoising was chosen to minimize the number of low-quality bases at the end of the reads, while maximizing the amount of overlap between the forward and reverse reads to optimize merging.

The amplicon sequence variants (ASVs) resulting from denoising were taxonomically assigned using a Naïve-Bayes classifier in QIIME2 with the Protist Ribosomal Reference Database (PR^2) (Guillou et al., 2013). Once classified, any ASV that had a classification confidence of lower than 95% was filtered out, removing low quality and low abundance ASVs from the dataset to help reduce noise in downstream analyses. The remaining ASVs were then filtered to isolate protist sequences from the Stramenopiles, Alveolates, and Rhizaria. These three groups were chosen specifically because they encompassed the most abundant and diverse microbial eukaryotic lineages from previous metabarcoding surveys across the globe (de Vargas et al., 2015).

Data analysis & statistics

The socio-environmental variables from the Marine Socio-Environmental Covariates (MSEC) database (Yeager et al., 2017) were used to assess how fishing pressure affects protist community structure across Indonesia on a large geographic scale. The variables, which included human population, distance to market, land area, and reef area were first tested for collinearity with one another to eliminate any variables that may negatively affect later analyses. Canonical Analysis of Principal Coordinates (CAP) was then run in R, using the *phyloseq* package, with the MSEC data as environmental variables (Anderson & Willis, 2003). To further assess impacts of fishing pressure on individual taxonomic groups, Pearson correlations were run in R, comparing the richness within the most abundant protist taxa to each socio-environmental variable. Results of these correlations were visualized in a heatmap, only showing significant correlations ($p < 0.05$).

Marine protected area designations were used to assess how protection schemes within individual sampling regions impacted protist community structure on a local geographic scale. Community composition changes between MPAs and non-MPAs were assessed using analysis of similarity (ANOSIM) with Bray-Curtis distances. These results were visualized using principal coordinates analysis (PCoA) ordinations, constructed using the *phyloseq* package in R (McMurdie & Holmes, 2013). These statistics were run both on the entire dataset and on subsets from individual sampling regions, to best discriminate the effects of MPAs on protist communities.

In addition to evaluating the impacts of fishing pressure and MPA designation on protist communities, we also evaluated the impacts those had on fish communities using our UVC data. First, comparisons between UVC data collected by different individuals at the same sites were done to ensure no bias based on the individual collector. These comparisons or counts from different observers were significant in some sampling regions (Lombok & Misool) and not significant in others (Waigeo) (Supplemental Table 1). Given that Wakatobi only had one observer, and the significant difference between observer counts in Lombok and Misool, only counts from one observer were used for each site. Comparisons of fish communities were done across sampling regions and between MPA designations broken down by trophic group to better understand how different levels of the food web were impacted by each of these variables. Similar comparisons were then run on protist alpha diversity data to identify how and if impacts of fishing pressure and management at upper trophic levels cascade through the food web.

RESULTS

Sequencing and filtering results

The amplicon data encompassed 293 samples and greater than 18 million total reads with an average of 63,668 reads per sample. After quality filtering, denoising, and merging we retained on average 84% of our reads. Our additional filtering, following taxonomic assignment, eliminated 16% of the remaining reads. That 16% was largely undefined, low abundance ASVs. The remaining sequences consisted of

48,656 ASVs, which classified to 1,387 taxa. The final filtering step isolated protist sequences, retaining only those classifying to Stramenopiles, Alveolates, and Rhizaria. This step eliminated 65% of the remaining data, resulted in 12,085 ASVs that classified to 839 taxa. While, this did not encompass all protist sequences, it did include the most diverse and abundant microbial eukaryotic lineages in the dataset. The majority of the reads eliminated classified to undefined eukaryotes (28%) and metazoans (24%). Remaining eukaryotic lineages included Amoebozoa (>1%), Apusozoa (>1%), Archaeplastida (5%), Hacrobia (1.5%), Excavata (>1%), and Fungi (5%) which together accounted for the remaining 13% of the reads.

Mock community

After taxonomic assignment of the sequences in our mock community, 95% of reads in from the were assigned to Stramenopiles. Most of the remaining 5% of reads were classified as undefined Eukaryotes (4.23%), while the remaining fraction of a percent came from various Opisthokonts (0.56%) and other protist groups (0.24%).

Within the Stramenopiles, all of the groups that were added to the mock community were recovered, in varying proportions. The majority of reads classified to Bacillariophyta (86.66%), followed distantly by Dictyochophyceae (3.62%), Chrysophyceae (2.38%), Phaeothamnion (2.05%), and Xanthophyceae (0.26%) (Chapter 1, Supplemental Figure 1). Within these groups, all of the expected species that were placed in the mock community were identified with the exception of the Xanthophyte, which classified to the correct genus but a different species than what we expected (*Tribonema ulotrichoides*). The misidentification of the Xanthophyte species, is likely the result with an error in identification of the species in culture or is reflective of a lack of Xanthophyte sequences in the reference database. The difference in the proportions of reads, despite starting with approximately equal concentrations of DNA, especially in the Bacillariophyta, is likely a result of variation in 18S copy number across different taxonomic groups or primer bias (Medinger et al., 2010; Zhu, Massana, Not, Marie, & Vaulot, 2005).

Effect of fishing on protist communities

The socio-environmental variables from the MSEC database, used here as proxy for fishing pressure, were poor explainers of broad-scale protist community composition changes. The CAP ordinations show those variables only explaining 7-12% of the variation across samples, despite showing significant correlations to axes on the CAP ordination (Figure 2). The ordinations also show that the variables explain slightly more variation in the water samples than the sediment samples. The 0.4µm water CAP axes explain 11.1% of the variation in samples while the 0.4µm sediment CAP axes only explain 8.5% of the variation in samples. Likewise, the 12µm water CAP axes explain 10.4% of the variation in samples, while the 12µm sediment CAP axes explain 7.3% of the variation in samples. While human population shows significant correlation with CAP axes in each ordination, the amount of variation explained by each of those axes is minimal suggesting that it is not a strong explainer of protist community variation. These variables, especially human population, also closely correspond with geographic position of sites, which also indicates spatial structure in these communities. Furthermore, the correlations between the socio-environmental variables and community composition are not significant when looking at data from individual sampling regions (ANOVA, $p > 0.05$).

Pearson correlations between relative abundance of the most abundant taxonomic groups and the same socio-environmental variables used above in the dataset were generally weak and inconsistent across sample type and filter size, suggesting that the human population, which we use as proxy for fishing pressure, was likely not differentially impacting individual protist groups (Figure 3). The correlations were also inconsistent across functional groups. While ciliates showed weak positive correlation with reef area (average $r = 0.30$), other small heterotrophic groups like cercozoans show no significant correlation with reef area, but instead show positive correlations with human population (average $r = 0.49$). For the autotrophic groups, the dinoflagellates show negative correlations with land area (average $r = -0.41$) and positive correlations with distance to market (average $r = 0.38$), while the diatoms show weak positive correlations with both land area (average $r = 0.35$) and distance to market (average $r = 0.45$).

Alpha diversity results further support that human population and shifts in abundance of fishes by region play a minimal role in structuring protist communities on a large geographic scale. The UVC data for fish communities supports previous studies showing significantly higher fish abundances in regions with low human population and therefore low fishing pressure (Misool and Waigeo) compared to regions with higher human population and fishing pressure (Lombok and Wakatobi) (Wilcoxon, $p < 0.05$). When we further break down the UVC data by trophic group we can see more clearly how different levels of the food web shift in abundances across the sampling regions and correlations between the shifts in abundances at each trophic level and human population (Figure 4). The planktivores, in particular, are significantly more abundant in the eastern regions of Misool and Waigeo where fishing pressure is low compared to the more intensely fished regions of Lombok and Waigeo in the west (Wilcoxon, $p < 0.05$) (Supplemental Table 2). In addition to showing significant shifts in abundance by sampling region, the planktivores are also the trophic level that show the strongest correlation with human population across Indonesia ($R = -0.26$, $p = 0.033$). Despite regional shifts in the lower trophic level fish, protist richness shows a significant shift in diversity between Waigeo and Misool (Wilcoxon, $p < 0.05$) (Chapter 1, Supplemental Table 1), the closest two sampling regions with the lowest fishing pressure and comparable planktivore abundances. The dramatic shift in diversity between these regions, despite comparable levels of human population and fish abundances again suggests that top-down pressure from upper trophic levels does not play a significant role in structuring protist communities.

Effects of management and MPA on protist communities

On a local geographic scale, MPA designation does not appear to impact alpha diversity in protist groups (Figure 5). Protist richness was not significantly different between MPA or open-access regions (Kruskal-Wallis, $p = 0.8433$). However, fish biomass and abundance of most fish trophic groups did vary significantly between MPA and open-access areas, across our sampling sites (Kruskal-Wallis, $p < 0.05$) (Figure 5) (Supplemental Table 3).

Additionally, protist community composition did not differ significantly between MPA and open-access areas for the 0.4μm samples in both water (ANOSIM; $R = 0.040349$, $p = 0.199$) and sediment samples (ANOSIM; $R = -0.022064$, $p = 0.62$). The p-values for the 12μm samples indicate significant differences between MPA and open-access sites, in both water (ANOSIM; R = 0.14726, p = 0.005) and sediment samples (ANOSIM; R = 0.096917, p = 0.03). However, the low R statistics indicate even distribution of high and low ranks between groups, which indicates lack of dissimilarity between protected and unprotected sites for the 12µm samples, despite the significant p-value. This is further supported by our PCoA ordinations, which have considerable overlap in community composition across MPA and open-access sites (Figure 6).

DISCUSSION

The negative impact of fishing pressure on biodiversity and biomass of marine animals is well documented across Indonesia (Campbell et al., 2020, 2014a; Pet-Soede et al., 2001). Likewise, there are a number of studies that document the benefits fishing gear restrictions and no-take reserves have in ecosystems, including increased biomass and fecundity of fish on coral reefs (Campbell et al., 2012a, 2018a; Hilborn et al., 2004; Lester & Halpern, 2008; Lester et al., 2009). Our data support the findings of these previous studies, showing that sites with lower human population and therefore fishing pressure and protected sites have higher fish abundances, especially planktivorous fish abundances than sites with no restrictions and higher human population and fishing pressure (Figure 4 & 5). We further expand on those studies by showing that protist communities at the base of the food web are minimally impacted by fishing pressure and fisheries management strategies on both broad and local geographic scales.

Impacts of fishing pressure on broad scale protist biogeography

Studies on the impacts of grazing and top-down pressures on microbial community structure focus primarily on the roles of microbial grazers like copepods and other protists (Benedetti et al., 2019; Prowe, Pahlow, Dutkiewicz, Follows, & Oschlies, 2012; Toullec et al., 2019; Ward, Dutkiewicz, & Follows, 2014). As a result, connectivity between the microbial food web and upper trophic levels is poorly understood. In particular, understanding how or if impacts on upper trophic levels cascade down to impact microbial communities at the base is limited.

Microbial communities play important roles in biogeochemical cycling and other ocean processes in coral reef ecosystems (Ainsworth, Thurber, & Gates, 2010; Falkowski, Barber, & Smetacek, 1998). Protists, in particular, play important roles as primary producers and also as consumers at the base of the food web and also participate in a wide spectrum of symbioses (Clerissi et al., 2018; Decelle, Colin, & Foster, 2015; Field, Behrenfeld, Randerson, & Falkowski, 1998). While broad scale biogeographic trends in protist community structure appear driven largely by a combination of environmental selection and dispersal limitation (Malviya et al., 2016; Richter et al., 2020; Sunagawa et al., 2015), top-down pressure from grazers appears to be more important in structuring communities on a local geographic scale (Sommeria-Klein, Watteaux, Iudicone, Bowler, & Morlon, 2020). Our data suggest that human population and subsequent effects on upper trophic levels have minimal effects on broad scale protist community structure in the tropical coastal waters of the Coral Triangle. Although the socio-environmental variables, used as proxy for fishing pressure, explained significant variation in community composition, the explanatory power on the CAP axes was low (7-12%), suggesting that these were not strong drivers of the observed community shifts across the region (Figure 2). Given the immense ecological and morphological diversity exhibited within protists, correlations between relative abundance of major groups and the socio-environmental variables were also run to evaluate whether human population differentially impacted certain protist groups. The correlations across all taxa were generally weak and inconsistent across filter sizes and sample types, further supporting that fishing pressure was a weak driver of protist community structure (Figure 3). Even when looking at correlations amongst specific functional groupings of protists (e.g. autotrophs, heterotrophs, etc.), the correlations remained weak and inconsistent across groups with similar ecologies further supporting that top-down pressures from human population and fish abundances plays a minimal role in shaping these communities on a large geographic scale.

The lack of impact the socio-environmental variables appears to play in structuring protist communities, leaves the question of what other variables may be driving the patterns observed in the data. While the CAP ordinations show that distance to market and human population explain significant variation along the first CAP axis, those variables also correlate closely with geographic region. The spatial structure in these communities could indicate that abiotic factors like dispersal limitation may play important roles in shaping these communities. The role dispersal limitation, and more specifically surface currents, plays in shaping protist community structure is further supported by alpha diversity results across sampling regions. Unlike fish abundances, which are highest in Waigeo and Misool where fishing pressure is low, protist diversity shows a significant change in richness between Waigeo and Misool, the two regions closest to one another (Figure 4). This shift in protist diversity is particularly interesting because not only is this happening on a relatively small geographic scale (~50km between Waigeo and Misool), this is also happening in regions with statistically similar fish abundances. When looking at the fish abundances broken down by trophic group, the planktivores specifically show the strongest correlation with human population and therefore fishing pressure and show highest abundance of planktivores with no significant shift between the two regions. Given that the planktivores sit low in the food web and most directly interact with protist communities relative to other fish trophic groups, the shift in protist diversity between Misool and Waigeo and lack of shift in planktivore abundances further support that fishing pressure has minimal role in structuring protist communities.

This disconnect in protist communities between Waigeo and Misool is further supported by evaluating ASV co-occurrence across sampling regions (Chapter 1, Figure 5). While each sampling region shows high numbers of ASVs unique to individual sampling regions, the overlap among regions shows a lack in overlap between Waigeo and the other three regions. The break between Waigeo and the rest of our sampling regions is consistent with genetic breaks observed in a number of invertebrate populations (Barber, Cheng, Erdmann, Tenggardjaja, & Ambariyanto, 2011; Crandall et al., 2008; DeBoer et al., 2008), which were hypothesized to have resulted from a dispersal barrier created by current patterns across the region (Carpenter et al., 2011). The significant shift in protist diversity from Misool to Waigeo, despite having comparable levels of fishing pressure and planktivore abundances, further suggests that shifts in upper levels of the food web have minimal impact on protist communities at the base. However, it is worth noting that comparing fish abundances to shifts in protist alpha diversity may not be a direct comparison. While fish abundances may be reflective of the amount of top-down pressure from those upper trophic levels on protist communities, richness is not reflective of absolute abundance of protists and amplicon data must be considered compositionally which limits our ability to compare community shifts in relation to fish biomass.

Fisheries management, MPAs, and protist communities

While fishing pressure shifts across Indonesia shape metazoan communities on a broad geographic scale (Campbell et al., 2020; Campbell, Mukminin, Kartawijaya, Huchery, & Cinner, 2014b; Pet-Soede et al., 2001), fisheries management strategies and MPAs affect metazoan communities on a more local geographic scale (Campbell et al., 2012b; Campbell, Edgar, Stuart-Smith, Soler, & Bates, 2018b; Lester & Halpern, 2008; Lester et al., 2009). While studies on protist community structure across the globe demonstrate the potential importance of biotic interactions and grazing in shaping protist communities on local geographic scales (Sommeria-Klein et al., 2020), studies on protist community structure in Indonesia has primarily focused on the impacts of water quality, temperature, and salinity in shaping these communities (Hasani, Adiwilaga, & Pratiwi, 2013; Likumahua et al., 2020; Nasution, Dian Takarina, & Thoha, 2021; Rahmadyani, Widiarti, & Hendrayanti, 2017; Syakti, Idris, Koenawan, Asyhar, & Apriadi, 2019). Therefore, understanding of how biotic interactions and particularly those with organisms at upper trophic levels shape protist communities on a local geographic scale is limited.

Beta diversity data suggest that MPA designation does not significantly impact protist community composition across all sampling regions. One factor that complicates this analysis is the variability in enforcement of protections across the country. Because Indonesia has decentralized management, each regency is responsible for patrolling and enforcing protections, which leads to a lack of standardization across regions (Satria & Matsuda, 2004). Furthermore, the variation in fishing pressure across the country means that management designations in lower fished areas like Waigeo and Misool may not be equivalent to those same management designations in Lombok and Wakatobi. Because of this, it is important to also evaluate these protections on a regional scale. While fishing is unrestricted at all sites in Wakatobi, and fishing is prohibited at all sites in Misool, MPA designation shows no significant impact on protist community composition in either Lombok or Waigeo where management varies across sites. The lack of impact these designations have in areas of high fishing pressure (Lombok) and areas of low fishing pressure (Waigeo) further emphasize the lack of effect MPA designation has on these communities and may also provide support for our hypothesis that abiotic factors like surface currents play an important role in structuring these communities.

More recently, MPA design has shifted towards protecting areas that have high connectivity to fished regions. The goal of this design is that protected areas will provide breeding grounds for target species and their larvae will disperse outwards to fished areas to seed new populations in fished areas (Gaines et al., 2010; Palumbi, 2003; Sale et al., 2005). Dispersal in these studies largely relies on biophysical modelling, larval ecology, and population genetics to understand how distant populations connect to one another via surface currents (Kool, Moilanen, & Treml, 2013). As a result of these changes in design strategy, unprotected and protected regions can be highly connected to one another, which would result in overlap in community composition between unprotected and protected regions in communities driven largely by dispersal limitation. This pattern is consistent with our PCoA plots and is supported by our beta diversity statistics showing no significant changes in community composition between protected and unprotected sites.

Our data support previous studies documenting the negative impacts of fishing pressure and beneficial impacts of management and MPAs on fish biomass in Indonesia, and further expands on those studies to investigate the impacts these strategies have on protist communities at the base of the food web. On a broad scale, protist community structure appears minimally impacted by fishing pressure, and instead appears likely driven by abiotic factors like surface currents across the region. On a local scale, management also appeared to play a negligible role in structuring protist communities. Despite higher fish biomass in regions where fishing was prohibited or gear was restricted, the impacts on the upper trophic levels do not appear to cascade down to the base of the food web. This result suggests a lack of connectivity between the microbial food web and higher trophic level organisms and further suggests that protist communities may not be essential to account for future management and MPA design.

REFERENCES

Ainsworth, T. D., Thurber, R. V., & Gates, R. D. (2010). The future of coral reefs: a microbial perspective. *Trends in Ecology and Evolution*, *25*(4), 233–240. https://doi.org/10.1016/j.tree.2009.11.001

Allen, G. R. (2008). Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*, 541–556. https://doi.org/10.1002/aqc

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). *A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes*. *4*(7), 1–9. https://doi.org/10.1371/journal.pone.0006372

Anderson, M. J., & Willis, T. J. (2003). Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. *Ecology*, *84*(2), 511–525. https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2

Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data*.

Asaad, I., Lundquist, C. J., Erdmann, M. V., & Costello, M. J. (2018). Delineating priority areas for marine biodiversity conservation in the Coral Triangle. *Biological Conservation*, *222*(March), 198–211. https://doi.org/10.1016/j.biocon.2018.03.037

Barber, P. H., Cheng, S. H., Erdmann, M. V, Tenggardjaja, K., & Ambariyanto.

(2011). Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea. *CrustIssues19*, *12*(17), 1–32.

Benedetti, F., Jalabert, L., Sourisseau, M., Beker, B., Cailliau, C., Desnos, C., … Pouline, P. (2019). The seasonal and inter-annual fluctuations of plankton abundance and community structure in a North Atlantic Marine Protected Area. *Frontiers in Marine Science*, *6*(APR), 1–16. https://doi.org/10.3389/fmars.2019.00214

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … 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

Bowen, B. W., Rocha, L. A., Toonen, R. J., & Karl, S. A. (2013). The origins of tropical marine biodiversity. *Trends in Ecology and Evolution*, *28*(6), 359–366. https://doi.org/10.1016/j.tree.2013.01.018

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2013). DADA2: High resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869.DADA2

Campbell, S. J., Cinner, J. E., Ardiwijaya, R. L., Pardede, S., Kartawijaya, T., Mukmunin, A., … Baird, A. H. (2012a). Avoiding conflicts and protecting coral reefs: Customary management benefits marine habitats and fish biomass. *Oryx*, *46*(4), 486–494. https://doi.org/10.1017/S0030605312000348

Campbell, S. J., Cinner, J. E., Ardiwijaya, R. L., Pardede, S., Kartawijaya, T., Mukmunin, A., … Baird, A. H. (2012b). Avoiding conflicts and protecting coral reefs: Customary management benefits marine habitats and fish biomass. *Oryx*, *46*(4), 486–494. https://doi.org/10.1017/S0030605312000348

Campbell, S. J., Darling, E. S., Pardede, S., Ahmadia, G., Mangubhai, S., Amkieltiela, … Maire, E. (2020). Fishing restrictions and remoteness deliver conservation outcomes for Indonesia's coral reef fisheries. *Conservation Letters*, *13*(2), 1–9. https://doi.org/10.1111/conl.12698

Campbell, S. J., Edgar, G. J., Stuart-Smith, R. D., Soler, G., & Bates, A. E. (2018a). Fishing-gear restrictions and biomass gains for coral reef fishes in marine protected areas. *Conservation Biology*, *32*(2), 401–410. https://doi.org/10.1111/cobi.12996

Campbell, S. J., Edgar, G. J., Stuart-Smith, R. D., Soler, G., & Bates, A. E. (2018b). Fishing-gear restrictions and biomass gains for coral reef fishes in marine protected areas. *Conservation Biology*, *32*(2), 401–410. https://doi.org/10.1111/cobi.12996

Campbell, S. J., Mukminin, A., Kartawijaya, T., Huchery, C., & Cinner, J. E. (2014a). Changes in a coral reef fishery along a gradient of fishing pressure in an Indonesian marine protected area. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(1), 92–103. https://doi.org/10.1002/aqc.2359

Campbell, S. J., Mukminin, A., Kartawijaya, T., Huchery, C., & Cinner, J. E. (2014b). Changes in a coral reef fishery along a gradient of fishing pressure in an

Indonesian marine protected area. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(1), 92–103. https://doi.org/10.1002/aqc.2359

Carpenter, K. E., Barber, P. H., Crandall, E. D., Ablan-lagman, M. C. A., Mahardika, G. N., Manjaji-matsumoto, B. M., … Toha, A. H. A. (2011). Comparative Phylogeography of the Coral Triangle and Implications for Marine Management. *Journal of Marine Biology*, *2011*. https://doi.org/10.1155/2011/396982

Clerissi, C., Brunet, S., Vidal-Dupiol, J., Adjeroud, M., Lepage, P., Guillou, L., … Toulza, E. (2018). Protists within corals: The hidden diversity. *Frontiers in Microbiology*, *9*(AUG), 1–13. https://doi.org/10.3389/fmicb.2018.02043

Crandall, E. D., Jones, M. E., Muñoz, M. M., Akinronbi, B., Erdmann, M. V., & Barber, P. H. (2008). Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle. *Molecular Ecology*, *17*(24), 5276–5290. https://doi.org/10.1111/j.1365-294X.2008.03995.x

Cruz-Trinidad, A., Aliño, P. M., Geronimo, R. C., & Cabral, R. B. (2014). Linking Food Security with Coral Reefs and Fisheries in the Coral Triangle. *Coastal Management*, *42*(2), 160–182. https://doi.org/10.1080/08920753.2014.877761

de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., … Karsenti, E. (2015). Eukaryotic plankton diversity in the sunlit ocean. *Science*, *348*(6237), 1261605-1/11. https://doi.org/10.1007/s13398-014-0173-7.2

DeBoer, T. S., Subia, M. D., Ambariyanto, Erdmann, M. V., Kovitvongsa, K., & Barber, P. H. (2008). Phylogeography and limited genetic connectivity in the endangered boring giant clam across the coral triangle. *Conservation Biology*, *22*(5), 1255–1266. https://doi.org/10.1111/j.1523-1739.2008.00983.x

Decelle, J., Colin, S., & Foster, R. A. (2015). Photosymbiosis in Marine Planktonic Protists. In *Marine Protists: Diversity and Dynamics* (pp. 465–500). https://doi.org/10.1007/978-4-431-55130-0

Dulvy, N. K., Freckleton, R. P., & Polunin, N. V. C. (2004). Coral reef cascades and the indirect effects of predator removal by exploitation. *Ecology Letters*, *7*(5), 410–416. https://doi.org/10.1111/j.1461-0248.2004.00593.x

Elliott, G., Mitchell, B., Wiltshire, B., Manan, I. A., & Wismer, S. (2001). Community participation in marine protected area management Wakatobi National Park, Sulawesi, Indonesia. *Coastal Management*, *29*(4), 295–316. https://doi.org/10.1080/089207501750475118

Falkowski, P. G., Barber, R. T., & Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science*, *281*(5374), 200–206. https://doi.org/10.1126/science.281.5374.200

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, *281*(5374), 237–240. https://doi.org/10.1126/science.281.5374.237

Foale, S., Adhuri, D., Aliño, P., Allison, E. H., Andrew, N., Cohen, P., … Weeratunge, N. (2013). Food security and the Coral Triangle Initiative. *Marine Policy*, *38*, 174–183. https://doi.org/10.1016/j.marpol.2012.05.033

Froese, R., & Pauly, D. (2021). FishBase. Retrieved from World Wide Web electronic publication website: www.fishbase.org

Gaines, S. D., White, C., Carr, M. H., & Palumbi, S. R. (2010). Designing marine reserve networks for both conservation and fisheries management. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(43), 18286–18293. https://doi.org/10.1073/pnas.0906473107

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., … Christen, R. (2013). The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, *41*(D1), 597–604. https://doi.org/10.1093/nar/gks1160

Halim A. (2002). *Adoption of cyanide fishing practice in Indonesia. Ocean Coastal Manage ; 45*, 45:313–23.

Halpern, B. S. (2003). The impact of marine reserves: Do reserves work and does reserve size matter? *Ecological Applications*, *13*(1 SUPPL.). https://doi.org/10.1890/1051-0761(2003)013[0117:tiomrd]2.0.co;2

Halpern, B. S., & Warner, R. R. (2003). Matching marine reserve design to reserve objectives. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1527), 1871–1878. https://doi.org/10.1098/rspb.2003.2405

Hasani, Q., Adiwilaga, E. M., & Pratiwi, N. T. M. (2013). The Relationship between the Harmful Algal Blooms (HABs) Phenomenon with Nutrients at Shrimp Farms and Fish Cage Culture Sites in Pesawaran District Lampung Bay. *MAKARA of Science Series*, *16*(3), 183–191. https://doi.org/10.7454/mss.v16i3.1480

Hilborn, R., Stokes, K., Maguire, J. J., Smith, T., Botsford, L. W., Mangel, M., … Walters, C. (2004). When can marine reserves improve fisheries management? *Ocean and Coastal Management*, *47*(3–4), 197–205. https://doi.org/10.1016/j.ocecoaman.2004.04.001

Hoeksema, B. W. (2007). Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity : Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity : The Coral Triangle. In W. Renema (Ed.), *Biogeography, Time, and Place: Distributions, Barriers, and Islands* (pp. 117–178). https://doi.org/10.1007/978-1-4020-6374-9

Kool, J. T., Moilanen, A., & Treml, E. A. (2013). Population connectivity: Recent advances and new perspectives. *Landscape Ecology*, *28*(2), 165–185. https://doi.org/10.1007/s10980-012-9819-z

Lester, S. E., & Halpern, B. S. (2008). Biological responses in marine no-take reserves versus partially protected areas. *Marine Ecology Progress Series*, *367*, 49–56. https://doi.org/10.3354/meps07599

Lester, S. E., Halpern, B. S., Grorud-Colvert, K., Lubchenco, J., Ruttenberg, B. I., Gaines, S. D., … Warner, R. R. (2009). Biological effects within no-take marine reserves: A global synthesis. *Marine Ecology Progress Series*, *384*, 33–46. https://doi.org/10.3354/meps08029

Likumahua, S., de Boer, M. K., Krock, B., Hehakaya, S., Imu, L., Müller, A., … Buma, A. G. J. (2020). Variability of dinoflagellates and their associated toxins in relation with environmental drivers in Ambon Bay, eastern Indonesia. *Marine*

Pollution Bulletin, *150*(November 2019), 110778. https://doi.org/10.1016/j.marpolbul.2019.110778

Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., … Bowler, C. (2016). Insights into global diatom distribution and diversity in the world's ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(11), E1516–E1525. https://doi.org/10.1073/pnas.1509523113

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 5–7.

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwälder, B., Schlötterer, C., & Boenigk, J. (2010). Diversity in a hidden world: Potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Molecular Ecology*, *19*(SUPPL. 1), 32–40. https://doi.org/10.1111/j.1365-294X.2009.04478.x

Mous, P. J., Pet, J. S., Arifin, Z., Djohani, R., Erdmann, M. V., Halim, A., … Wiadnya, G. (2005). Policy needs to improve marine capture fisheries management and to define a role for marine protected areas in Indonesia. *Fisheries Management and Ecology*, *12*(4), 259–268. https://doi.org/10.1111/j.1365-2400.2005.00448.x

Nasution, A. K., Dian Takarina, N., & Thoha, H. (2021). The presence and abundance of harmful dinoflagellate algae related to water quality in Jakarta Bay, Indonesia. *Biodiversitas Journal of Biological Diversity*, *22*(5), 2909–2917. https://doi.org/10.13057/biodiv/d220556

Palumbi, S. R. (2003). Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, *13*(1 SUPPL.), 146–158. https://doi.org/10.1890/1051-0761(2003)013[0146:pgdcat]2.0.co;2

Pet-Soede, C., Cesar, H. S. ., & Pet, J. S. (2012). An economic analysis of blast fishing on Indonesian coral reefs An economic analysis of blast fishing on Indonesian coral reefs. *Environmental Conservation*, *26*(May 2002), 83–93.

Pet-Soede, C., Van Densen, W. L. T., Pet, J. S., & Machiels, M. A. M. (2001). Impact of Indonesian coral reef fisheries on fish community structure and the resultant catch composition. *Fisheries Research*, *51*(1), 35–51. https://doi.org/10.1016/S0165-7836(00)00236-8

Prowe, A. E. F., Pahlow, M., Dutkiewicz, S., Follows, M., & Oschlies, A. (2012). Top-down control of marine phytoplankton diversity in a global ecosystem model. *Progress in Oceanography*, *101*(1), 1–13. https://doi.org/10.1016/j.pocean.2011.11.016

Rahmadyani, R. F., Widiarti, R., & Hendrayanti, D. (2017). Distribution analysis on cyst abundance of HAB causing dinoflagellates at green mussel (Perna viridis) culture area in Kamal Muara , North Jakarta. *Proceeding – ICM-MBT*, (November), 014–021. Retrieved from http://pksplipb.or.id/; email: journal@pksplipb.or.id

Richter, D., Watteaux, R., Vannier, T., Leconte, J., Reygondeau, G., Maillet, N., … Frémont, P. (2020). Genomic evidence for global ocean plankton biogeography shaped by large-scale current systems. *HAL Archives-Ouvertes*.

Sadovy, Y. (2005). Trouble on the reef: The imperative for managing vulnerable and valuable fisheries. *Fish and Fisheries*, *6*(3), 167–185. https://doi.org/10.1111/j.1467-2979.2005.00186.x

Sale, P. F., Cowen, R. K., Danilowicz, B. S., Jones, G. P., Kritzer, J. P., Lindeman, K. C., … Steneck, R. S. (2005). Critical science gaps impede use of no-take fishery reserves. *Trends in Ecology and Evolution*, *20*(2), 74–80. https://doi.org/10.1016/j.tree.2004.11.007

Satria, A., & Matsuda, Y. (2004). Decentralization of fisheries management in Indonesia. *Marine Policy*, *28*(5), 437–450. https://doi.org/10.1016/j.marpol.2003.11.001

Siry, H. Y. (2007). Making Decentralized Coastal Zone Management Work for the Southeast Asian Region: Comparative Perspectives. In *Oceans and Law of Sea*.

Sommeria-Klein, G., Watteaux, R., Iudicone, D., Bowler, C., & Morlon, H. (2020). Global drivers of eukaryotic plankton biogeography in the sunlit ocean. *BioRxiv*. Retrieved from https://www.biorxiv.org/content/10.1101/2020.09.08.287524v1.abstract

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., … Lepoivre, C. (2015). *Structure and function of the global ocean microbiome*. *348*(6237), 1–10.

Syakti, A. D., Idris, F., Koenawan, C. J., Asyhar, R., & Apriadi, T. (2019). Biological pollution potential in the water of Bintan-Riau Islands Province, Indonesia: First appearance of harmful algal bloom species. *Egyptian Journal of Aquatic Research*, *45*(2), 117–122. https://doi.org/10.1016/j.ejar.2019.04.002

Teh, L. S. L., Teh, L. C. L., & Sumaila, U. R. (2013). A Global Estimate of the Number of Coral Reef Fishers. *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0065397

Toullec, J., Vincent, D., Frohn, L., Miner, P., Le Goff, M., Devesa, J., & Moriceau, B. (2019). Copepod Grazing Influences Diatom Aggregation and Particle Dynamics. *Frontiers in Marine Science*, *6*(December). https://doi.org/10.3389/fmars.2019.00751

Veron, J. E. N., Turak, E., Stafford-Smith, M., Stuart, K., Devantier, L. M., Green, A. L., & Peterson, N. (2009). Delineating the Coral Triangle. *Galaxea, Journal of Coral Reef Studies*, *11*(2), 91–100. https://doi.org/10.3755/galaxea.11.91

Ward, B. A., Dutkiewicz, S., & Follows, M. J. (2014). Modelling spatial and temporal patterns in size-structured marine plankton communities: Top-down and bottom-up controls. *Journal of Plankton Research*, *36*(1), 31–47. https://doi.org/10.1093/plankt/fbt097

Yeager, L. A., Marchand, P., Gill, D. A., Baum, J. K., & McPherson, J. M. (2017). Marine Socio-Environmental Covariates: queryable global layers of environmental and anthropogenic variables for marine ecosystem studies. *Ecology*, *98*(7), 1976. https://doi.org/10.1002/ecy.1884

Zhu, F., Massana, R., Not, F., Marie, D., & Vaulot, D. (2005). Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiology Ecology*, *52*(1), 79–92. https://doi.org/10.1016/j.femsec.2004.10.006

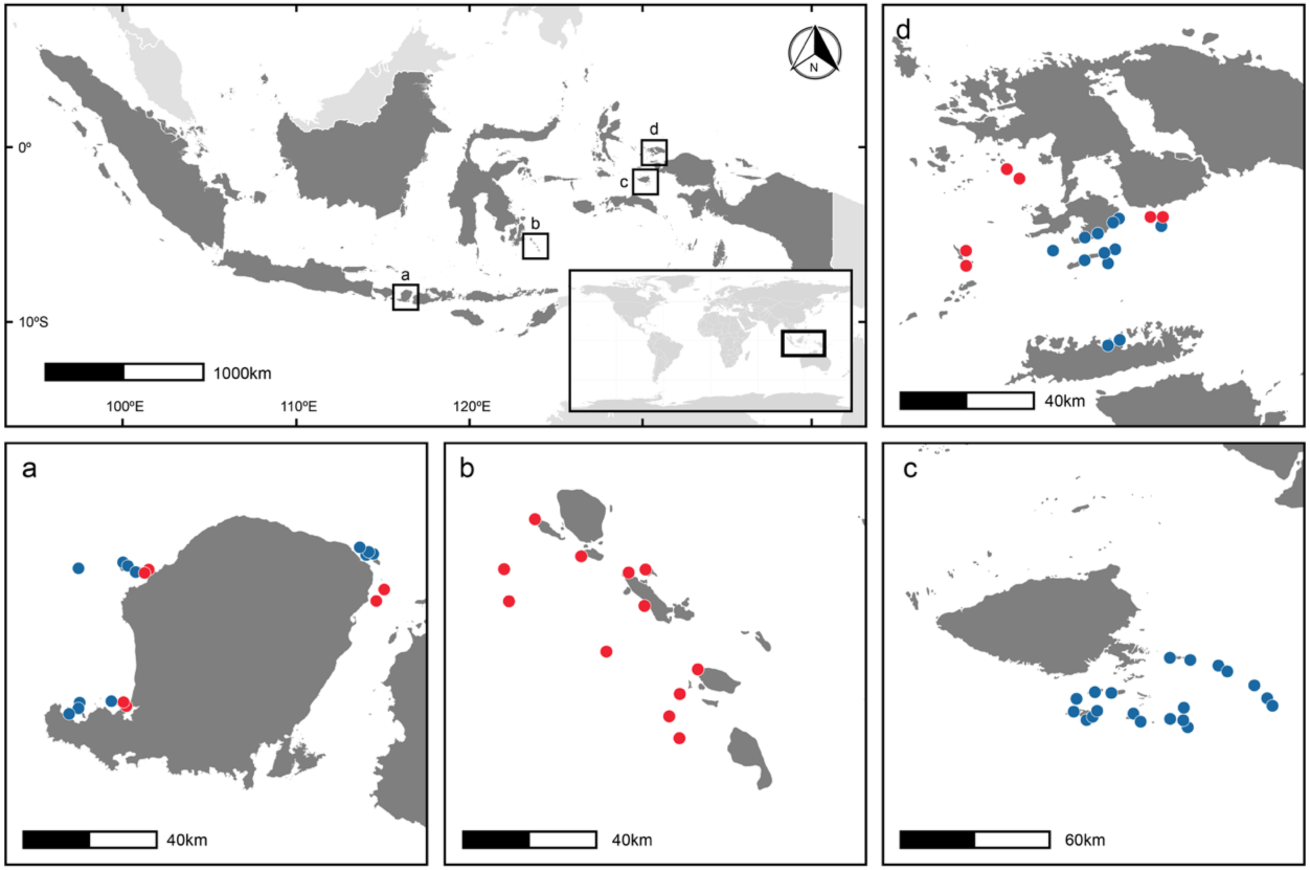


Figure 1. Map of Indonesia and individual sampling regions (a. Lombok, b. Wakatobi, c. Misool, d. Waigeo) with sites colored to show protection (red = open access; blue = marine protected area).

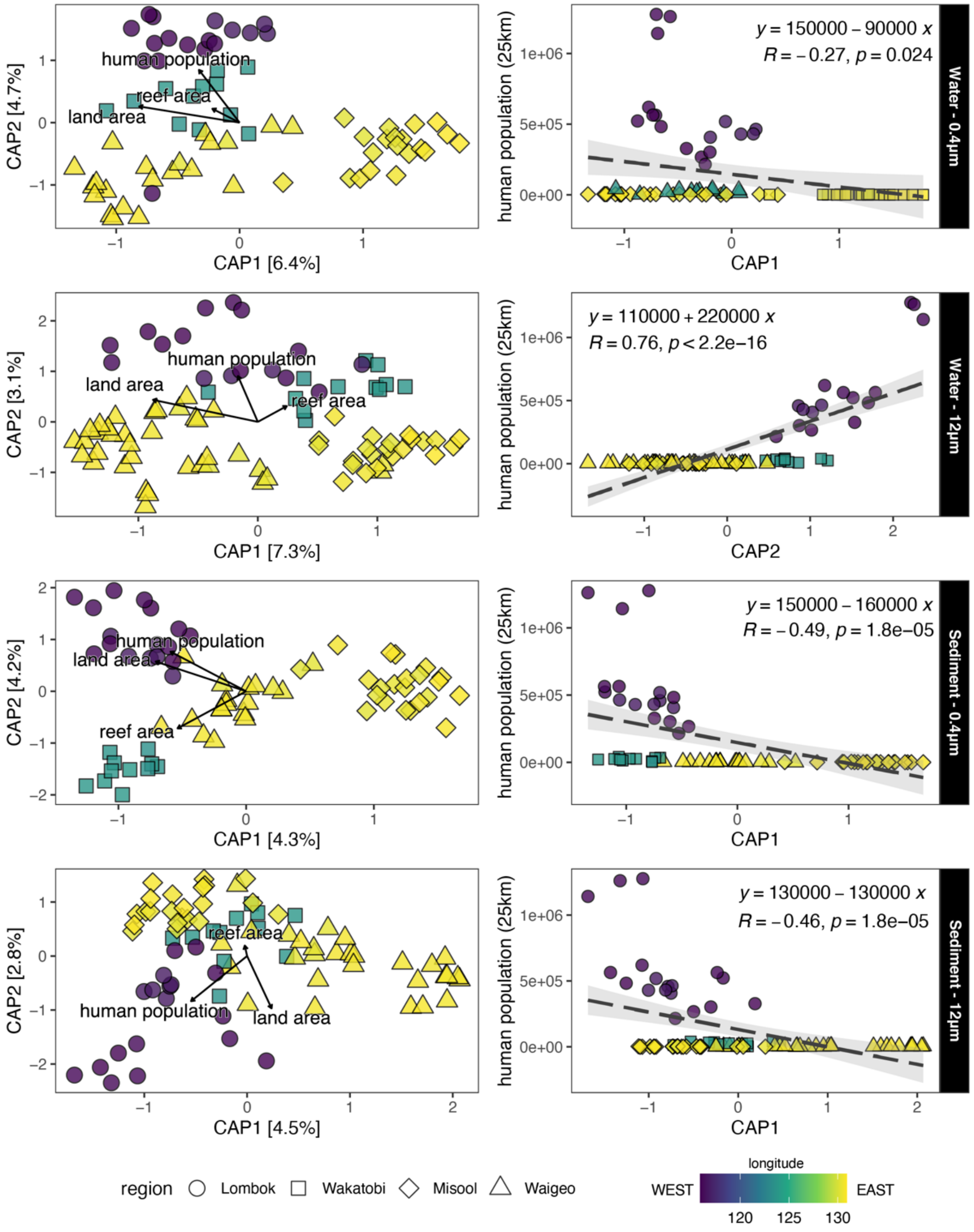


Figure 2. Canonical analysis of principal coordinates (CAP) 0.4µm samples run using socio-environmental variables as proxy for fishing pressure with regressions showing significant correlation between axes and human population and distance to market. Points colored by longitude to indicate geographic location.

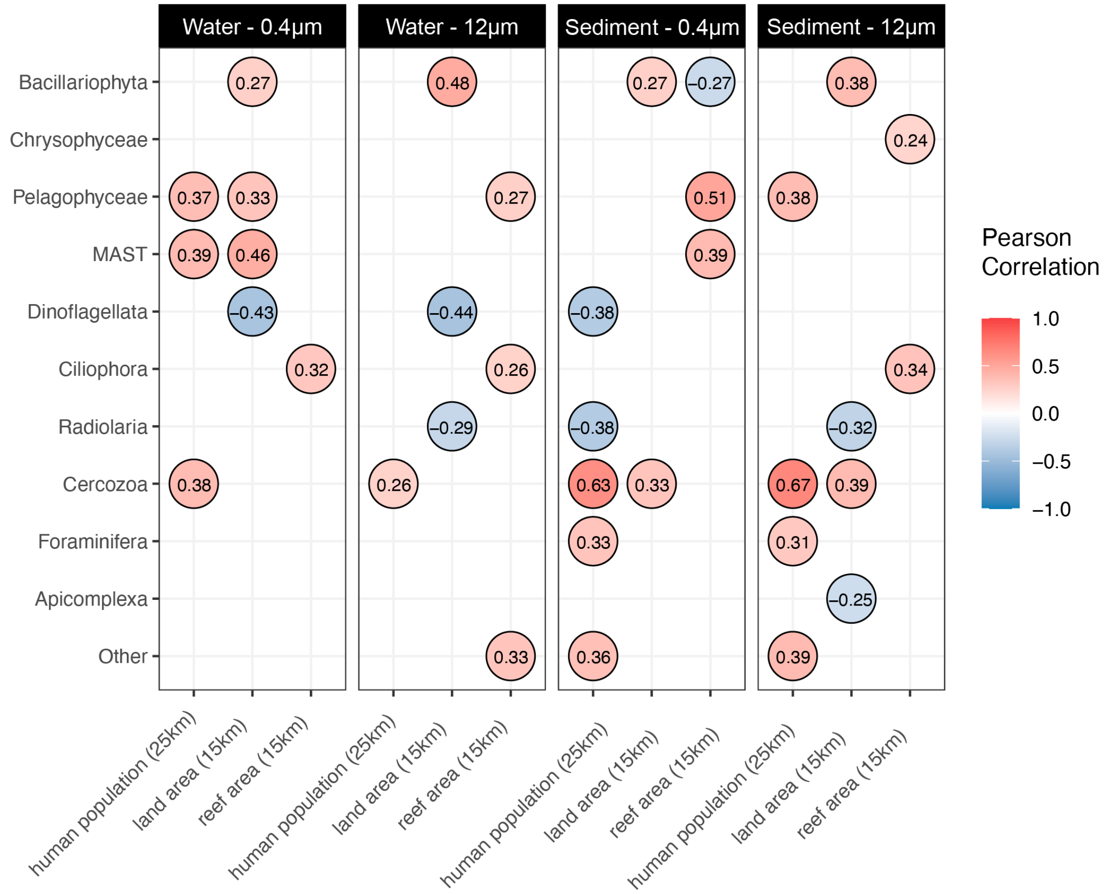


Figure 3. Heatmap showing significant Pearson correlations between relative abundance of the ten most abundant protist groups from the dataset and socio-environmental variables.

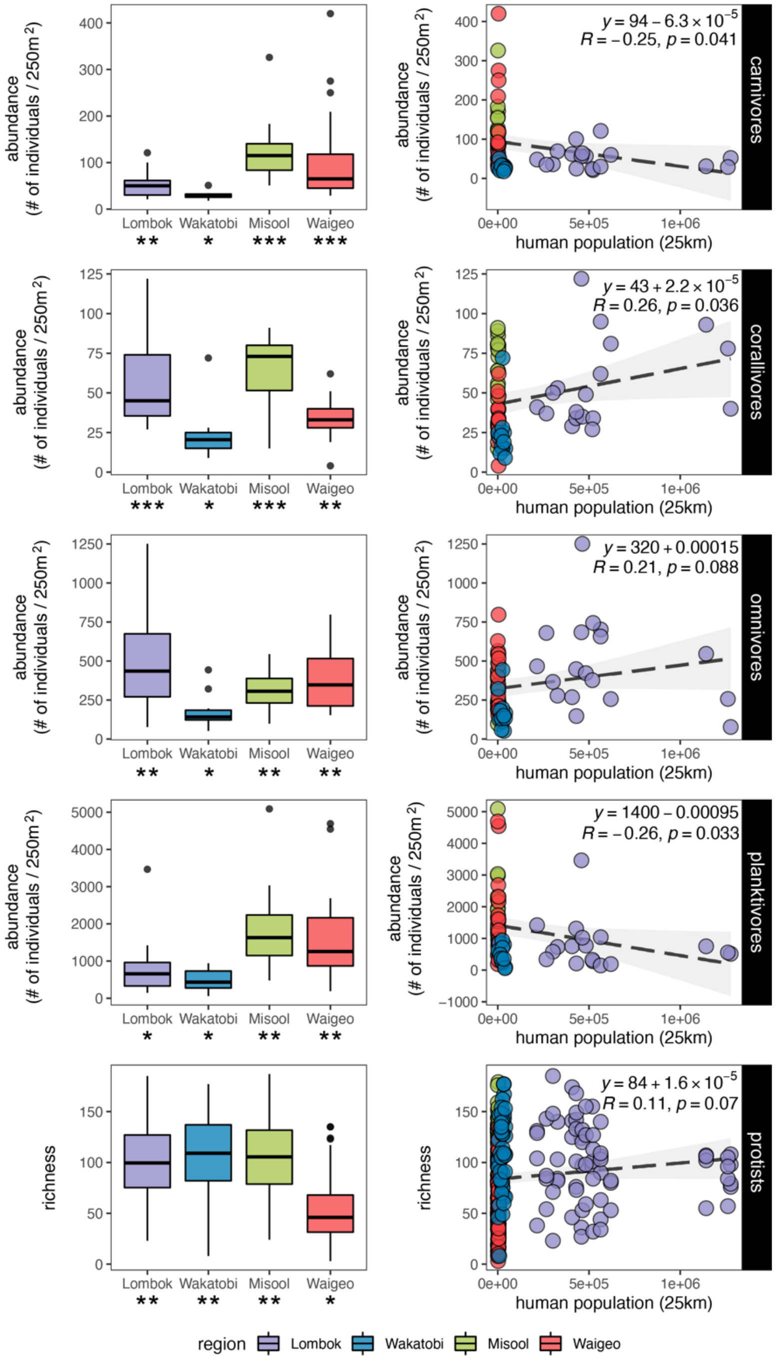


Figure 4. (left) Fish abundances (number of individuals per 250m^2) by trophic level and protist richness by region. Asterisks (*) below the region names indicate statistically similar groupings (Wilcoxon, $p < 0.05$). Full Wilcoxon pairwise statistics by region in Supplemental Table 1.

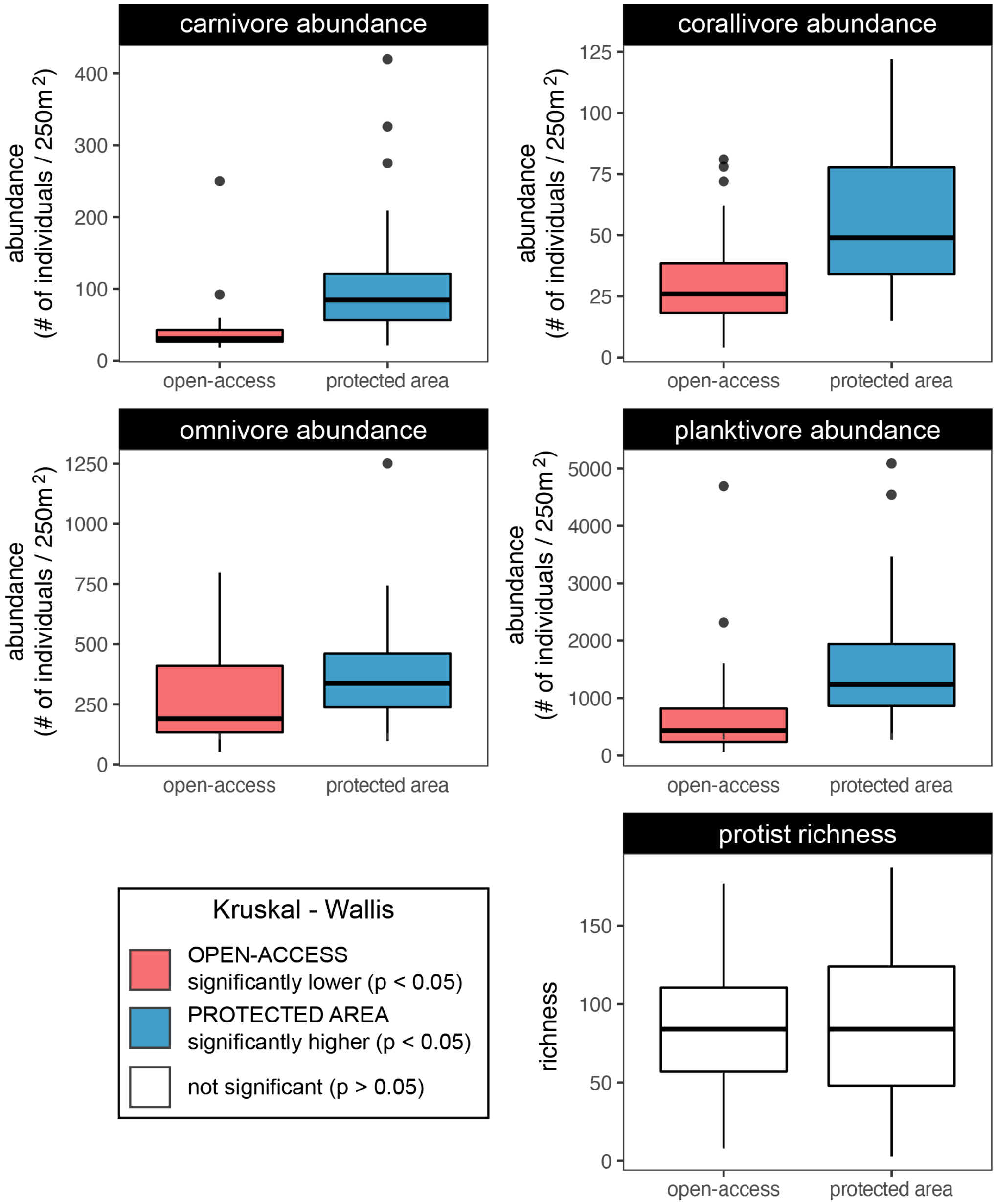


Figure 5. Fish trophic group abundances (number of individuals per 250m^2) and protist richness by protected area. Fish abundance was significantly higher in protected areas across most trophic groups (Kruskal-Wallis, $p < 0.05$). Protist diversity showed no significant changes across those same designations (Kruskal-Wallis, $p > 0.05$).

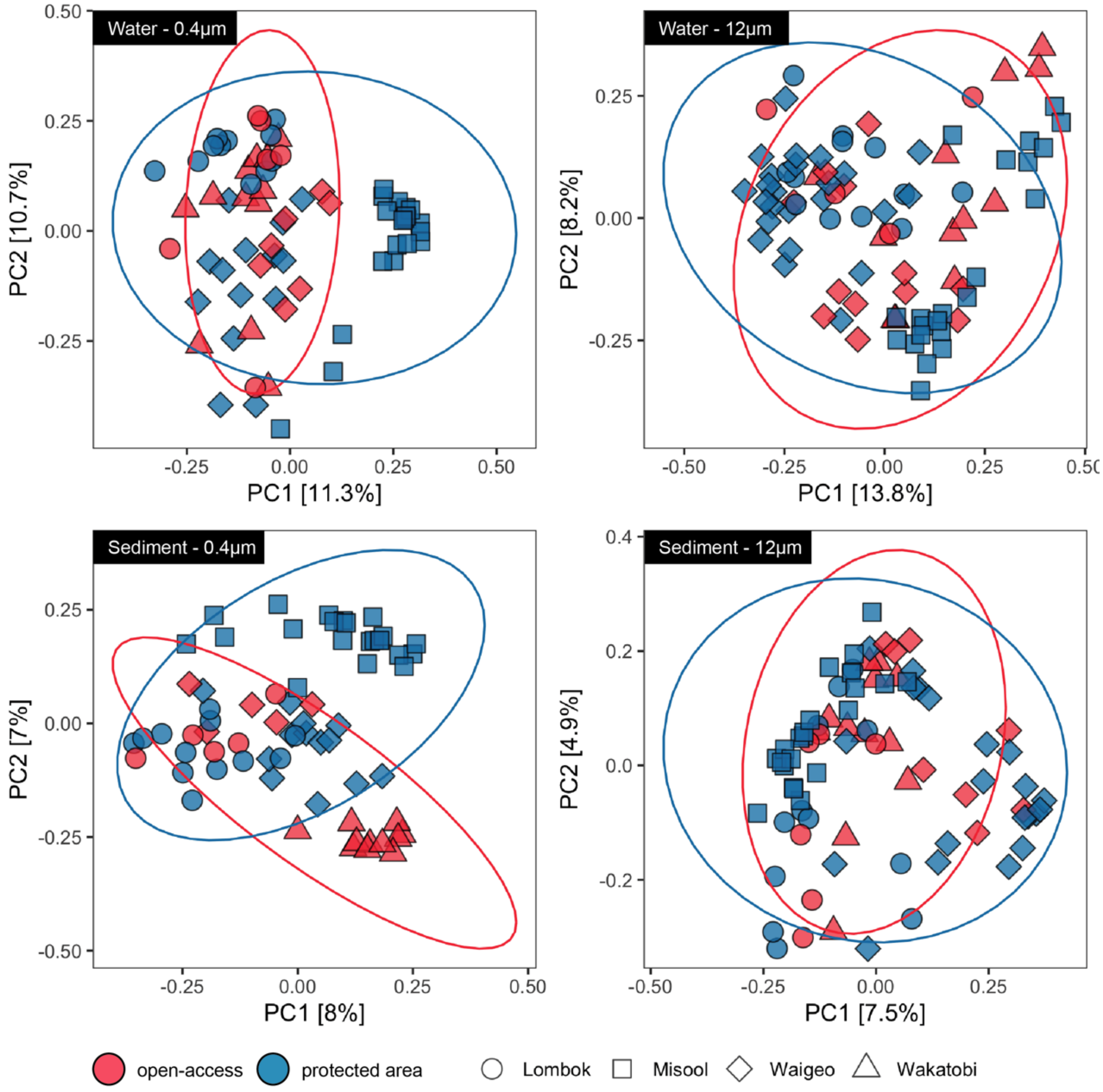


Figure 6. Principal coordinates analysis (PCoA) constructed using Bray-Curtis dissimilarity matrix, with points colored according to protection (red = open access; blue = marine protected area).

Supplemental Table 1. T-test results comparing UVC counts at each sampling site between different observers.

region	siteID	average1	average2	p-value
Misool	MIS_01	68.3333333	51	<0.001*
	MIS_02	72.6666667	49.3333333	<0.001*
	MIS_03	42.6666667	20	<0.001*
	MIS_04	84.6666667	52.3333333	<0.001*
	MIS_05	57	54.3333333	<0.001*
	MIS_06	65.3333333	50.3333333	<0.001*
	MIS_07	67.3333333	53.3333333	<0.001*
	MIS_08	75	51	<0.001*
	MIS_09	58.3333333	40.6666667	<0.001*
	MIS_10	58.6666667	53	0.001*
	MIS_11	61	52.3333333	<0.001*
	MIS_12	64	45.3333333	0.002*
	MIS_13	57	51.3333333	<0.001*
	MIS_14	75	51	<0.001*
	MIS_15	57.3333333	46.6666667	<0.001*
	MIS_16	50	37.3333333	<0.001*
	MIS_17	64.3333333	83.3333333	<0.001*
	MIS_19	75	51.6666667	<0.001*
	MIS_20	72	49.3333333	<0.001*
	MIS_21	81.3333333	51	<0.001*
Lombok	LBK_01	80	55	0.016*
	LBK_02	40.3333333	27	0.202
	LBK_03	72	31.6666667	0.002*
	LBK_04	107.333333	88.6666667	0.023*
	LBK_05	35.3333333	81	0.013*
	LBK_06	40.3333333	94	0.006*
	LBK_07	69	97.6666667	0.088
	LBK_08	21	59.3333333	0.068
	LBK_09	51.3333333	70.3333333	0.139
	LBK_10	92	93.3333333	0.922

	LBK_11	61.6666667	90.6666667	0.083
	LBK_12	57	94	0.023*
	LBK_13	71	76.33	0.781
	LBK_14	81	93.33	0.066
	LBK_15	54	86	0.076
	LBK_16	69	22	<0.001*
	LBK_17	82.67	24	0.008*
	LBK_18	79	31.33	0.004*
Waigeo	WGO_02	88.5	44.5	0.075
	WGO_04	76	62	0.410
	WGO_05	69	66.5	0.841
	WGO_06	81	80	0.939
	WGO_08	65.33	81	0.191
	WGO_10	68	83.5	0.126
	WGO_11	77	76.5	0.975
	WGO_12	73.33	78	0.735
	WGO_16	50	87	0.021*
	WGO_17	95	107.5	0.557
	WGO_18	63.67	82	0.010*
	WGO_19	81.33	102.5	0.012*

Supplemental Table 2. Wilcoxon pairwise statistics for regional comparisons of fish abundances by trophic group. Asterisks (*) indicate significant p-values ($\alpha = 0.05$)

	region 1	region 2	p-value
carnivore	Lombok	Wakatobi	0.010*
		Misool	<0.001*
		Waigeo	0.041*
	Wakatobi	Misool	<0.001*
		Waigeo	<0.001*
	Misool	Waigeo	0.054
benthic invertivore	Lombok	Wakatobi	0.019*
		Misool	0.012*
		Waigeo	0.028*
	Wakatobi	Misool	0.351
		Waigeo	0.003*
	Misool	Waigeo	<0.001*
corallivore	Lombok	Wakatobi	<0.001*
		Misool	0.176
		Waigeo	0.005*
	Wakatobi	Misool	<0.001*
		Waigeo	0.005*
	Misool	Waigeo	<0.001*
omnivore	Lombok	Wakatobi	0.002*
		Misool	0.050
		Waigeo	0.193
	Wakatobi	Misool	0.002*
		Waigeo	0.002*
	Misool	Waigeo	0.735

herbivore	Lombok	Wakatobi	0.005*
		Misool	0.800
		Waigeo	0.344
	Wakatobi	Misool	0.005*
		Waigeo	0.028*
	Misool	Waigeo	0.423
planktivore	Lombok	Wakatobi	0.294
		Misool	<0.001*
		Waigeo	0.009*
	Wakatobi	Misool	<0.001*
		Waigeo	<0.001*
	Misool	Waigeo	0.294
detritivore	Lombok	Wakatobi	0.006*
		Misool	0.008*
		Waigeo	<0.001*
	Wakatobi	Misool	0.139
		Waigeo	0.090
	Misool	Waigeo	0.007*

Supplemental Table 3. Kruskal-Wallis statistics comparing abundances of fish at each trophic level between protected (MPA) and unprotected (non-MPA) regions. Asterisks (*) indicate significant p-value (α = 0.05).

trophic group	p-value
carnivore	<0.001*
benthic invertivore	<0.001*
corallivore	<0.001*
omnivore	0.027*
herbivore	<0.001*
planktivore	<0.001*
detritivore	0.584

CHAPTER 4:

RUBBLE FIELDS SHAPE PLANKTONIC PROTIST COMMUNITIES IN INDONESIA ON A LOCAL GEOGRAPHIC SCALE

ABSTRACT

The Coral Triangle encompasses nearly 30% of the world's coral reefs and is widely considered the epicenter of marine biodiversity. Destructive fishing practices and natural disturbances common to this region like earthquakes, tsunamis, and storms can damage these reefs leaving behind fields of coral rubble. The damage to these ecosystems can have long-term ecological and economic impacts for countries like Indonesia that rely on these reefs. While the impacts of these disturbances are well documented on metazoans that live on the reefs, we have a poor understanding of how they impact microbial communities at the base of the food web. In particular, we have a poor understanding of how these disturbances impact protist communities which play important roles in biogeochemical cycling and other ocean processes. Our study shows that rubble coverage and net primary productivity (NPP) are the strongest explainers of variation in protist community composition across the island of Lombok in Indonesia. Rubble fields are characterized by an increase in small heterotrophic protists including ciliates and cercozoans. In addition to shifts in heterotrophic protist communities, we also observed increases in diatom relative abundance in sites with high rubble coverage, which also correspond to sites with higher NPP. These results are the first to characterize protist community shifts in tropical marine rubble fields and provide insight on what environmental factors may be driving these shifts on a local scale.

INTRODUCTION

The Coral Triangle encompasses nearly 30% of the world's coral reefs and is widely considered the epicenter of marine biodiversity (Allen, 2008; Bowen, Rocha, Toonen, & Karl, 2013; Burke, Reytar, Spalding, & Perry, 2012; Hoeksema, 2007; Veron et al., 2009). The coral reef ecosystems in this region are vital food and economic resources for countries across the Indo-Pacific (Cruz-Trinidad, Aliño, Geronimo, & Cabral, 2014; Foale et al., 2013). Indonesia, the largest country in this region, is home to 1.7 million coral reef fishers and nearly 60% of its total population lives in coastal areas (Siry, 2007; Teh, Teh, & Sumaila, 2013). While fisheries management strategies aim to protect these ecosystems and promote sustainable fishing practices (Gaines, White, Carr, & Palumbi, 2010), overfishing and destructive fishing practices like cyanide and blast fishing across the country can pose threats to coral reef ecosystems (Dulvy, Freckleton, & Polunin, 2004; Halim A., 2002; Langfelder & Horvath, 2008; Pet-Soede & Erdmann, 1998). These threats to reefs are of particular concern in southwestern Indonesia where human population and fishing pressure are highest.

Natural disturbances like earthquakes, storms, and tsunamis can also cause damage reefs in this region. While the damage caused by natural disturbances is often patchy across impacted reefs (Foster et al., 2006; Hagan, Foster, Perera, & Aji, 2007), these disturbances also contribute to formation of rubble fields and can exacerbate existing damage from destructive fishing practices (Campbell et al., 2007). The formation of rubble fields by these various disturbances leads to a sudden drop in diversity and dramatic shifts in food web dynamics on the impacted reefs (Edinger, Jompa, Limmon, Widjatmoko, & Risk, 1998; McManus, Nanola, Reyes, Kesner, & Mcmanus, 1997). The coral fragments that do manage to survive these disturbances often die within a month (Fox, Pet, Dahuri, & Caldwell, 2003).

Studies on recovery and response of organisms to these disturbances in the Indo-Pacific largely center around macroscopic organisms including hard coral, soft coral, and macroalgae (Connell, Hughes, & Wallace, 1997; Fox, Mous, Pet, Muljadi, & Caldwell, 2005; Fox et al., 2003; Sawall, Jompa, Litaay, Maddusila, & Richter, 2013; Williams et al., 2019). These studies have documented how unstable substrate left behind in rubble fields makes settlement of hard coral larvae difficult by essentially creating a “killing field” for coral recruits (Fox et al., 2003; Sawall et al., 2013). However, both models and experimental data suggest the extent of the damage on the reef is also an important factor in evaluating potential for recovery of these reefs (Fox & Caldwell, 2006; Saila, Kocic, & McManus, 1993).

Despite our understanding of the responses of macroorganisms in rubble fields, we lack data on microbial communities and their responses to such disturbances. Microorganisms play important roles in biogeochemical cycling across marine environments and also play important roles as symbionts to organisms in coral reef ecosystems (Ainsworth, Thurber, & Gates, 2010; Falkowski, Barber, & Smetacek, 1998; Glasl et al., 2019). Protists in particular play important roles as both primary producers and consumers in the microbial food web and engage in a wide spectrum of symbioses (Clerissi et al., 2018; Decelle, Colin, & Foster, 2015; Field, Behrenfeld, Randerson, & Falkowski, 1998). Understanding the impacts these disturbances have on protist communities and how protist communities respond to such dramatic environmental change is therefore important for understanding connectivity across different levels of the food web and for understanding how these disturbances might potentially impact nutrient cycling and other microbial processes in coral reef ecosystems.

Our study focuses on protist communities across the island of Lombok in Indonesia. These communities encompass phytoplankton, grazers, and symbionts, many of which play essential roles in coral reef ecosystems. We use metabarcoding data to evaluate protist community composition across sites of differing fisheries management schemes and benthic profiles, and attempt to demonstrate how rubble fields may impact protist community structure across the island. Our data show that rubble cover and net primary productivity (NPP) were the strongest explainers of protist community structure across Lombok. Rubble fields were characterized by increased relative abundance of small grazers including ciliates and cercozoans. Additionally, we show that sites with high rubble cover also have increased net primary productivity (NPP) and relative abundance of diatoms, which could potentially reflect shifting nutrient levels across the island, allowing diatoms to be more successful at those sites.

METHODS

Sample collection and preservation

Samples were collected from 18 sites around the island of Lombok, Indonesia, in July 2018 (Figure 1a). The sites group into three regions around the island (northeast, northwest, and southwest) and were chosen to span various fisheries management zones, including open access zones with no restrictions on fishing, gear-restricted zones where some fishing gear is restricted and no-take zones. At each site water was collected at 5m depth and surface sediment with water was collected at 10m in 4L containers by SCUBA. After collection, water and sediment samples were each filtered over 12µm followed by 0.4µm polycarbonate filters (Sterlitech) using a peristaltic pump until filters clogged. The entire 4L water sample was filtered, but the sediment samples often clogged after 1-2L were filtered. Following filtering, filters were cut in half and placed in 2mL cryovials with 1mL of DNA/RNA Shield (Zymo Research). The samples were then stored at room temperature until transported back to the lab where they were stored at 4°C.

In addition to water and sediment at each site, underwater visual census (UVC) data was collected on fish communities and benthic coverage. At each site a total of six 50m transects were surveyed by two observers (three transects each). For fish counts, the observers counted fish and estimated their size within 2.5m on either side of the transect for each of the transects. The fish were classified as close to species level as possible and were later organized into trophic and functional groups for data analysis. Benthic coverage data was estimated along each transect using the point-intercept method every 0.5m. The benthic categories were organized into, hard coral (classified to the genus level), soft coral, macroalgae, rubble, and other. To further supplement the UVC data and get more metadata for our sites, socio-environmental variables including mean net primary productivity (NPP), reef area, land area, and others were downloaded from the Marine Socio-Environmental Covariates dataset (Yeager, Marchand, Gill, Baum, & McPherson, 2017).

DNA extraction, PCR, and sequencing

DNA was extracted from each filter using the Zymo Biomics DNA Miniprep Kit following the manufacturer's protocol. Two sets of eukaryotic primers were used to amplify different regions of DNA using polymerase chain reaction (PCR) (Table 1). The primers used for V9 hypervariable region of 18S rDNA were used primarily to target protists and other microbial eukaryotic groups, while the cox1 primers were used to target metazoan groups. All PCRs were set up using Bioline MyTaq Red Mix following the volume and concentration recommendations from the manufacturer for both primers and DNA template. Successful amplification and amplicon size were confirmed using gel electrophoresis. Library prep was done by the University of Rhode Island Genomics and Sequencing Center and the amplicons were then sequenced on the Illumina MiSeq using paired end sequencing.

In addition to samples from the field, a mock community was prepared using DNA samples from cultures available in the lab as a control on the sequencing run. The cultures used spanned different Stramenopile lineages, including *Fragilariopsis cylindrus* (Bacillariophyta), *Apedinella radians* (Dictyochophyceae), *Phaeothamnion confervicola* (Phaeothamniophyceae), *Chrysosaccus* sp. (Chrysophyceae), and *Tribonema minus* (Xanthophyceae). The DNA from each culture was added in equal concentrations to a single tube, and the resulting DNA sample was amplified using the same primers and PCR methods described above. The amplicons were then sequenced on the Illumina MiSeq on the same run as the environmental samples.

Bioinformatics

Forward and reverse reads were initially quality assessed in FastQC (Andrews, 2010). Primer sequences were trimmed from the paired reads using Cutadapt (v1.9.1) (Martin, 2011). After trimming, the reads were imported into QIIME2 (v2020.6) and were filtered, denoised, merged, and chimera checked using the DADA2 plugin in QIIME2 (Bolyen et al., 2019; Callahan et al., 2013). Truncation length for denoising was chosen to minimize the number of low-quality bases at the end of the reads while maximizing the amount of overlap between the forward and reverse reads to optimize merging.

The amplicon sequence variants (ASVs) resulting from denoising were then clustered into OTUs at 97% similarity using the vsearch plugin in QIIME2 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) taxonomically assigned using a Naïve-Bayes classifier in QIIME2. The V9 reads were classified using the Protist Ribosomal Reference Database (PR^2) (Guillou et al., 2013) and the cox1 reads were classified using the MIDORI database (Leray, Ho, Lin, & Machida, 2018). Once classified, any ASV that had a classification of lower than 95% confidence at any level was filtered out to remove low quality and low abundance ASVs from the dataset to help reduce noise in downstream analyses. Finally, to obtain the protist community data for further analysis, the resulting ASV table was filtered to only include the sequences classifying to Stramenopiles, Alveolates, and Rhizaria. This filtering step allows us to eliminate undefined eukaryotic reads and reads classifying to metazoans, but also allows us to filter out other low abundance eukaryotic groups to reduce noise in downstream analyses. Furthermore, the Stramenopiles, Alveolates, and Rhizaria, encompass some of the most diverse and abundant protist lineages, and have been shown to be the dominant protists in ocean sampling across the globe (de Vargas et al., 2015).

Data analysis and statistics

Alpha and Beta diversity statistics were calculated in R using the *vegan* package. Richness was used as the alpha diversity metric to avoid potential 18S copy number bias in the V9 dataset across taxonomic groups. Kruskal-Wallis and Wilcoxon-pairwise tests were used to test differences across the three geographic groupings of sites (NE, NW, and SW).

Canonical Analysis of Principal coordinates (CAP) was used to evaluate potential drivers of protist community composition across Lombok (Anderson & Willis, 2003). The analysis and ordinations were done using the *phyloseq* package in R (McMurdie & Holmes, 2013). The environmental variables used in the analyses were a combination of UVC data from each site including benthic coverage percentages and fish abundances across different trophic groups, as well as socio-environmental data from the MSEC dataset. Sequence data was also used from the cox1 and V9 datasets we generated to fill in gaps in the food web and better understand potential biotic drivers at play. To do this, copepod data was filtered from the V9 dataset and invertebrate data including bivalves, anthozoans, and sponges were filtered from the cox1 datasets for each site.

Co-occurrence networks were constructed using the WGCNA package in R (Langfelder & Horvath, 2008). A network was constructed encompassing all sites and then separate subsequent networks were constructed for the NE, NW, and SW sites in order to observe how co-occurrence patterns shifted across the island (Figure 5). The edges for each network were used to construct circos plots showing which taxonomic groups most closely co-occurred with one another (Figure 6). The thickness of the links between different taxa and the thickness of the outer bands on the plots are proportional to the number and weights of the edges in that network (the thicker the links, the more co-occurrence between those groups). The colors of the different groups were also chosen to indicate ecological roles. Blues are indicative of heterotrophs, reds indicative of parasites, and greens indicative of autotrophs.

RESULTS

Sequencing and filtering results

The V9 sequencing for these sites was part of a larger dataset encompassing 293 samples and greater than 18 million total reads with an average of 63,668 reads per sample. After quality filtering, denoising, and merging we retained on average 84% of reads. Additional filtering following taxonomic assignment eliminated 16% of the remaining reads, which were largely undefined, low abundance ASVs. The remaining sequences consisted of 48,656 ASVs, which classified to 1,387 taxa. The final filtering step isolated protist sequences, retaining only those classifying to Stramenopiles, Alveolates, and Rhizaria. This step eliminated 65% of the remaining data, resulted in 12,085 ASVs that classified to 839 taxa. The 65% of reads that were filtered out were primarily undefined eukaryotes (28%) and metazoans (24%), and the remaining eukaryotic groups including Fungi, Archaeplastida, Hacrobia, Excavata, Amoebozoa, and Apusozoa each made up 0-5% of the total reads.

The cox1 sequencing data for the Lombok sites was also part of a larger sequencing run encompassing 166 samples with an average of 50,049 sequences per sample. After quality filtering, denoising, and merging, we retained 63% of our total reads. The resulting dataset consisted of 40,772 ASVs that classified to 639 taxa. Sequences classifying to filter-feeders were filtered out to be used as metadata variables in later analysis. These sequences only made up on average 5% of reads per sample.

Mock community

After taxonomic assignment of the sequences in our mock community, 95% of reads in from the sample were assigned to Stramenopiles. Most of the remaining 5% of reads were classified as undefined Eukaryotes, while the remaining fraction of a percent came from various Opisthokonts and protist groups.

Within the Stramenopiles, we saw all of the groups that were added to the mock community in varying proportions. The majority of reads came back classified to Bacillariophyta (86.66%), followed distantly by Dictyochophyceae (3.62%), Chrysophyceae (2.38%), Phaeothamnion (2.05%), and Xanthophyceae (0.26%) (supplemental fig). The species identified within each of these groups were consistent with the species we expected to find with the exception of a large percentage of undefined Bacillariophyta (85.30%) and the Xanthophyte, which classified to the correct genus but a different species than what we expected (*Tribonema* *ulotrichoides*). The misidentification at the species level for the Xanthophyte is likely a result of limited Xanthophyte species available in the database. On the other hand the difference in the proportions of reads with the diatoms despite starting with approximately equal concentrations of DNA, could be a result of variation in 18S copy number across different taxa, primer bias, or a combination of both (Medinger et al., 2010; Zhu, Massana, Not, Marie, & Vaulot, 2005). Another explanation for this discrepancy in proportions could be contamination of the original *Fragilariopsis* culture with other diatom species that the DNA was then extracted from.

UVC site data

The three collection site regions show distinct benthic profiles from one another (Figure 1b). While coral cover does not statistically differ by these regions (Kruskal-Wallis, $p > 0.05$), rubble percent and available substrate do. Rubble is significantly higher in the NW and SW sites than it is in the NE sites (Wilcoxon pairwise, $p < 0.05$). Coral cover shows the opposite trend, where it is significantly higher in the NE compared to the NW (Wilcoxon, $p < 0.05$). The SW was not significantly different from either the NE or NW coral cover, but that is likely due to a single site (10), which had much higher coral cover than the other sites in that region. When site 10 is excluded from the analysis, NW and SW sites have significantly higher rubble percent than NE sites. benthic profiles correlated with changes in fish abundance per site, with rubble percent, in particular, showing a significant negative correlation with fish abundance per site ($R = -0.58$, $p = 0.016$).

Protist community composition

Protist community composition appears to vary by geography across Lombok with significant differences in community composition between North and South Lombok across all samples and further separation between northwest and northeast sites in the 0.4μm water samples and 12μm sediment samples (ANOSIM, $p < 0.05$). In particular, the diatoms (Bacillariophyta), ciliates (Ciliophora), and cercozoans (Cercozoa), appear to increase in relative abundance in the southwest and certain northwest sites (Figure 2). Despite these shifts in relative abundance and Beta diversity, Alpha diversity remains steady in all protist groups across Lombok (Wilcoxon pairwise, $p > 0.05$) (Figure 3).

Canonical analysis of principal coordinates (CAP)

CAP ordinations constructed using Bray-Curtis dissimilarity provide insight into potential drivers of these community shifts (Figure 4). The percent variation explained in each of the ordinations reveals that these variables explain a higher amount of variation in the water samples than in the sediment samples. Furthermore, the percent explained by the CAP axes in the 0.4μm water samples is 10.4% higher than the percent explained on the CAP axes for the 12μm water samples, suggesting that smaller planktonic protist communities are more strongly impacted by the variables used in these models. The sediment ordinations on the other hand only explained 23.9% and 27.2% of variation in the 0.4μm and 12μm samples respectively. This drop in percent explanation between water in sediment samples suggests that benthic communities were not as strongly impacted by the variables used in these models as planktonic communities. The difference could also be reflective of the community differences between water and sediment samples. The sediment samples have a much larger proportion of low abundance taxa that individually contributed to less than 1% of each sample but collectively contributed to on average 10-20% more of the reads in sediment samples than they did in water samples at the same sites.

Across all of the CAP ordinations, the two variables with the strongest correlation to the first CAP axis, and therefore explaining the most variation, are mean NPP and rubble percent (Figure 4). Other variables that explain significant correlation along the first CAP axis include copepod relative abundance (V9 data), herbivore and corallivore abundance (UVC data), as well as sponge and anthozoan relative abundance (COI data).

Co-occurrence networks

Given the significance of copepod relative abundance in explaining variation among certain samples in the CAP ordinations, the V9 dataset was re-filtered to include both protist and copepod ASVs, in order to evaluate co-occurrence among copepods and various protist groups. The network constructed using all of the collection sites had a total of 88 edges, which is considerably less than the networks constructed for each subset of sites. The networks for the NE, NW, and SW sites had 311, 461, and 280 edges respectively, suggesting high spatial structuring of communities across the island. The networks for each individual grouping of sites have distinct patterns of co-occurrence among taxonomic groups further supporting the high spatial structuring of these communities. More specifically, there are a higher proportion of edges involving heterotrophic groups in the SW and the NW where rubble percent is higher (Figure 5). This shift is most notable in the ciliates, which have low co-occurrence in NE sites (111 edges, 36% of network edges), higher co-occurrence in SW sites (129 edges, 46% of network edges), and highest co-occurrence in NW sites (223 edges, 48% of network edges) (Figure 5a). Cercozoans also show higher number of edges involving other heterotrophic groups in the NW and SW sites compared to NE sites (Figure 5b).

Co-occurrence between the ciliates and cercozoans alone also showed a similar pattern, with the greatest number of edges between these groups occurring in the SW sites (23 edges), followed closely by the NW sites (19 edges), and the least number of edges in the NE sites (7 edges). That taxa within the ciliates and cercozoans that co-occurred in each of these site groupings also varied from most edges occurring between bacterivorous species and species that feed on small phytoplankton in the NW and SW to various functional groups in the NE including parasites, bacterivores, and other heterotrophic groups. While co-occurrence does not necessarily indicate ecological interactions, it may suggest that their distributions on a local scale are driven by similar environmental variables, which in this case appears to be rubble cover.

DISCUSSION

Variation in protist community composition across Lombok was best explained by rubble percent and mean NPP per site (Figure 4). While other variables, like anthozoa and sponge percent contributed to the percent variation explained on the

CAP axes, rubble cover and mean NPP were the only variables explaining significant variation in 0.4µm and 12µm size fractions in both water and sediment samples. The sites with higher rubble percent and mean NPP were characterized by increased relative abundance in ciliates (Ciliophora), cercozoans (Cercozoa), and diatoms (Bacillariophyta) (Figure 2). Despite these shifts in relative abundance, the alpha diversity in all protist groups showed no significant change across all site groupings (Figure 3). The lack of change in alpha diversity suggests that the observed shifts in relative abundance were likely driven by certain species taking advantage of environmental differences across sites, rather than new species coming into certain sites and establishing themselves. The correlation between these shifts in community composition and rubble coverage suggest that disturbances resulting in rubble fields, and the subsequent changes in food web dynamics, likely play a significant role in structuring protist communities across Lombok. In addition to rubble percent, the increase in diatom relative abundance at sites with high rubble cover, and significance of NPP in explaining community variation, also suggests that shifting nutrient levels, in connection to both rubble fields and human activity across the island, may also play a role in structuring these communities.

Water vs. sediment communities

Given the wide range of ecological roles protists serve in marine environments, water and surface sediment protist communities have distinct compositions, despite their proximity to one another. While community composition in our water samples remains fairly consistent within groupings of sampling sites, the sediment samples show much more variation across all sites. Our sediment samples show higher relative abundance of parasitic groups, like apicomplexans, compared to water samples, which is consistent with sediment communities sampled across other parts of the globe, including Antarctic marine sediments (Cleary & Durbin, 2016) and neotropical soils (Mahé et al., 2017). However, while apicomplexans are the dominant parasitic group in Antarctic marine sediments and neotropical soils, the dominant parasitic group in our dataset is the Syndiniales dinoflagellates. Our data also show on average 10-20% more undefined reads (encompassed in "Other" in Figure 2) in sediment samples compared to water samples. This high level of unknown diversity in marine sediments is consistent with other metabarcoding surveys across the globe (Forster et al., 2016; Massana et al., 2015; Santoferrara et al., 2020).

While sediment samples appeared to have more variation in community composition from site to site than water samples, the variables used to construct the CAP ordinations only explained a minimal amount the variation across sediment samples. More specifically, the CAP ordinations for sediment explained half as much variation in the 0.4μm samples and a tenth of the variation in the 12μm samples compared to water samples in those same size fractions (Figure 4). The lack of variation explained in the sediment ordinations suggests that disturbances resulting in rubble fields, and the subsequent effects on the pelagic food web, have a minimal impact on benthic protist communities. These ordinations also suggest that we are missing other environmental variables that play more important roles in structuring benthic protist communities. The variability in benthic communities and low percent explanation of the CAP axes could also be reflective of variations in life history across protists. Benthic environments often act as a seed for planktonic diversity as planktonic protists transition between benthic and pelagic life stages (Massana et al., 2015). Populations of planktonic species that have benthic cyst stages are also hard to predict, with some lasting days, while others can last years, and some occur on seasonal cycles (Satta et al., 2010). This variability in benthic stages makes these organisms difficult to study and further complicates understanding variables driving benthic protist community structure.

Response of heterotrophic protists in rubble fields

Despite current knowledge of the importance of both free-living and symbiotic microbial communities on coral reefs, our understanding of how these communities respond to disturbances largely comes from studies on bacterial communities and their roles and responses to coral disease and bleaching events (D. G. Bourne et al., 2009; D. Bourne, Iida, Uthicke, & Smith-Keune, 2008; Mao-Jones, Ritchie, Jones, & Ellner, 2010). However, protists play important roles as both producers and consumers in cycling nutrients in these ecosystems and also play a role in controlling bacterial growth on reefs (Silveira et al., 2017). Additionally, given the ability of microbial organisms to respond rapidly to changing environmental conditions (Glasl et al., 2019), protists could provide important insights on how these disturbances impact biogeochemical cycling and microbial food web dynamics in these ecosystems on a local scale.

Ciliates and cercozoans both play important roles as top-down controls on microbial communities across marine environments by consuming bacteria and other small protists (Flues, Bass, & Bonkowski, 2017; Glücksman, Bell, Griffiths, & Bass, 2010; Gonzalez, Sherr, & Sherr, 1990; Hall, Barrett, & James, 1993). While there have been no studies on these groups and their response in rubble fields, there have been studies on the associations between ciliates and some of the most widespread coral diseases (Katz, Pollock, Bourne, & Willis, 2014; M. Sweet & Bythell, 2012; M. J. Sweet, Croquer, & Bythell, 2014). While some of the ciliates associated with these diseases have been identified as bacterivores, others have been identified as ciliatovores and are likely feeding on the ciliates drawn to the skeletons by bacteria (M. J. Sweet & Séré, 2016). A similar dynamic could be happening in rubble fields. Ciliates are initially drawn to bacteria breaking down organic material in the rubble field, which triggers a chain reaction attracting more ciliates, and other small grazers, to feed on them. The increase in co-occurrence among small heterotrophic protists in sites with high rubble cover further suggests that the spatial structure in these groups is likely driven by the same environmental variables (Figure 5). In the case of the NW and SW sites, those variables appear to be rubble percent and mean NPP, as indicated in the CAP ordinations. However, it is important to note, the high co-occurrence among these heterotrophic groups at those sites does not necessarily indicate that they are directly interacting with one another.

In addition to highlighting potential environmental drivers of community composition shifts, the CAP ordinations also revealed differential impact of those variables across size fractions. In water samples, CAP axes explained 47.8% of the variation in 0.4μm samples and only 37.4% of variation in the 12μm samples (Figure 4). This difference in percent explanation between size fractions suggests that rubble percent and the other variables used in the analysis had stronger effects on smaller protists like ciliates and cercozoans than they did on larger protists like diatoms.

Phytoplankton dynamics in rubble fields

The two dominant phytoplankton groups in our samples were the dinoflagellates (Dinophyceae) and the diatoms (Bacillariophyta), with the dinoflagellates dominating the 0.4µm samples and the diatoms dominating the 12µm samples. The decrease in percent explanation on the CAP axes from the 0.4µm to the 12µm samples suggests that the variables used to construct the ordinations likely did not have as strong of an impact on the larger size fraction protists like diatoms (Figure 4). This drop in percent explanation could suggest that the variables used have an indirect, and therefore weaker impact, on these communities. It could also suggest that other variables we are unable to account for better explain the increase in diatom relative abundance we see in NW and SW sites.

Both diatoms and dinoflagellates have been studied extensively across the globe, with dinoflagellates typically most successful in oligotrophic regions and diatoms more successful in nutrient-rich regions (Edwards, Thomas, Klausmeier, & Litchman, 2015; Litchman, Klausmeier, Schofield, & Falkowski, 2007). On a broad scale, the biogeography of these groups appears driven by abiotic factors like environmental selection and ocean circulation patterns, but biotic factors like grazing can also play an important role in structuring these communities on a local scale (Malviya et al., 2016; Richter et al., 2020; Sommeria-Klein, Watteaux, Iudicone, Bowler, & Morlon, 2020; Sunagawa et al., 2015). However, given the size difference between most diatoms and dinoflagellates, these groups face different pressures when it comes to grazing. Diatoms, for example, are the primary food source for copepods (Jagadeesan, Jyothibabu, Arunpandi, & Parthasarathi, 2017; Liu, Chen, Zhu, &

Harrison, 2016), while dinoflagellates face grazing pressure from smaller plankton like ciliates (Pierce & Turner, 1992).

The increase in small grazers, including ciliates and cercozoans, could create additional top-down pressure on dinoflagellate communities. In addition to the increased grazing pressure on smaller phytoplankton at these sites, there is also lower abundance of larger grazers like planktivorous fish. This may, in turn, remove some grazing pressure on larger phytoplankton like diatoms, allowing for them to be more successful at those sites (Figure 6). The increased relative abundance in diatoms could also help explain why copepod relative abundance also appears connected to rubble percent and mean NPP in the CAP ordinations (Figure 4). Both diatoms and ciliates are important components of copepod diets (Calbet & Saiz, 2005; Jagadeesan et al., 2017), and the increased relative abundance of both of these groups, combined with decrease in abundance of higher trophic level predators at sites with high rubble percent, could allow for copepods to be more successful there. While grazing pressure and competition may play a role in structuring phytoplankton communities across Lombok, it is also likely that shifts in nutrient levels across the island contribute to the increase diatom relative abundance at sites in the NW and SW.

Although dinoflagellates are typically more successful competitors in oligotrophic environments, diatoms can often respond quickly to increased nutrient levels in oligotrophic coastal ecosystems (Kafouris et al., 2019). In Indonesia, the most common example of an influx of nutrients into coastal waters comes during monsoon season where there is increased runoff in the western portion of the country (Lee, Fournier, Gordon, & Sprintall, 2019). However, our sampling took place during the dry season and therefore runoff is unlikely a major source of nutrients across Lombok for our samples. The differential success of diatoms across Lombok also suggests other potential sources of nutrients that could allow for their increased success in the NW and SW sites as opposed to in the NE sites.

One explanation for diatom success in the NW and SW sites is the high rubble coverage at those sites. The absence of corals and other metazoans at these sites could potentially free up nutrients allowing for diatoms to be more successful. However, sewage effluent could also play a role in supplying nutrients to certain areas across Lombok. According to a 2017 report on development and tourism across Lombok, only 58.49% of households in Lombok Utara (encompassing NW sites), 52.51% of households in Lombok Barat (encompassing SW sites), and 55.46% of households in Lombok Timur (encompassing NE sites) had access to proper wastewater and sanitation (Horvath HTL, 2017). Furthermore, Lombok Utara (NW) and Lombok Barat (SW) were both characterized as high flood risk (Horvath HTL, 2017). The lack of access to proper wastewater treatments combined with increased flood risks in these regions could provide nutrients to these areas that allow for diatoms to be more successful in the NW and SW as opposed to the NE. Another source of nutrients to the western site along Lombok could also be upwelling along the Lombok Strait, which is a passage on the western side of Lombok that the Indonesian throughflow. Upwelling along the western coast, could also provide increased nutrients to the NW and SW sites allowing for diatoms to be more successful there as opposed to the NE sites.

Characterizing protist community response to varying environmental conditions and disturbances on a small geographic scale is important for understanding how those disturbances potentially impact the microbial food web, and in turn, biogeochemical cycling in the ecosystem. Our study shows that sites with high rubble coverage are characterized by increased relative abundance of small grazers, including ciliates and cercozoans. In addition to small grazers, these sites were also characterized by increased relative abundance of diatoms indicating a likely influx of nutrients at sites where rubble cover was highest. These results provide insight on how protist communities on coral reefs respond to sudden and dramatic ecosystem disturbances and also provide insight on what environmental factors appear most important in shaping these communities on a local level.

REFERENCES

Ainsworth, T. D., Thurber, R. V., & Gates, R. D. (2010). The future of coral reefs: a microbial perspective. *Trends in Ecology and Evolution*, *25*(4), 233–240. https://doi.org/10.1016/j.tree.2009.11.001

Allen, G. R. (2008). Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*, 541–556. https://doi.org/10.1002/aqc

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). *A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes*. *4*(7), 1–9. https://doi.org/10.1371/journal.pone.0006372

Anderson, M. J., & Willis, T. J. (2003). Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. *Ecology*, *84*(2), 511–525. https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2

Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data*.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … 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

Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W., & Harvell, C.

D. (2009). Microbial disease and the coral holobiont. *Trends in Microbiology*, *17*(12), 554–562. https://doi.org/10.1016/j.tim.2009.09.004

Bourne, D., Iida, Y., Uthicke, S., & Smith-Keune, C. (2008). Changes in coral-associated microbial communities during a bleaching event. *ISME Journal*, *2*(4), 350–363. https://doi.org/10.1038/ismej.2007.112

Bowen, B. W., Rocha, L. A., Toonen, R. J., & Karl, S. A. (2013). The origins of tropical marine biodiversity. *Trends in Ecology and Evolution*, *28*(6), 359–366. https://doi.org/10.1016/j.tree.2013.01.018

Burke, L., Reytar, K., Spalding, M., & Perry, A. (2012). Reefs at Risk Revisited in the Coral Triangle. In *Defenders* (Vol. 74). Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3150666&tool=pmcentrez&rendertype=abstract

Calbet, A., & Saiz, E. (2005). The ciliate-copepod link in marine ecosystems. *Aquatic Microbial Ecology*, *38*(2), 157–167. Retrieved from http://www.researchgate.net/publication/230660217_The_ciliate-copepod_link_in_marine_ecosystems/file/5046351ac501e1b0be.pdf%0Ahttp://www.int-res.com/articles/ame2004/38/a038p157.pdf%0Apapers2://publication/uuid/A856D2A0-BE63-453B-9487-C56B6B5C3E2A

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2013). DADA2: High resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583.

https://doi.org/10.1038/nmeth.3869.DADA2

Campbell, S. J., Pratchett, M. S., Anggoro, A. W., Ardiwijaya, R. L., Fadli, N., Herdiana, Y., … Baird, A. H. (2007). Disturbance to coral reefs in Aceh, Northern Sumatra: Impacts of the Sumatra-Andaman tsunami and pre-tsunami degradation. *Atoll Research Bulletin*, (544), 55–78.

Cleary, A. C., & Durbin, E. G. (2016). Unexpected prevalence of parasite 18S rDNA sequences in winter among Antarctic marine protists. *Journal of Plankton Research*, *38*(3), 401–417. https://doi.org/10.1093/plankt/fbw005

Clerissi, C., Brunet, S., Vidal-Dupiol, J., Adjeroud, M., Lepage, P., Guillou, L., … Toulza, E. (2018). Protists within corals: The hidden diversity. *Frontiers in Microbiology*, *9*(AUG), 1–13. https://doi.org/10.3389/fmicb.2018.02043

Connell, J. H., Hughes, T. P., & Wallace, C. C. (1997). A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. *Ecological Monographs*, *67*(4), 461–488. https://doi.org/10.1890/0012-9615(1997)067[0461:AYSOCA]2.0.CO;2

Cruz-Trinidad, A., Aliño, P. M., Geronimo, R. C., & Cabral, R. B. (2014). Linking Food Security with Coral Reefs and Fisheries in the Coral Triangle. *Coastal Management*, *42*(2), 160–182. https://doi.org/10.1080/08920753.2014.877761

de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., … Karsenti, E. (2015). Eukaryotic plankton diversity in the sunlit ocean. *Science*, *348*(6237), 1261605-1/11. https://doi.org/10.1007/s13398-014-0173-7.2

Decelle, J., Colin, S., & Foster, R. A. (2015). Photosymbiosis in Marine Planktonic Protists. In *Marine Protists: Diversity and Dynamics* (pp. 465–500). https://doi.org/10.1007/978-4-431-55130-0

Dulvy, N. K., Freckleton, R. P., & Polunin, N. V. C. (2004). Coral reef cascades and the indirect effects of predator removal by exploitation. *Ecology Letters*, *7*(5), 410–416. https://doi.org/10.1111/j.1461-0248.2004.00593.x

Edinger, Jompa, J., Limmon, G., Widjatmoko, W., & Risk, M. (1998). Reef degradation and coral biodiversity in indonesia: Effects of land-based pollution, destructive …. *Marine Pollution Bulletin*, *36*(8), 617–630. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0025326X98000472%5Cnpapers3://publication/uuid/F990175E-78F1-4674-979C-0ED7C4DE7A10

Edwards, K. F., Thomas, M. K., Klausmeier, C. A., & Litchman, E. (2015). Light and growth in marine phytoplankton: Allometric, taxonomic, and environmental variation. *Limnology and Oceanography*, *60*(2), 540–552. https://doi.org/10.1002/lno.10033

Falkowski, P. G., Barber, R. T., & Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science*, *281*(5374), 200–206. https://doi.org/10.1126/science.281.5374.200

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, *281*(5374), 237–240. https://doi.org/10.1126/science.281.5374.237

Flues, S., Bass, D., & Bonkowski, M. (2017). Grazing of leaf-associated Cercomonads

(Protists: Rhizaria: Cercozoa) structures bacterial community composition and function. *Environmental Microbiology*, *19*(8), 3297–3309. https://doi.org/10.1111/1462-2920.13824

Foale, S., Adhuri, D., Aliño, P., Allison, E. H., Andrew, N., Cohen, P., … Weeratunge, N. (2013). Food security and the Coral Triangle Initiative. *Marine Policy*, *38*, 174–183. https://doi.org/10.1016/j.marpol.2012.05.033

Forster, D., Dunthorn, M., Mahé, F., Dolan, J. R., Audic, S., Bass, D., … Stoeck, T. (2016). Benthic protists: The under-charted majority. *FEMS Microbiology Ecology*, *92*(8), 1–11. https://doi.org/10.1093/femsec/fiw120

Foster, R., Hagan, A., Perera, N., Gunawan, C. A., Silaban, I., Yaha, Y., … Hodgson, G. (2006). Tsunami and earthquake damage to coral reefs of Aceh, Indonesia. In *Reef Check Foundation, Pacific Palisades, California, USA* (Vol. 33).

Fox, H. E., & Caldwell, R. L. (2006). Recovery from blast fishing on coral reefs: A tale of two scales. *Ecological Applications*, *16*(5), 1631–1635.

Fox, H. E., Mous, P. J., Pet, J. S., Muljadi, A. H., & Caldwell, R. L. (2005). Experimental assessment of coral reef rehabilitation following blast fishing. *Conservation Biology*, *19*(1), 98–107. https://doi.org/10.1111/j.1523-1739.2005.00261.x

Fox, H. E., Pet, J. S., Dahuri, R., & Caldwell, R. L. (2003). Recovery in rubble fields: Long-term impacts of blast fishing. *Marine Pollution Bulletin*, *46*(8), 1024–1031. https://doi.org/10.1016/S0025-326X(03)00246-7

Gaines, S. D., White, C., Carr, M. H., & Palumbi, S. R. (2010). Designing marine reserve networks for both conservation and fisheries management. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(43), 18286–18293. https://doi.org/10.1073/pnas.0906473107

Glasl, B., Bourne, D. G., Frade, P. R., Thomas, T., Schaffelke, B., & Webster, N. S. (2019). Microbial predictors of environmental perturbations in coral reef ecosystems. *Microbiome*, *7*(1), 1–13. https://doi.org/10.1101/524173

Glücksman, E., Bell, T., Griffiths, R. I., & Bass, D. (2010). Closely related protist strains have different grazing impacts on natural bacterial communities. *Environmental Microbiology*, *12*(12), 3105–3113. https://doi.org/10.1111/j.1462-2920.2010.02283.x

Gonzalez, J. M., Sherr, E. B., & Sherr, B. F. (1990). Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Applied and Environmental Microbiology*, *56*(3), 583–589. https://doi.org/10.1128/aem.56.3.583-589.1990

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., … Christen, R. (2013). The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, *41*(D1), 597–604. https://doi.org/10.1093/nar/gks1160

Hagan, A. B., Foster, R., Perera, N., & Aji, C. (2007). Tsunami impacts in Aceh Province and North Sumatra, Indonesia. *Atoll Research Bulletin*, *544*, 37–54.

Halim A. (2002). *Adoption of cyanide fishing practice in Indonesia. Ocean Coastal*

Manage ; 45, 45:313–23.

Hall, J. A., Barrett, D. P., & James, M. R. (1993). The importance of phytoflagellate, heterotrophic flagellate and ciliate grazing on bacteria and picophytoplankton sized prey in a coastal marine environment. *Journal of Plankton Research*, *15*(9), 1075–1086. https://doi.org/10.1093/plankt/15.9.1075

Hoeksema, B. W. (2007). Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity : Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity : The Coral Triangle. In W. Renema (Ed.), *Biogeography, Time, and Place: Distributions, Barriers, and Islands* (pp. 117–178). https://doi.org/10.1007/978-1-4020-6374-9

Horvath HTL. (2017). *Lombok baseline supply, market demand forecasts and investment needs*.

Jagadeesan, L., Jyothibabu, R., Arunpandi, N., & Parthasarathi, S. (2017). Copepod grazing and their impact on phytoplankton standing stock and production in a tropical coastal water during the different seasons. *Environmental Monitoring and Assessment*, *189*(3). https://doi.org/10.1007/s10661-017-5804-y

Kafouris, S., Smeti, E., Spatharis, S., Tsirtsis, G., Economou-Amilli, A., & Danielidis, D. B. (2019). Nitrogen as the main driver of benthic diatom composition and diversity in oligotrophic coastal systems. *Science of the Total Environment*, *694*, 1–9. https://doi.org/10.1016/j.scitotenv.2019.133773

Katz, S. M., Pollock, F. J., Bourne, D. G., & Willis, B. L. (2014). Crown-of-thorns starfish predation and physical injuries promote brown band disease on corals.

Coral Reefs, *33*(3), 705–716. https://doi.org/10.1007/s00338-014-1153-2

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*. https://doi.org/10.1186/1471-2105-9-559

Lee, T., Fournier, S., Gordon, A. L., & Sprintall, J. (2019). Maritime Continent water cycle regulates low-latitude chokepoint of global ocean circulation. *Nature Communications*, *10*(1), 1–13. https://doi.org/10.1038/s41467-019-10109-z

Leray, M., Ho, S. L., Lin, I. J., & Machida, R. J. (2018). MIDORI server: a webserver for taxonomic assignment of unknown metazoan mitochondrial-encoded sequences using a curated database. *Bioinformatics (Oxford, England)*, *34*(21), 3753–3754. https://doi.org/10.1093/bioinformatics/bty454

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., … Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, *10*(1), 1–14. https://doi.org/10.1186/1742-9994-10-34

Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. *Ecology Letters*, *10*(12), 1170–1181. https://doi.org/10.1111/j.1461-0248.2007.01117.x

Liu, H., Chen, M., Zhu, F., & Harrison, P. J. (2016). Effect of diatom silica content on copepod grazing, growth and reproduction. *Frontiers in Marine Science*, *3*(JUN), 1–7. https://doi.org/10.3389/fmars.2016.00089

Mahé, F., Vargas, C. De, Bass, D., Czech, L., Stamatakis, A., Lara, E., … Dunthorn, M. (2017). Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nature Publishing Group*, *1*(March), 1–8. https://doi.org/10.1038/s41559-017-0091

Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., … Bowler, C. (2016). Insights into global diatom distribution and diversity in the world's ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(11), E1516–E1525. https://doi.org/10.1073/pnas.1509523113

Mao-Jones, J., Ritchie, K. B., Jones, L. E., & Ellner, S. P. (2010). How microbial community composition regulates coral disease development. *PLoS Biology*, *8*(3), 22–26. https://doi.org/10.1371/journal.pbio.1000345

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 5–7.

Massana, R., Gobet, A., Audic, S., Bass, D., Bittner, L., Boutte, C., … de Vargas, C. (2015). Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing. *Environmental Microbiology*, *17*(10), 4035–4049. https://doi.org/10.1111/1462-2920.12955

McManus, J. W., Nanola, C. L., Reyes, R. B., Kesner, K. N., & Mcmanus, J. W. (1997). Resource Ecology of the Bolinao Coral Reef System\rEffects of some destructive fishing methods on coral cover and potential rates of recovery. *Environmental Management*, *21*(1), 69–78.

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061217

Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwälder, B., Schlötterer, C., & Boenigk, J. (2010). Diversity in a hidden world: Potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Molecular Ecology*, *19*(SUPPL. 1), 32–40. https://doi.org/10.1111/j.1365-294X.2009.04478.x

Pet-Soede, L., & Erdmann, M. V. (1998). Blast fishing in Southwest Sulawesi, Indonesia. *Naga, The ICLARM Quarterly*, *21*(2), 4–9.

Pierce, R. W., & Turner, J. T. (1992). Ecology of Planktonic Ciliates in Marine Food Webs. *Reviews in Aquatic Sciences*, *6*(January), 139–181.

Richter, D., Watteaux, R., Vannier, T., Leconte, J., Reygondeau, G., Maillet, N., … Frémont, P. (2020). Genomic evidence for global ocean plankton biogeography shaped by large-scale current systems. *HAL Archives-Ouvertes*.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *2016*(10), 1–22. https://doi.org/10.7717/peerj.2584

Saila, S. B., Kocic, V. L., & McManus, J. W. (1993). Modelling the effects practices on tropical coral reefs. *Marine Ecology Progress Series*, *94*(1), 51–60.

Santoferrara, L., Burki, F., Filker, S., Logares, R., Dunthorn, M., & McManus, G. B.

(2020). Perspectives from Ten Years of Protist Studies by High-Throughput Metabarcoding. *Journal of Eukaryotic Microbiology*, *67*(5), 612–622. https://doi.org/10.1111/jeu.12813

Satta, C. T., Anglès, S., Garcés, E., Lugliè, A., Padedda, B. M., & Sechi, N. (2010). Dinoflagellate cysts in recent sediments from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *57*(3–4), 256–267. https://doi.org/10.1016/j.dsr2.2009.09.013

Sawall, Y., Jompa, J., Litaay, M., Maddusila, A., & Richter, C. (2013). Coral recruitment and potential recovery of eutrophied and blast fishing impacted reefs in Spermonde Archipelago, Indonesia. *Marine Pollution Bulletin*, *74*(1), 374–382. https://doi.org/10.1016/j.marpolbul.2013.06.022

Silveira, C. B., Cavalcanti, G. S., Walter, J. M., Silva-Lima, A. W., Dinsdale, E. A., Bourne, D. G., … Thompson, F. L. (2017). Microbial processes driving coral reef organic carbon flow. *FEMS Microbiology Reviews*, *41*(4), 575–595. https://doi.org/10.1093/femsre/fux018

Siry, H. Y. (2007). Making Decentralized Coastal Zone Management Work for the Southeast Asian Region: Comparative Perspectives. In *Oceans and Law of Sea*.

Sommeria-Klein, G., Watteaux, R., Iudicone, D., Bowler, C., & Morlon, H. (2020). Global drivers of eukaryotic plankton biogeography in the sunlit ocean. *BioRxiv*. Retrieved from https://www.biorxiv.org/content/10.1101/2020.09.08.287524v1.abstract

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., … Lepoivre, C. (2015). *Structure and function of the global ocean microbiome*. *348*(6237), 1–10.

Sweet, M., & Bythell, J. (2012). Ciliate and bacterial communities associated with White Syndrome and Brown Band Disease in reef-building corals. *Environmental Microbiology*, *14*(8), 2184–2199. https://doi.org/10.1111/j.1462-2920.2012.02746.x

Sweet, M. J., Croquer, A., & Bythell, J. C. (2014). Experimental antibiotic treatment identifies potential pathogens of white band disease in the endangered Caribbean coral Acropora cervicornis. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1788). https://doi.org/10.1098/rspb.2014.0094

Sweet, M. J., & Séré, M. G. (2016). Ciliate communities consistently associated with coral diseases. *Journal of Sea Research*, *113*, 119–131. https://doi.org/10.1016/j.seares.2015.06.008

Teh, L. S. L., Teh, L. C. L., & Sumaila, U. R. (2013). A Global Estimate of the Number of Coral Reef Fishers. *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0065397

Veron, J. E. N., Turak, E., Stafford-Smith, M., Stuart, K., Devantier, L. M., Green, A. L., & Peterson, N. (2009). Delineating the Coral Triangle. *Galaxea, Journal of Coral Reef Studies*, *11*(2), 91–100. https://doi.org/10.3755/galaxea.11.91

Williams, S. L., Sur, C., Janetski, N., Hollarsmith, J. A., Rapi, S., Barron, L., … Mars, F. (2019). Large-scale coral reef rehabilitation after blast fishing in Indonesia.

Restoration Ecology, *27*(2), 447–456. https://doi.org/10.1111/rec.12866

Yeager, L. A., Marchand, P., Gill, D. A., Baum, J. K., & McPherson, J. M. (2017). Marine Socio-Environmental Covariates: queryable global layers of environmental and anthropogenic variables for marine ecosystem studies. *Ecology*, *98*(7), 1976. https://doi.org/10.1002/ecy.1884

Zhu, F., Massana, R., Not, F., Marie, D., & Vaulot, D. (2005). Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiology Ecology*, *52*(1), 79–92. https://doi.org/10.1016/j.femsec.2004.10.006

Table 1. Primer sequences and PCR cycles used to amplify V9 hypervariable region of 18S rDNA (121bp) and portion of COI (313bp).

Target region	Primer name/direction	Primer Sequence (5’-3’)	Reference	PCR Cycles
18S (V9)	1389F (Forward)	TTGTACACACCGCCC	(Amaral-Zettler, McCliment, Ducklow, & Huse, 2009)	**Start:** 3min denaturation at 94°C **Cycles (x35):** Denaturation at 94°C for 45sec, Annealing at 48°C for 30sec, Elongation at 72°C for 30sec **End:** 5min elongation at 72°C
	1510R (Reverse)	CCTTCYGCAGGTTCACCTAC		
COI	mICOIintF (Forward)	GGWACWGGWTGAACWGTWTAYCCYCC	(Leray et al., 2013)	**Start:** 5min denaturation at 95°C **Cycles (x35):** Denaturation at 95°C for 1min, Annealing at 48°C for 45sec, Elongation at 72°C for 30sec **End:** 10min elongation at 72°C
	dgHCO2198 (Reverse)	TAAACTTCAGGGTGACCAAARAAYCA		

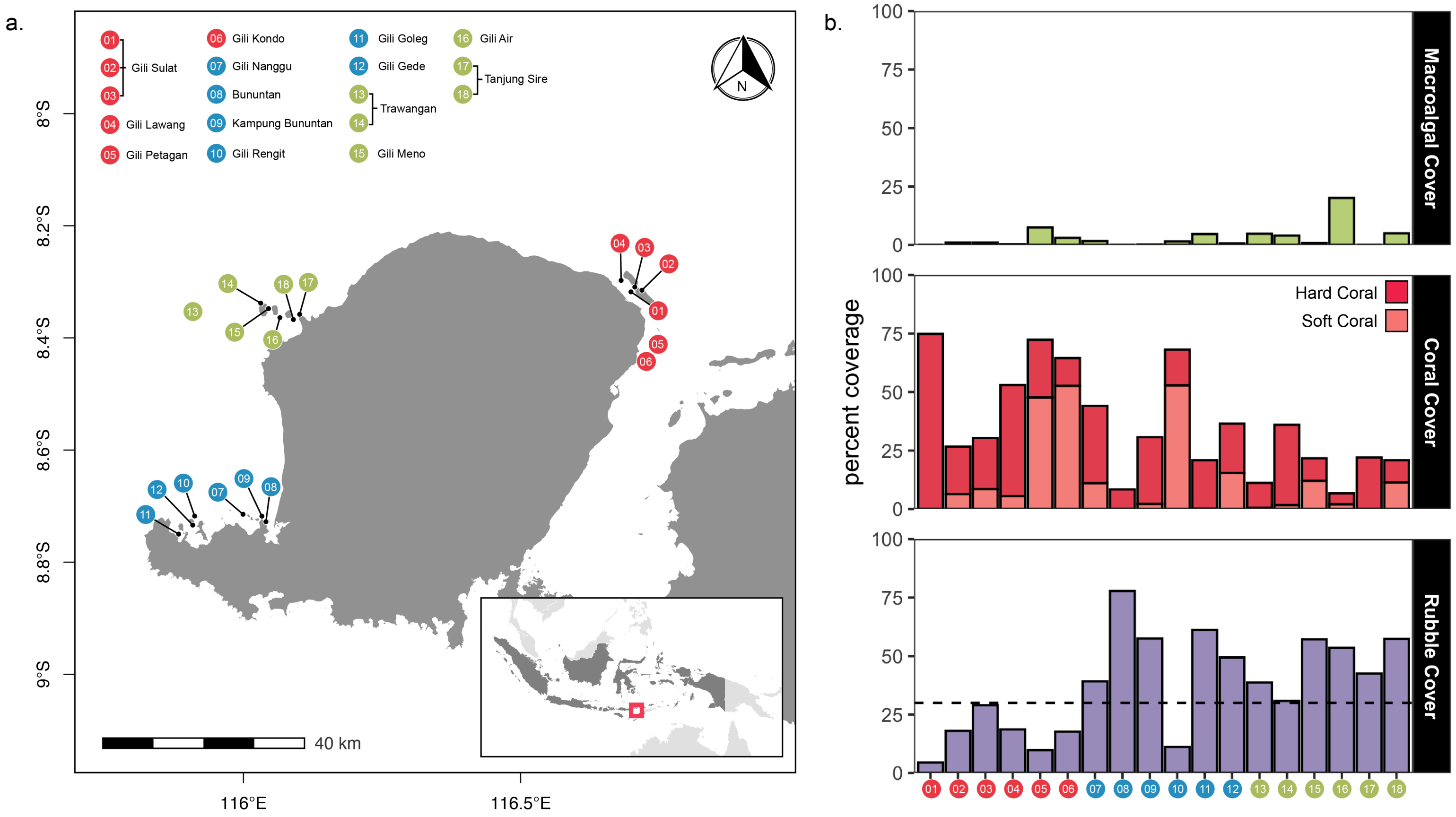


Figure 1. (a) Map of collection sites on the island of Lombok, Indonesia, and (b) corresponding benthic coverage data for each collection site as evaluated by UVC. Horizontal line across rubble coverage indicates 30% threshold used in Sawall, et al. 2013 to denote reefs impacted by blast fishing.

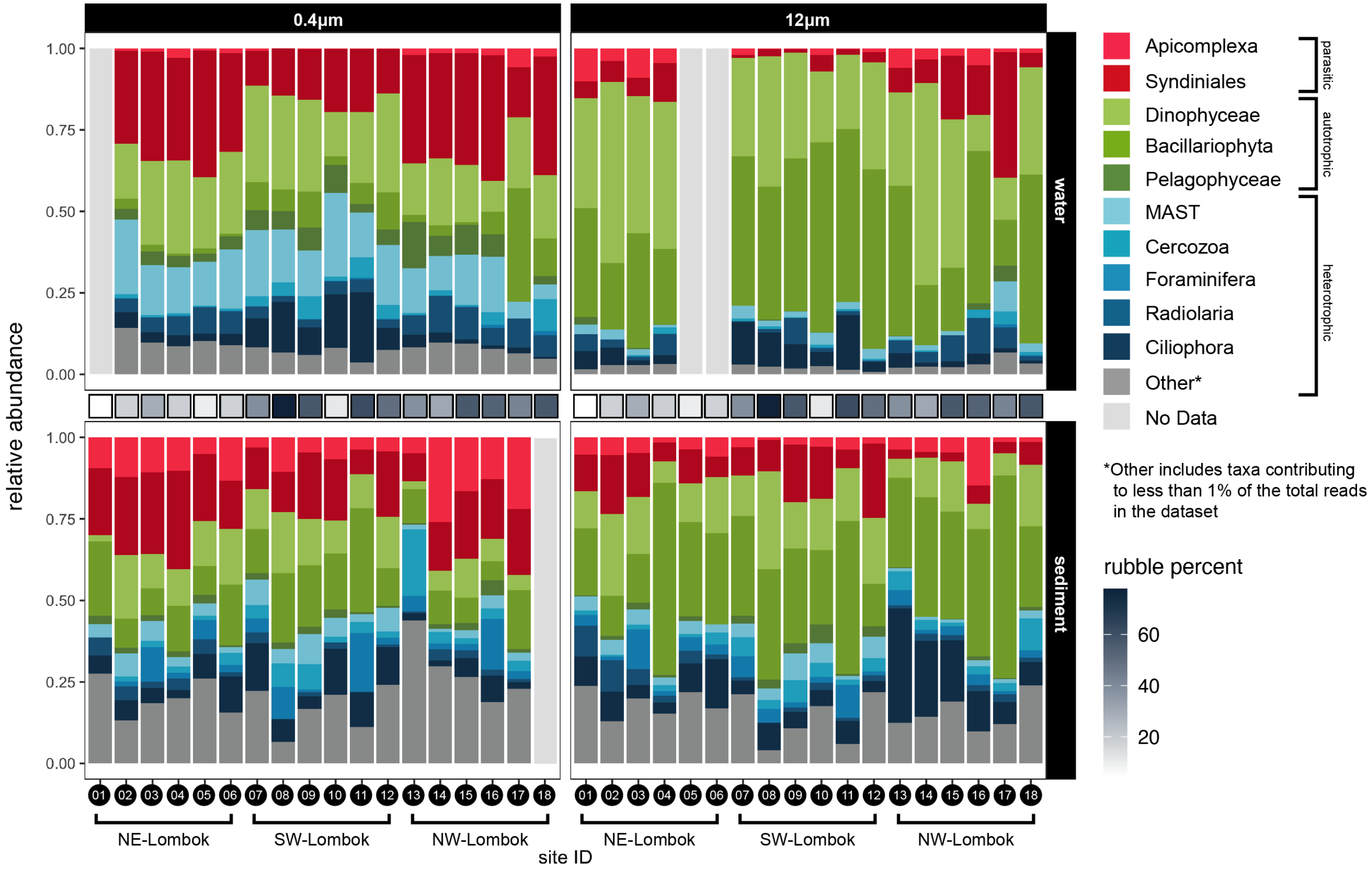


Figure 2. Protist community composition by collection site for water and sediment sample of both size fractions. Squares between water and sediment plots represent, rubble percent per site.

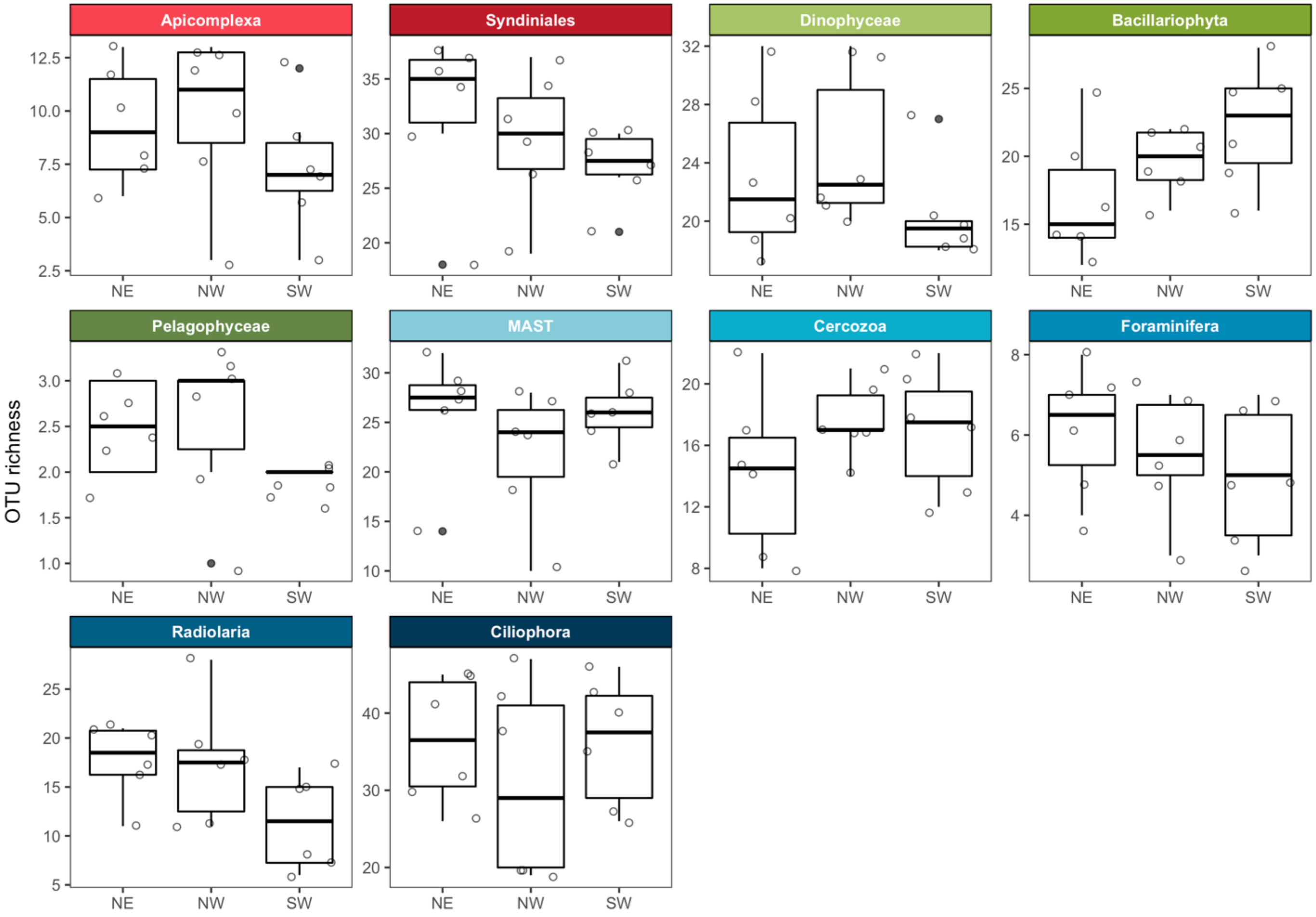


Figure 3. OTU richness by geographic groupings of collection sites (NE = sites 01-06, SW = sites 07-12, NW = sites 13-18). Richness did not significantly differ by groups of site in any protist groups (Wilcoxon pairwise, $p < 0.05$).

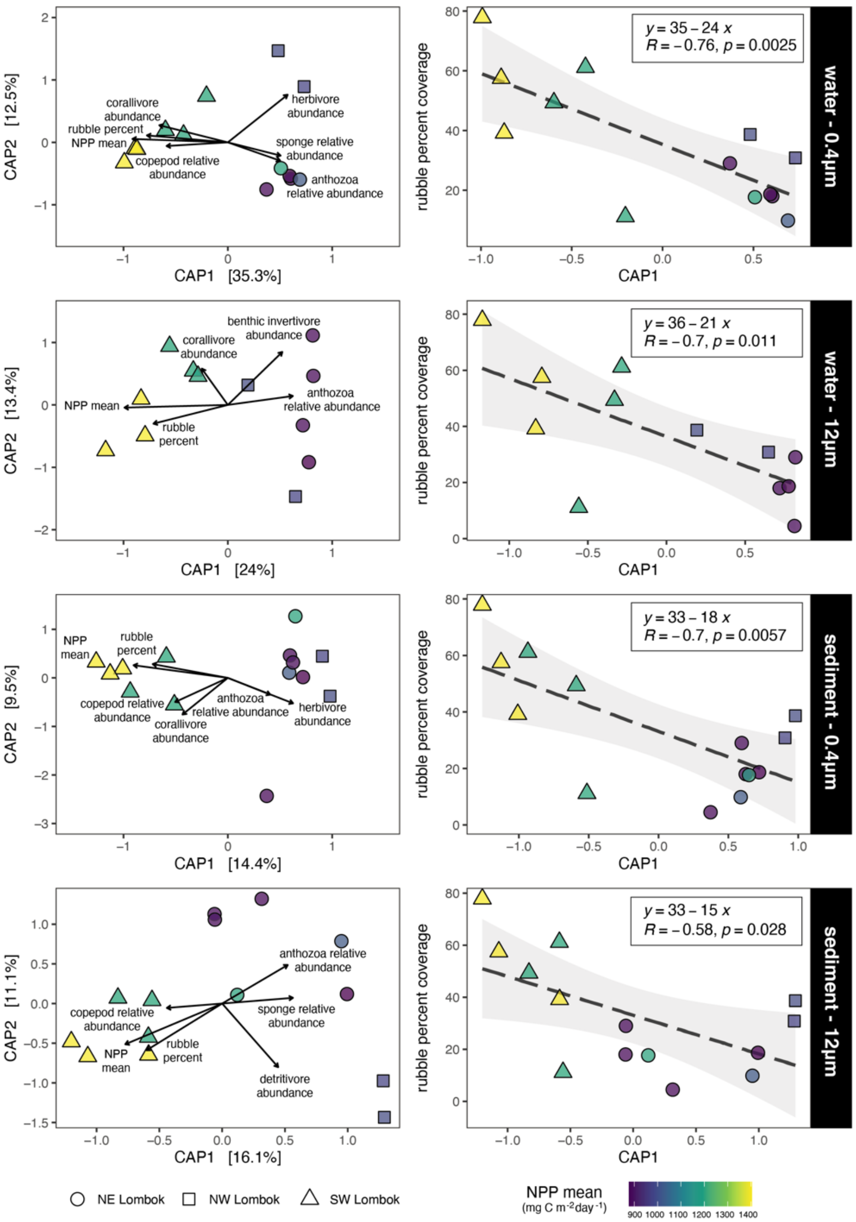


Figure 4. CAP ordinations of V9 samples constructed using Bray-Curtis dissimilarity, and regressions showing significant correlation between CAP axes and rubble percent at each given site. Fish trophic level abundance data and rubble percent came from UVC data, mean NPP came from MSEC, copepod relative abundance data came from V9 sequence data, and sponge and anthozoan relative abundance came from cox1 sequence data.

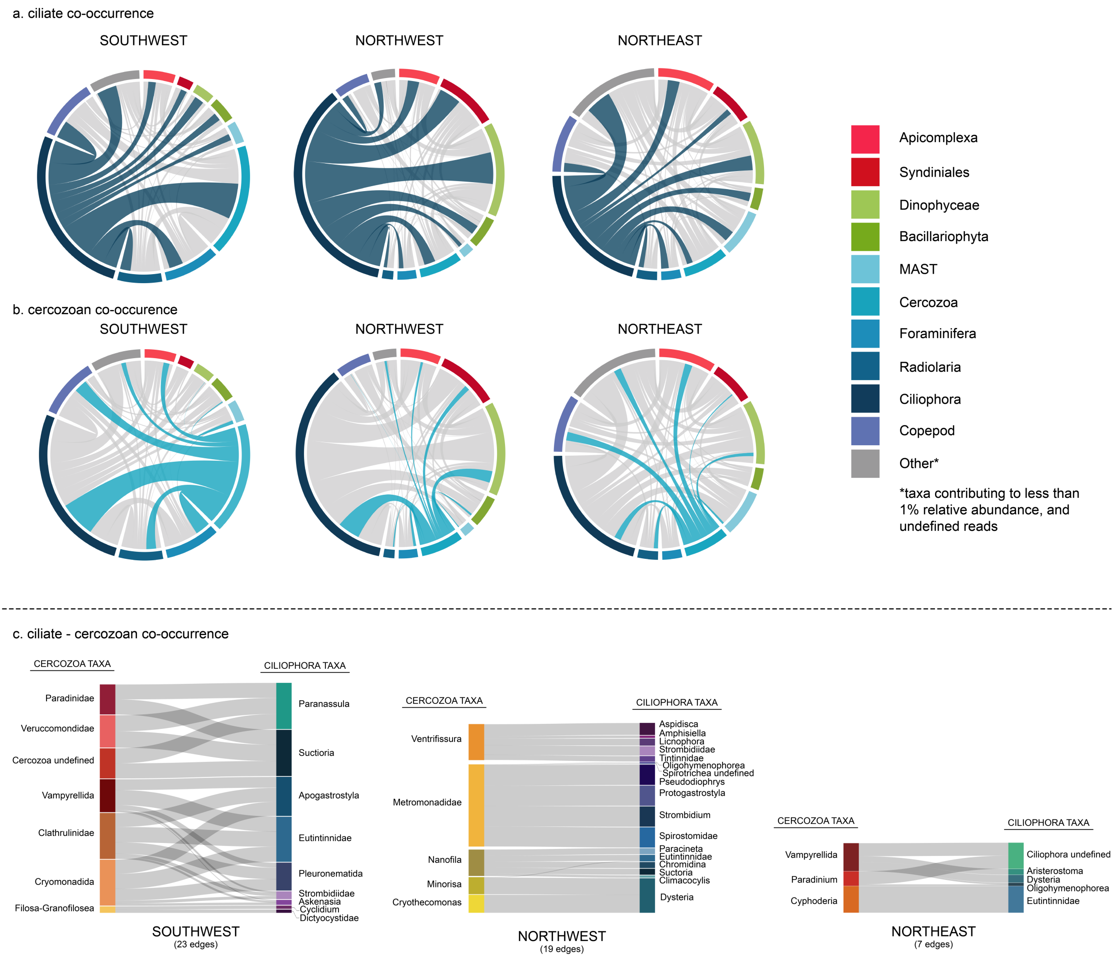


Figure 5. Co-occurrence networks constructed using WGCNA with (a) ciliate edges highlighted and (b) cercozoan edges highlighted, and (c) he co-occurrence between ciliates and cercozoans at lower taxonomic level, showing the stronger co-occurrence between these groups in the northwest and southwest regions. Edge thickness corresponds to the number of edges between two groups.

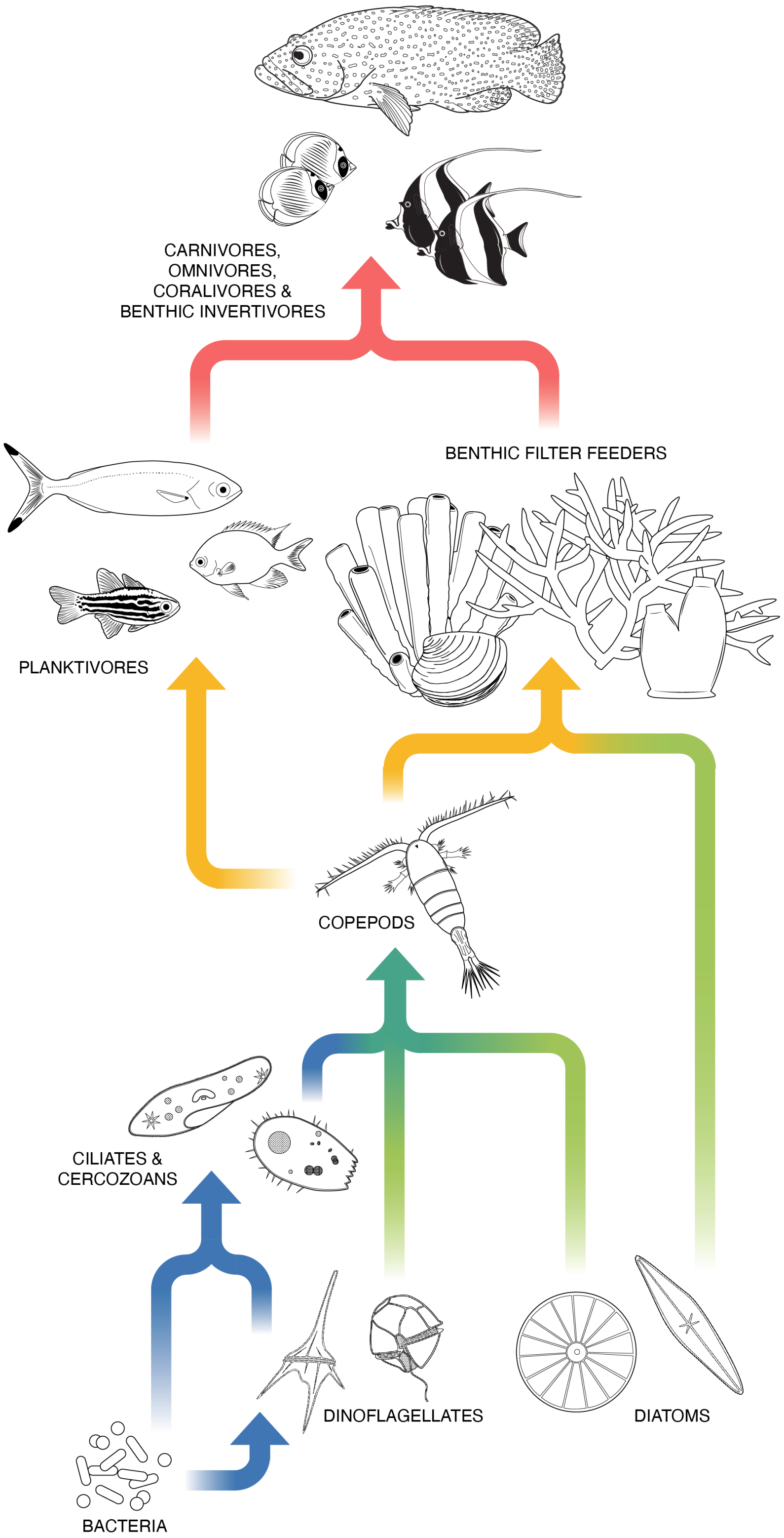


Figure 6. Diagram showing energy flow through the food web.

CHAPTER 5: CONCLUSION

Protist communities across the globe

Protists exhibit immense morphological and ecological diversity and serve important roles in biogeochemical cycling and other ocean processes across the globe (Falkowski, Barber, & Smetacek, 1998; Field, Behrenfeld, Randerson, & Falkowski, 1998). Despite this vast diversity and their importance to ecosystems across the globe, what we currently know about these groups is only scratching the surface. Development of high-throughput sequencing and global sampling efforts in recent years has enabled us to better understand the composition and function of protist communities across the globe, as well as better understand environmental drivers behind protist community structure.

Expeditions like Tara Oceans (Pesant et al., 2015; Sunagawa et al., 2020) and Malaspina (Duarte, 2015), have used both high-throughput sequencing and visual methods to characterize protist communities across the world's oceans. These expeditions have revealed a vast amount of unknown protistan diversity previously overlooked by visual methods alone, especially in small and low abundant groups (Forster et al., 2016; Le Bescot et al., 2016; López-García, Rodriguez-Valera, Pedrós-Alló, & Moreira, 2001; Massana et al., 2015; Santoferrara et al., 2020). Furthermore, these expeditions have expanded our understanding of the relative roles dispersal limitation and environmental selection play in structuring protist communities on a global scale. Not only do these data support that protist communities are shaped by a combination of both dispersal limitation and environmental selection on a broad geographic scale (Sunagawa et al., 2015), but they also highlight that top-down selection by grazers may play an important role in shaping these communities on a local scale (Sommeria-Klein, Watteaux, Iudicone, Bowler, & ..., 2020). While these expeditions have greatly expanded our knowledge on global protist biodiversity and protist community structure across the world's oceans, difficulty with permitting has led to a lack of data on these communities in the Indo-Pacific (Pansiot, 2017).

Protist communities across Indonesia

The Indo-Pacific is home to some of the most biodiverse ecosystems in the world (Allen, 2008; Hoeksema, 2007; Veron et al., 2009), and, as its name indicates, is the region where the Pacific and Indian Oceans converge. These two factors combine to make this region ideal for studying both abiotic and biotic drivers of protist community structure. While this region has a long history of biodiversity and biogeography research, research on protist communities in the Indo-Pacific, and in Indonesia in particular, have relied heavily on visual based surveys conducted on relatively small geographic scales. These studies highlight the important roles, water quality, runoff, and other human activities have on protist communities at a local scale (Nasution, Dian Takarina, & Thoha, 2021; Sidabutar et al., 2016; Suteja et al., 2021; Syakti, Idris, Koenawan, Asyhar, & Apriadi, 2019; Thoha et al., 2015), but they do not address how these communities are structured on a broad scale across the transition from the Pacific to the Indian Ocean.

The work presented in this dissertation is the first to characterize protist communities across the Indo-Pacific using metabarcoding data. In addition to metabarcoding data, this work also incorporates underwater visual census (UVC) data of fish and benthic communities to better understand connectivity across different levels of the food web. Chapters one and two explore how dispersal limitation and fishing pressure shape protist community structure across the region. While fishing pressure correlates with fish biomass across Indonesia, it does not appear to strongly impact protist community structure. Instead, protist communities show a significant shift in diversity between the two eastern regions with low fishing pressure and comparable fish biomass. The shift in diversity from dinoflagellate dominated communities in one region to diatom dominated communities in the other could potentially be explained by surface current patterns across the region. The change in community composition and diversity between two regions with similar diversity and biomass of metazoans further suggests a lack of connectivity between upper trophic level organisms and the microbial food web.

Chapter 2 further explores the impacts of fishing on protist communities on a local scale. Fisheries management strategies including prohibition of fishing and fishing gear restrictions in certain areas have been shown to correlate with higher fish biomass and metazoan diversity across Indonesia (Campbell et al., 2020; Campbell, Edgar, Stuart-Smith, Soler, & Bates, 2018; Lester et al., 2009). Our data support those previous findings and further show that despite the impacts these protections have on upper trophic levels, they do not appear to impact protist communities at the base of the food web. These results suggest a disconnect between upper trophic levels and the microbial food web and demonstrate that effects of management and fishing pressure are not strong enough to carry down to the base of the food web. Furthermore, these results indicate that due to the lack of impact management has on protists, these communities should likely not have strong influence in future management design.

Chapter 3 focuses on protist communities across the island of Lombok, the most heavily fished of our sampling regions. The sites across Lombok encompassed various management schemes, allowing us to evaluate how management potentially impacted protist communities across the island. While management, did not impact these communities, rubble cover and net primary productivity (NPP) both appeared to best explain shifts in protist community composition across the region. In particular, sites with high rubble cover were characterized by higher relative abundance of small grazers including ciliates and cercozoans and by higher relative abundance of diatoms. While the increase in small grazers is likely indicative of increased bacterial growth in rubble fields, the increase in relative abundance of diatoms at these sites could be indicative of nutrient inputs from waste water across different parts of the island. These results not only provide insight on response of protist communities to disturbances on small geographic scales, but they also provide insight into potential differences in biogeochemical cycling in protist communities across relatively small areas.

Overall, the work in this dissertation informs our understanding of protist community structure across the globe, and highlights the lack of impact fishing pressure and management have in structuring these communities from the top-down on both large and small geographic scales. In addition to the significance this work has for management and protist biogeography research in this region, it has also provided a foundation for future protist biodiversity research and highlighted the value of continued capacity building for molecular research in this region.

Future directions for protist research and capacity building in Indonesia

The Indo-Pacific provides a unique setting for studying top-down and bottom-up drivers of protist community structure. While the data presented in this dissertation begin to explore the roles dispersal limitation, environmental selection, and human activity play in shaping these communities, there is still a lot to build on. In particular, the seasonal variations in surface current patterns and water chemistry across the region as a result of monsoons (Gordon & Fine, 1996; Gordon, Susanto, & Vranes, 2003; Lee, Fournier, Gordon, & Sprintall, 2019), poses an interesting opportunity for understanding the relative roles dispersal limitation and environmental selection play in seasonal community shifts. Additionally, metabarcoding data can better help us understand these seasonal dynamics, by enabling researchers to identify small, low abundance species that would otherwise be overlooked by visual surveys.

The data presented in this dissertation not only provide information on protist community structure across the Indo-Pacific, but they also allow us to examine these communities in a global context. More specifically, these data allow us to fill gaps left by global sampling expeditions like Tara Oceans and Malaspina. While drivers of biogeographic structure in dominant protist groups like diatoms and dinoflagellates are well studied across the globe, the drivers of biogeographic structure of other protists are poorly understood. One group in particular where biogeographic structure is poorly understood is the Apicomplexans. Apicomplexans are a group of parasitic protists that have been found to be globally distributed across a wide array of environments (Del Campo et al., 2019) and have been found in particularly in high diversity and high relative abundance in neotropical soils (Mahé et al., 2017) and

Antarctic marine sediments (Cleary & Durbin, 2016). However, in our dataset, Apicomplexans are low diversity and low relative abundance when they are present at all. This contrast in diversity and abundance of Apicomplexans across different marine and terrestrial environments is just one example of how little we know about the biogeography of many protist groups across the globe, and emphasizes the value of metabarcoding data in exploring the structure of these communities.

Research in biodiversity hotspots is often complicated by cost, permitting, and limited resources (Pitman, 2010). In Indonesia specifically, a limiting factor is often the lack of field stations and labs across the country equipped for molecular lab work (Barber et al., 2014). However, as cost of high-throughput sequencing continues to drop, and sequencing platforms like the MinION which allow for sequencing in the field, continue to become more accessible, protist biodiversity research in these hotspots can continue to grow. In addition to growth of and increased accessibility to sequencing technologies, development and improvement of open-source tools for data analysis will further support molecular research and capacity building of local scientists in these regions, enabling more equitable research and collaborations in these countries in the future.

REFERENCES

Allen, G. R. (2008). Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*, 541–556. https://doi.org/10.1002/aqc

Barber, P. H., Ablan-Lagman, M. C. A., Berlinck, R. G., Cahyani, D., Crandall, E. D., Ravago-Gotanco, R., … Willette, D. A. (2014). Advancing biodiversity research in developing countries: the need for changing paradigms. *Bulletin of Marine Science*, *90*(1), 187–210. https://doi.org/10.5343/bms.2012.1108

Campbell, S. J., Darling, E. S., Pardede, S., Ahmadia, G., Mangubhai, S., Amkieltiela, … Maire, E. (2020). Fishing restrictions and remoteness deliver conservation outcomes for Indonesia’s coral reef fisheries. *Conservation Letters*, *13*(2), 1–9. https://doi.org/10.1111/conl.12698

Campbell, S. J., Edgar, G. J., Stuart-Smith, R. D., Soler, G., & Bates, A. E. (2018). Fishing-gear restrictions and biomass gains for coral reef fishes in marine protected areas. *Conservation Biology*, *32*(2), 401–410. https://doi.org/10.1111/cobi.12996

Cleary, A. C., & Durbin, E. G. (2016). Unexpected prevalence of parasite 18S rDNA sequences in winter among Antarctic marine protists. *Journal of Plankton Research*, *38*(3), 401–417. https://doi.org/10.1093/plankt/fbw005

Del Campo, J., Heger, T. J., Rodríguez-Martínez, R., Worden, A. Z., Richards, T. A., Massana, R., & Keeling, P. J. (2019). Assessing the diversity and distribution of apicomplexans in host and free-living environments using high-throughput amplicon data and a phylogenetically informed reference framework. *Frontiers in Microbiology*, *10*(OCT), 1–15. https://doi.org/10.3389/fmicb.2019.02373

Duarte, C. M. (2015). Seafaring in the 21st century: The Malaspina 2010 circumnavigation expedition. *Limnology and Oceanography Bulletin*, *24*(1), 11–14. https://doi.org/10.1002/lob.10008

Falkowski, P. G., Barber, R. T., & Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science*, *281*(5374), 200–206. https://doi.org/10.1126/science.281.5374.200

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, *281*(5374), 237–240. https://doi.org/10.1126/science.281.5374.237

Forster, D., Dunthorn, M., Mahé, F., Dolan, J. R., Audic, S., Bass, D., … Stoeck, T. (2016). Benthic protists: The under-charted majority. *FEMS Microbiology Ecology*, *92*(8), 1–11. https://doi.org/10.1093/femsec/fiw120

Gordon, A. L., & Fine, R. A. (1996). Pathways of water between the Pacific and Indian oceans in the Indonesian seas. *Letters to Nature*, *379*(6561), 146–149. https://doi.org/10.1038/379146a0

Gordon, A. L., Susanto, R. D., & Vranes, K. (2003). Cool Indonesian throughflow as a consequence of restricted surface layer flow. *Letters to Nature*, *425*(6960), 821–824. https://doi.org/10.1038/nature02013

Hoeksema, B. W. (2007). *Delineation of the Indo-Malayan Centre of Maximum*

Marine Biodiversity: The Coral Triangle. https://doi.org/10.1007/978-1-4020-6374-9_5

Le Bescot, N., Mahé, F., Audic, S., Dimier, C., Garet, M. J., Poulain, J., … Siano, R. (2016). Global patterns of pelagic dinoflagellate diversity across protist size classes unveiled by metabarcoding. *Environmental Microbiology*, *18*(2), 609–626. https://doi.org/10.1111/1462-2920.13039

Lee, T., Fournier, S., Gordon, A. L., & Sprintall, J. (2019). Maritime Continent water cycle regulates low-latitude chokepoint of global ocean circulation. *Nature Communications*, *10*(1), 1–13. https://doi.org/10.1038/s41467-019-10109-z

Lester, S. E., Halpern, B. S., Grorud-Colvert, K., Lubchenco, J., Ruttenberg, B. I., Gaines, S. D., … Warner, R. R. (2009). Biological effects within no-take marine reserves: A global synthesis. *Marine Ecology Progress Series*, *384*, 33–46. https://doi.org/10.3354/meps08029

López-García, P., Rodriguez-Valera, F., Pedrós-Alló, C., & Moreira, D. (2001). Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Letters to Nature*, *409*(February), 603–607.

Mahé, F., Vargas, C. De, Bass, D., Czech, L., Stamatakis, A., Lara, E., … Dunthorn, M. (2017). Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nature Publishing Group*, *1*(March), 1–8. https://doi.org/10.1038/s41559-017-0091

Massana, R., Gobet, A., Audic, S., Bass, D., Bittner, L., Boutte, C., … de Vargas, C. (2015). Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing. *Environmental Microbiology*, *17*(10), 4035–4049. https://doi.org/10.1111/1462-2920.12955

Nasution, A. K., Dian Takarina, N., & Thoha, H. (2021). The presence and abundance of harmful dinoflagellate algae related to water quality in Jakarta Bay, Indonesia. *Biodiversitas Journal of Biological Diversity*, *22*(5), 2909–2917. https://doi.org/10.13057/biodiv/d220556

Pansiot, N. (2017). Change of desitination: Tara will not go to Indonesia.

Pesant, S., Not, F., Picheral, M., Kandels-Lewis, S., Le Bescot, N., Gorsky, G., … Searson, S. (2015). Open science resources for the discovery and analysis of Tara Oceans data. *Scientific Data*, *2*(Lmd), 1–16. https://doi.org/10.1038/sdata.2015.23

Pitman, N. C. A. (2010). Research in biodiversity hotspots should be free. *Trends in Ecology and Evolution*, *25*(7), 381. https://doi.org/10.1016/j.tree.2010.04.002

Santoferrara, L., Burki, F., Filker, S., Logares, R., Dunthorn, M., & McManus, G. B. (2020). Perspectives from Ten Years of Protist Studies by High-Throughput Metabarcoding. *Journal of Eukaryotic Microbiology*, *67*(5), 612–622. https://doi.org/10.1111/jeu.12813

Sidabutar, T., Thoha, H., Bayu, M. D., Rachman, A., Sianturi, O. R., Fitriya, N., … Masseret, E. (2016). Occurrence of Pyrodinium bahamense blooms related to cyst accumulation in the bottom sediments in the bays at Ambon, Lampung and Jakarta, Indonesia. *Harmful Algae News*, (52), 8–9. Retrieved from www.ioc-unesco.org/hab

Sommeria-Klein, G., Watteaux, R., Iudicone, D., Bowler, C., & ... (2020). Global drivers of eukaryotic plankton biogeography in the sunlit ocean. *BioRxiv*. Retrieved from https://www.biorxiv.org/content/10.1101/2020.09.08.287524v1.abstract

Sunagawa, S., Acinas, S. G., Bork, P., Bowler, C., Acinas, S. G., Babin, M., … de Vargas, C. (2020). Tara Oceans: towards global ocean ecosystems biology. *Nature Reviews Microbiology*, *18*(8), 428–445. https://doi.org/10.1038/s41579-020-0364-5

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., … Lepoivre, C. (2015). *Structure and function of the global ocean microbiome*. *348*(6237), 1–10.

Suteja, Y., Dirgayusa, I. G. N. P., Afdal, Cordova, M. R., Rachman, A., Rintaka, W. E., … Purwiyanto, A. I. S. (2021). Identification of potentially harmful microalgal species and eutrophication status update in Benoa Bay, Bali, Indonesia. *Ocean & Coastal Management*, *210*(April), 105698. https://doi.org/10.1016/j.ocecoaman.2021.105698

Syakti, A. D., Idris, F., Koenawan, C. J., Asyhar, R., & Apriadi, T. (2019). Biological pollution potential in the water of Bintan-Riau Islands Province, Indonesia: First appearance of harmful algal bloom species. *Egyptian Journal of Aquatic Research*, *45*(2), 117–122. https://doi.org/10.1016/j.ejar.2019.04.002

Thoha, H., Rachman, A., Sidabutar, T., Fitriya, N., Bayu, M. D., Iwataki, M., … Masseret, E. (2015). First record of the harmful dinoflagellate Cochlodinium polykrikoides in Lampung Bay, Indonesia. *Harmful Algae News*, (50), 14–15. Retrieved from www.ioc-unesco.org/hab

Veron, J. E. N., Turak, E., Stafford-Smith, M., Stuart, K., Devantier, L. M., Green, A. L., & Peterson, N. (2009). Delineating the Coral Triangle. *Galaxea, Journal of Coral Reef Studies*, *11*(2), 91–100. https://doi.org/10.3755/galaxea.11.91

BIBLIOGRAPHY

Ainsworth, T. D., Thurber, R. V., & Gates, R. D. (2010). The future of coral reefs: a microbial perspective. *Trends in Ecology and Evolution*, *25*(4), 233–240. https://doi.org/10.1016/j.tree.2009.11.001

Allen, A. E., Vardi, A., & Bowler, C. (2006). An ecological and evolutionary context for integrated nitrogen metabolism and related signaling pathways in marine diatoms. *Current Opinion in Plant Biology*, *9*(3), 264–273. https://doi.org/10.1016/j.pbi.2006.03.013

Allen, G. R. (2008). Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*, 541–556. https://doi.org/10.1002/aqc

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). *A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes*. *4*(7), 1–9. https://doi.org/10.1371/journal.pone.0006372

Amato, A., Kooistra, W. H. C. F., Levialdi Ghiron, J. H., Mann, D. G., Pröschold, T., & Montresor, M. (2007). Reproductive Isolation among Sympatric Cryptic Species in Marine Diatoms. *Protist*, *158*(2), 193–207. https://doi.org/10.1016/j.protis.2006.10.001

Anderson, M. J., & Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, *83*(4), 557–574. https://doi.org/10.1890/12-

2010.1

Anderson, M. J., & Willis, T. J. (2003). Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. *Ecology*, *84*(2), 511–525. https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2

Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data*.

Armbrust, E. V. (2009). The life of diatoms in the world's oceans. *Nature*, *459*(7244), 185–192. https://doi.org/10.1038/nature08057

Asaad, I., Lundquist, C. J., Erdmann, M. V., & Costello, M. J. (2018). Delineating priority areas for marine biodiversity conservation in the Coral Triangle. *Biological Conservation*, *222*(March), 198–211. https://doi.org/10.1016/j.biocon.2018.03.037

Avise, J. C. (2009). Phylogeography: Retrospect and prospect. *Journal of Biogeography*, *36*(1), 3–15. https://doi.org/10.1111/j.1365-2699.2008.02032.x

Bahonak, A. J. (1999). Dispersal , Gene Flow , and Population Structure. *The Quarterly Review of Biology*, *74*(1), 21–45.

Baker, A. C. (2003). Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 661–689. https://doi.org/10.1146/annurev.ecolsys.34.011802.132417

Barber, P. H. (2009). The challenge of understanding the Coral Triangle biodiversity hotspot. *Journal of Biogeography*, *36*(10), 1845–1846. https://doi.org/10.1111/j.1365-2699.2009.02198.x

Barber, P. H., Ablan-Lagman, M. C. A., Berlinck, R. G., Cahyani, D., Crandall, E. D., Ravago-Gotanco, R., … Willette, D. A. (2014). Advancing biodiversity research in developing countries: the need for changing paradigms. *Bulletin of Marine Science*, *90*(1), 187–210. https://doi.org/10.5343/bms.2012.1108

Barber, P. H., Cheng, S. H., Erdmann, M. V, Tenggardjaja, K., & Ambariyanto. (2011). Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea. *CrustIssues19*, *12*(17), 1–32.

Barber, P. H., Erdmann, M. V., & Palumbi, S. R. (2006). Comparative phylogeography of three codistributed stomatopods: Origins and timing of regional lineage diversification in the Coral Triangle. *Evolution*, *60*(9), 1825–1839.

Barber, P. H., Palumbi, S. R., Erdmann, M. V., & Moosa, M. K. (2000). A marine Wallace’s line? *Nature Brief Communications*, *406*, 692–693.

Barraclough, T. G. (1998). Revealing the factors that promote speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *353*(1366), 241–249. https://doi.org/10.1098/rstb.1998.0206

Benedetti, F., Jalabert, L., Sourisseau, M., Beker, B., Cailliau, C., Desnos, C., … Pouline, P. (2019). The seasonal and inter-annual fluctuations of plankton abundance and community structure in a North Atlantic Marine Protected Area. *Frontiers in Marine Science*, *6*(APR), 1–16.

https://doi.org/10.3389/fmars.2019.00214

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … 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

Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W., & Harvell, C. D. (2009). Microbial disease and the coral holobiont. *Trends in Microbiology*, *17*(12), 554–562. https://doi.org/10.1016/j.tim.2009.09.004

Bourne, D., Iida, Y., Uthicke, S., & Smith-Keune, C. (2008). Changes in coral-associated microbial communities during a bleaching event. *ISME Journal*, *2*(4), 350–363. https://doi.org/10.1038/ismej.2007.112

Bowen, B. W., Rocha, L. A., Toonen, R. J., & Karl, S. A. (2013). The origins of tropical marine biodiversity. *Trends in Ecology and Evolution*, *28*(6), 359–366. https://doi.org/10.1016/j.tree.2013.01.018

Briggs, J. C. (1999a). Coincident Biogeographic Patterns: Indo-West Pacific Ocean. *Evolution*, *53*(2), 326. https://doi.org/10.2307/2640770

Briggs, J. C. (1999b). Extinction and replacement in the Indo-West Pacific Ocean. *Journal of Biogeography*, *26*(4), 777–783. https://doi.org/10.1046/j.1365-2699.1999.00322.x

Briggs, J. C. (1999c). Modes of speciation: Marine Indo-West Pacific. *Bulletin of Marine Science*, *65*(3), 645–656.

Briggs, J. C. (2003). Marine centres of origin as evolutionary engines. *Journal of Biogeography*, *30*, 1–18. Retrieved from http://dx.doi.org/10.1046/j.1365-2699.2003.00810.x

Brzezinski, M. A., Villareal, T. A., & Lipschultz, F. (1998). Silica production and the contribution of diatoms to new and primary production in the central North Pacific. *Marine Ecology Progress Series*, *167*, 89–104. https://doi.org/10.3354/meps167089

Burke, L., Reytar, K., Spalding, M., & Perry, A. (2012). Reefs at Risk Revisited in the Coral Triangle. In *Defenders* (Vol. 74). Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3150666&tool=pmcentrez&rendertype=abstract

Bush, G. L. (1975). Modes of Animal Speciation. *Annual Review of Ecology and Systematics*, *6*(1), 339–364. https://doi.org/10.1146/annurev.es.06.110175.002011

Calbet, A., & Saiz, E. (2005). The ciliate-copepod link in marine ecosystems. *Aquatic Microbial Ecology*, *38*(2), 157–167. Retrieved from http://www.researchgate.net/publication/230660217_The_ciliate-copepod_link_in_marine_ecosystems/file/5046351ac501e1b0be.pdf%0Ahttp://www.int-res.com/articles/ame2004/38/a038p157.pdf%0Apapers2://publication/uuid/A856D2A0-BE63-453B-9487-C56B6B5C3E2A

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., &

Holmes, S. P. (2013). DADA2: High resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869.DADA2

Campbell, S. J., Cinner, J. E., Ardiwijaya, R. L., Pardede, S., Kartawijaya, T., Mukmunin, A., … Baird, A. H. (2012a). Avoiding conflicts and protecting coral reefs: Customary management benefits marine habitats and fish biomass. *Oryx*, *46*(4), 486–494. https://doi.org/10.1017/S0030605312000348

Campbell, S. J., Cinner, J. E., Ardiwijaya, R. L., Pardede, S., Kartawijaya, T., Mukmunin, A., … Baird, A. H. (2012b). Avoiding conflicts and protecting coral reefs: Customary management benefits marine habitats and fish biomass. *Oryx*, *46*(4), 486–494. https://doi.org/10.1017/S0030605312000348

Campbell, S. J., Darling, E. S., Pardede, S., Ahmadia, G., Mangubhai, S., Amkieltiela, … Maire, E. (2020). Fishing restrictions and remoteness deliver conservation outcomes for Indonesia's coral reef fisheries. *Conservation Letters*, *13*(2), 1–9. https://doi.org/10.1111/conl.12698

Campbell, S. J., Edgar, G. J., Stuart-Smith, R. D., Soler, G., & Bates, A. E. (2018a). Fishing-gear restrictions and biomass gains for coral reef fishes in marine protected areas. *Conservation Biology*, *32*(2), 401–410. https://doi.org/10.1111/cobi.12996

Campbell, S. J., Edgar, G. J., Stuart-Smith, R. D., Soler, G., & Bates, A. E. (2018b). Fishing-gear restrictions and biomass gains for coral reef fishes in marine protected areas. *Conservation Biology*, *32*(2), 401–410.

https://doi.org/10.1111/cobi.12996

Campbell, S. J., Mukminin, A., Kartawijaya, T., Huchery, C., & Cinner, J. E. (2014a). Changes in a coral reef fishery along a gradient of fishing pressure in an Indonesian marine protected area. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(1), 92–103. https://doi.org/10.1002/aqc.2359

Campbell, S. J., Mukminin, A., Kartawijaya, T., Huchery, C., & Cinner, J. E. (2014b). Changes in a coral reef fishery along a gradient of fishing pressure in an Indonesian marine protected area. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(1), 92–103. https://doi.org/10.1002/aqc.2359

Campbell, S. J., Pratchett, M. S., Anggoro, A. W., Ardiwijaya, R. L., Fadli, N., Herdiana, Y., … Baird, A. H. (2007). Disturbance to coral reefs in Aceh, Northern Sumatra: Impacts of the Sumatra-Andaman tsunami and pre-tsunami degradation. *Atoll Research Bulletin*, (544), 55–78.

Caron, D. A., Countway, P. D., Jones, A. C., Kim, D. Y., & Schnetzer, A. (2012). Marine protistan diversity. *Annual Review of Marine Science*, *4*, 467–493. https://doi.org/10.1146/annurev-marine-120709-142802

Carpenter, K. E., Barber, P. H., Crandall, E. D., Ablan-Lagman, M. C. A., Ambariyanto, Mahardika, G. N., … Toha, A. H. A. (2011). Comparative phylogeography of the coral triangle and implications for marine management. *Journal of Marine Biology*, *2011*, 1–14. https://doi.org/10.1155/2011/396982

Carpenter, K. E., Barber, P. H., Crandall, E. D., Ablan-lagman, M. C. A., Mahardika, G. N., Manjaji-matsumoto, B. M., … Toha, A. H. A. (2011). Comparative

Phylogeography of the Coral Triangle and Implications for Marine Management. *Journal of Marine Biology*, *2011*. https://doi.org/10.1155/2011/396982

Carvalho, P. G., Setiawan, F., Fahlevy, K., Subhan, B., Madduppa, H., Zhu, G., & Humphries, A. T. (2021). Fishing and habitat condition differentially affect size spectra slopes of coral reef fishes. *Ecological Applications*, *0*(0), 1–14. https://doi.org/10.1002/eap.2345

Casteleyn, G., Leliaert, F., Backeljau, T., Debeer, A. E., Kotaki, Y., Rhodes, L., … Vyverman, W. (2010). Limits to gene flow in a cosmopolitan marine planktonic diatom. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(29), 12952–12957. https://doi.org/10.1073/pnas.1001380107

Cermeño, P., de Vargas, C., Abrantes, F., & Falkowski, P. G. (2010). Phytoplankton biogeography and community stability in the ocean. *PLoS ONE*, *5*(4). https://doi.org/10.1371/journal.pone.0010037

Cermeño, P., & Falkowski, P. G. (2009). Controls on Diatom Biogeography in the Ocean. *Science*, *325*(September), 1539–1542.

Chust, G., Irigoien, X., Chave, J., & Harris, R. P. (2013). Latitudinal phytoplankton distribution and the neutral theory of biodiversity. *Global Ecology and Biogeography*, *22*(5), 531–543. https://doi.org/10.1111/geb.12016

Clayton, S., Nagai, T., & Follows, M. J. (2014). Fine scale phytoplankton community structure across the Kuroshio Front. *Journal of Plankton Research*, *36*(4), 1017–1030. https://doi.org/10.1093/plankt/fbu020

Cleary, A. C., & Durbin, E. G. (2016a). Unexpected prevalence of parasite 18S rDNA sequences in winter among Antarctic marine protists. *Journal of Plankton Research*, *38*(3), 401–417. https://doi.org/10.1093/plankt/fbw005

Cleary, A. C., & Durbin, E. G. (2016b). Unexpected prevalence of parasite 18S rDNA sequences in winter among Antarctic marine protists. *Journal of Plankton Research*, *38*(3), 401–417. https://doi.org/10.1093/plankt/fbw005

Clerissi, C., Brunet, S., Vidal-Dupiol, J., Adjeroud, M., Lepage, P., Guillou, L., … Toulza, E. (2018). Protists within corals: The hidden diversity. *Frontiers in Microbiology*, *9*(AUG), 1–13. https://doi.org/10.3389/fmicb.2018.02043

Connell, J. H., Hughes, T. P., & Wallace, C. C. (1997). A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. *Ecological Monographs*, *67*(4), 461–488. https://doi.org/10.1890/0012-9615(1997)067[0461:AYSOCA]2.0.CO;2

Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, *1*, 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

Crandall, E. D., Jones, M. E., Muñoz, M. M., Akinronbi, B., Erdmann, M. V., & Barber, P. H. (2008). Comparative phylogeography of two seastars and their ectosymbionts within the Coral Triangle. *Molecular Ecology*, *17*(24), 5276–5290. https://doi.org/10.1111/j.1365-294X.2008.03995.x

Crandall, E. D., Riginos, C., Bird, C. E., Liggins, L., Treml, E., Beger, M., … Gaither, M. R. (2019). The molecular biogeography of the Indo-Pacific: Testing hypotheses with multispecies genetic patterns. *Global Ecology and Biogeography*, *28*(7), 943–960. https://doi.org/10.1111/geb.12905

Cruz-Trinidad, A., Aliño, P. M., Geronimo, R. C., & Cabral, R. B. (2014). Linking Food Security with Coral Reefs and Fisheries in the Coral Triangle. *Coastal Management*, *42*(2), 160–182. https://doi.org/10.1080/08920753.2014.877761

De Luca, D., Piredda, R., Sarno, D., & Kooistra, W. H. C. F. (2021). Resolving cryptic species complexes in marine protists: phylogenetic haplotype networks meet global DNA metabarcoding datasets. *ISME Journal*, 16–18. https://doi.org/10.1038/s41396-021-00895-0

De Vargas, Colomban, Norris, R., Zaninetti, L., Gibb, S. W., & Pawlowski, J. (1999). Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(6), 2864–2868. https://doi.org/10.1073/pnas.96.6.2864

de Vargas, Columban, Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., … Karsenti, E. (2015). Eukaryotic plankton diversity in the sunlit ocean. *Science*, *348*(6237), 1261605-1/11. https://doi.org/10.1007/s13398-014-0173-7.2

DeBoer, T. S., Baker, A. C., Erdmann, M. V., Ambariyanto, Jones, P. R., & Barber, P. H. (2012). Patterns of Symbiodinium distribution in three giant clam species across the biodiverse Bird's Head region of Indonesia. *Marine Ecology Progress Series*, *444*(Baker 2003), 117–132. https://doi.org/10.3354/meps09413

DeBoer, T. S., Subia, M. D., Ambariyanto, Erdmann, M. V., Kovitvongsa, K., &

Barber, P. H. (2008a). Phylogeography and limited genetic connectivity in the endangered boring giant clam across the coral triangle. *Conservation Biology*, *22*(5), 1255–1266. https://doi.org/10.1111/j.1523-1739.2008.00983.x

DeBoer, T. S., Subia, M. D., Ambariyanto, Erdmann, M. V., Kovitvongsa, K., & Barber, P. H. (2008b). Phylogeography and limited genetic connectivity in the endangered boring giant clam across the coral triangle. *Conservation Biology*, *22*(5), 1255–1266. https://doi.org/10.1111/j.1523-1739.2008.00983.x

Decelle, J., Colin, S., & Foster, R. A. (2015). Photosymbiosis in Marine Planktonic Protists. In *Marine Protists: Diversity and Dynamics* (pp. 465–500). https://doi.org/10.1007/978-4-431-55130-0

Del Campo, J., Heger, T. J., Rodríguez-Martínez, R., Worden, A. Z., Richards, T. A., Massana, R., & Keeling, P. J. (2019). Assessing the diversity and distribution of apicomplexans in host and free-living environments using high-throughput amplicon data and a phylogenetically informed reference framework. *Frontiers in Microbiology*, *10*(OCT), 1–15. https://doi.org/10.3389/fmicb.2019.02373

Draredja, M. A., Frihi, H., Boualleg, C., Gofart, A., Abadie, E., & Laabir, M. (2019). Seasonal variations of phytoplankton community in relation to environmental factors in a protected meso-oligotrophic southern Mediterranean marine ecosystem (Mellah lagoon, Algeria) with an emphasis of HAB species. *Environmental Monitoring and Assessment*, *191*(10). https://doi.org/10.1007/s10661-019-7708-5

Duarte, C. M. (2015). Seafaring in the 21st century: The Malaspina 2010 circumnavigation expedition. *Limnology and Oceanography Bulletin*, *24*(1), 11–14. https://doi.org/10.1002/lob.10008

Dulvy, N. K., Freckleton, R. P., & Polunin, N. V. C. (2004). Coral reef cascades and the indirect effects of predator removal by exploitation. *Ecology Letters*, *7*(5), 410–416. https://doi.org/10.1111/j.1461-0248.2004.00593.x

Edinger, Jompa, J., Limmon, G., Widjatmoko, W., & Risk, M. (1998). Reef degradation and coral biodiversity in indonesia: Effects of land-based pollution, destructive …. *Marine Pollution Bulletin*, *36*(8), 617–630. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0025326X98000472%5Cnpapers3://publication/uuid/F990175E-78F1-4674-979C-0ED7C4DE7A10

Edwards, K. F., Thomas, M. K., Klausmeier, C. A., & Litchman, E. (2015). Light and growth in marine phytoplankton: Allometric, taxonomic, and environmental variation. *Limnology and Oceanography*, *60*(2), 540–552. https://doi.org/10.1002/lno.10033

Elliott, G., Mitchell, B., Wiltshire, B., Manan, I. A., & Wismer, S. (2001). Community participation in marine protected area management Wakatobi National Park, Sulawesi, Indonesia. *Coastal Management*, *29*(4), 295–316. https://doi.org/10.1080/089207501750475118

Falkowski, P. G., Barber, R. T., & Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science*, *281*(5374), 200–206. https://doi.org/10.1126/science.281.5374.200

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, *281*(5374), 237–240. https://doi.org/10.1126/science.281.5374.237

Flues, S., Bass, D., & Bonkowski, M. (2017). Grazing of leaf-associated Cercomonads (Protists: Rhizaria: Cercozoa) structures bacterial community composition and function. *Environmental Microbiology*, *19*(8), 3297–3309. https://doi.org/10.1111/1462-2920.13824

Foale, S., Adhuri, D., Aliño, P., Allison, E. H., Andrew, N., Cohen, P., … Weeratunge, N. (2013). Food security and the Coral Triangle Initiative. *Marine Policy*, *38*, 174–183. https://doi.org/10.1016/j.marpol.2012.05.033

Forster, D., Dunthorn, M., Mahé, F., Dolan, J. R., Audic, S., Bass, D., … Stoeck, T. (2016). Benthic protists: The under-charted majority. *FEMS Microbiology Ecology*, *92*(8), 1–11. https://doi.org/10.1093/femsec/fiw120

Foster, R., Hagan, A., Perera, N., Gunawan, C. A., Silaban, I., Yaha, Y., … Hodgson, G. (2006). Tsunami and earthquake damage to coral reefs of Aceh, Indonesia. In *Reef Check Foundation, Pacific Palisades, California, USA* (Vol. 33).

Fox, H. E., & Caldwell, R. L. (2006). Recovery from blast fishing on coral reefs: A tale of two scales. *Ecological Applications*, *16*(5), 1631–1635.

Fox, H. E., Mous, P. J., Pet, J. S., Muljadi, A. H., & Caldwell, R. L. (2005). Experimental assessment of coral reef rehabilitation following blast fishing. *Conservation Biology*, *19*(1), 98–107. https://doi.org/10.1111/j.1523-1739.2005.00261.x

Fox, H. E., Pet, J. S., Dahuri, R., & Caldwell, R. L. (2003). Recovery in rubble fields: Long-term impacts of blast fishing. *Marine Pollution Bulletin*, *46*(8), 1024–1031. https://doi.org/10.1016/S0025-326X(03)00246-7

Froese, R., & Pauly, D. (2021). FishBase. Retrieved from World Wide Web electronic publication website: www.fishbase.org

Futuyma, D. J., & Mayer, G. C. (1980). Non-Allopatric Speciation in Animals. *Systematic Zoology*, *29*(3), 254. https://doi.org/10.2307/2412661

Gaines, S. D., White, C., Carr, M. H., & Palumbi, S. R. (2010). Designing marine reserve networks for both conservation and fisheries management. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(43), 18286–18293. https://doi.org/10.1073/pnas.0906473107

Gaither, M. R., Bowen, B. W., Bordenave, T. R., Rocha, L. A., Newman, S. J., Gomez, J. A., … Craig, M. T. (2011). Phylogeography of the reef fish Cephalopholis argus (Epinephelidae) indicates Pleistocene isolation across the indo-pacific barrier with contemporary overlap in the coral triangle. *BMC Evolutionary Biology*, *11*(1). https://doi.org/10.1186/1471-2148-11-189

Gaither, M. R., & Rocha, L. A. (2013). Origins of species richness in the Indo-Malay-Philippine biodiversity hotspot: Evidence for the centre of overlap hypothesis. *Journal of Biogeography*, *40*, 1638–1648. https://doi.org/10.1111/jbi.12126

Gasinaite, Z. R., Cardoso, A. C., Heiskanen, A. S., Henriksen, P., Kauppila, P., Olenina, I., … Wasmund, N. (2005). Seasonality of coastal phytoplankton in the Baltic Sea: Influence of salinity and eutrophication. *Estuarine, Coastal and Shelf*

Science, *65*(1–2), 239–252. https://doi.org/10.1016/j.ecss.2005.05.018

Gilg, M. R., & Hilbish, T. J. (2003). The geography of marine larval dispersal: Coupling genetics with fine-scale physical oceanography. *Ecology*, *84*(11), 2989–2998. https://doi.org/10.1890/02-0498

Glasl, B., Bourne, D. G., Frade, P. R., Thomas, T., Schaffelke, B., & Webster, N. S. (2019). Microbial predictors of environmental perturbations in coral reef ecosystems. *Microbiome*, *7*(1), 1–13. https://doi.org/10.1101/524173

Glücksman, E., Bell, T., Griffiths, R. I., & Bass, D. (2010). Closely related protist strains have different grazing impacts on natural bacterial communities. *Environmental Microbiology*, *12*(12), 3105–3113. https://doi.org/10.1111/j.1462-2920.2010.02283.x

Gonzalez, J. M., Sherr, E. B., & Sherr, B. F. (1990). Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Applied and Environmental Microbiology*, *56*(3), 583–589. https://doi.org/10.1128/aem.56.3.583-589.1990

Gordon, A. L., & Fine, R. A. (1996). Pathways of water between the Pacific and Indian oceans in the Indonesian seas. *Letters to Nature*, *379*(6561), 146–149. https://doi.org/10.1038/379146a0

Gordon, A. L., Susanto, R. D., & Vranes, K. (2003). Cool Indonesian throughflow as a consequence of restricted surface layer flow. *Letters to Nature*, *425*(6960), 821–824. https://doi.org/10.1038/nature02013

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., … Christen, R. (2013). The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, *41*(D1), 597–604. https://doi.org/10.1093/nar/gks1160

Hagan, A. B., Foster, R., Perera, N., & Aji, C. (2007). Tsunami impacts in Aceh Province and North Sumatra, Indonesia. *Atoll Research Bulletin*, *544*, 37–54.

Halim A. (2002). *Adoption of cyanide fishing practice in Indonesia. Ocean Coastal Manage ; 45*, 45:313–23.

Hall, J. A., Barrett, D. P., & James, M. R. (1993). The importance of phytoflagellate, heterotrophic flagellate and ciliate grazing on bacteria and picophytoplankton sized prey in a coastal marine environment. *Journal of Plankton Research*, *15*(9), 1075–1086. https://doi.org/10.1093/plankt/15.9.1075

Halpern, B. S. (2003). The impact of marine reserves: Do reserves work and does reserve size matter? *Ecological Applications*, *13*(1 SUPPL.). https://doi.org/10.1890/1051-0761(2003)013[0117:tiomrd]2.0.co;2

Halpern, B. S., & Warner, R. R. (2003). Matching marine reserve design to reserve objectives. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1527), 1871–1878. https://doi.org/10.1098/rspb.2003.2405

Hasani, Q., Adiwilaga, E. M., & Pratiwi, N. T. M. (2013). The Relationship between the Harmful Algal Blooms (HABs) Phenomenon with Nutrients at Shrimp Farms and Fish Cage Culture Sites in Pesawaran District Lampung Bay. *MAKARA of Science Series*, *16*(3), 183–191. https://doi.org/10.7454/mss.v16i3.1480

He, L., Liu, F., Karuppiah, V., Ren, Y., & Li, Z. (2014). Comparisons of the Fungal and Protistan Communities among Different Marine Sponge Holobionts by Pyrosequencing. *Microbial Ecology*, *67*(4), 951–961. https://doi.org/10.1007/s00248-014-0393-6

Hilborn, R., Stokes, K., Maguire, J. J., Smith, T., Botsford, L. W., Mangel, M., … Walters, C. (2004). When can marine reserves improve fisheries management? *Ocean and Coastal Management*, *47*(3–4), 197–205. https://doi.org/10.1016/j.ocecoaman.2004.04.001

Hoeksema, B. W. (2007a). Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity : Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity : The Coral Triangle. In W. Renema (Ed.), *Biogeography, Time, and Place: Distributions, Barriers, and Islands* (pp. 117–178). https://doi.org/10.1007/978-1-4020-6374-9

Hoeksema, B. W. (2007b). *Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity: The Coral Triangle*. https://doi.org/10.1007/978-1-4020-6374-9_5

Horvath HTL. (2017). *Lombok baseline supply, market demand forecasts and investment needs*.

Huxley, T. H. (1868). On the classification and distribution of Alectromorphae and Heteromorphae. *Proceedings of the Zoological Society of London*, *36*(1), 294–319.

Ibarbalz, F. M., Henry, N., Brandão, M. C., Martini, S., Busseni, G., Byrne, H., …

Zinger, L. (2019). Global Trends in Marine Plankton Diversity across Kingdoms of Life. *Cell*, *179*(5), 1084–1097. https://doi.org/10.1016/j.cell.2019.10.008

Jagadeesan, L., Jyothibabu, R., Arunpandi, N., & Parthasarathi, S. (2017). Copepod grazing and their impact on phytoplankton standing stock and production in a tropical coastal water during the different seasons. *Environmental Monitoring and Assessment*, *189*(3). https://doi.org/10.1007/s10661-017-5804-y

Jokiel, P., & Martinelli, F. J. (1992). The Vortex Model of Coral Reef Biogeography. *Journal of Biogeography*, *19*(4), 449–458.

Jones, G P, Milicich, M. J., Emslie, M. J., & Lunow, C. (1999). Self-recruitment in a coral reef fish population. *Environmental Protection*, *402*(December), 802–804.

Jones, Geoffrey P., Planes, S., & Thorrold, S. R. (2005). Coral reef fish larvae settle close to home. *Current Biology*, *15*(14), 1314–1318. https://doi.org/10.1016/j.cub.2005.06.061

Kafouris, S., Smeti, E., Spatharis, S., Tsirtsis, G., Economou-Amilli, A., & Danielidis, D. B. (2019). Nitrogen as the main driver of benthic diatom composition and diversity in oligotrophic coastal systems. *Science of the Total Environment*, *694*, 1–9. https://doi.org/10.1016/j.scitotenv.2019.133773

Katz, S. M., Pollock, F. J., Bourne, D. G., & Willis, B. L. (2014). Crown-of-thorns starfish predation and physical injuries promote brown band disease on corals. *Coral Reefs*, *33*(3), 705–716. https://doi.org/10.1007/s00338-014-1153-2

Kooistra, W. H. C. F., Sarno, D., Balzano, S., Gu, H., Andersen, R. A., & Zingone, A.

(2008). Global Diversity and Biogeography of Skeletonema Species (Bacillariophyta). *Protist*, *159*(2), 177–193. https://doi.org/10.1016/j.protis.2007.09.004

Kool, J. T., Moilanen, A., & Treml, E. A. (2013). Population connectivity: Recent advances and new perspectives. *Landscape Ecology*, *28*(2), 165–185. https://doi.org/10.1007/s10980-012-9819-z

Ladd, H. S. (1960). Origin of the Pacific island molluscan fauna. *American Journal of Science*, Vol. 258-A, pp. 137–150.

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*. https://doi.org/10.1186/1471-2105-9-559

Le Bescot, N., Mahé, F., Audic, S., Dimier, C., Garet, M. J., Poulain, J., … Siano, R. (2016a). Global patterns of pelagic dinoflagellate diversity across protist size classes unveiled by metabarcoding. *Environmental Microbiology*, *18*(2), 609–626. https://doi.org/10.1111/1462-2920.13039

Le Bescot, N., Mahé, F., Audic, S., Dimier, C., Garet, M. J., Poulain, J., … Siano, R. (2016b). Global patterns of pelagic dinoflagellate diversity across protist size classes unveiled by metabarcoding. *Environmental Microbiology*, *18*(2), 609–626. https://doi.org/10.1111/1462-2920.13039

Lee, T., Fournier, S., Gordon, A. L., & Sprintall, J. (2019). Maritime Continent water cycle regulates low-latitude chokepoint of global ocean circulation. *Nature Communications*, *10*(1), 1–13. https://doi.org/10.1038/s41467-019-10109-z

Leray, M., Ho, S. L., Lin, I. J., & Machida, R. J. (2018). MIDORI server: a webserver for taxonomic assignment of unknown metazoan mitochondrial-encoded sequences using a curated database. *Bioinformatics (Oxford, England)*, *34*(21), 3753–3754. https://doi.org/10.1093/bioinformatics/bty454

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., … Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, *10*(1), 1–14. https://doi.org/10.1186/1742-9994-10-34

Lester, S. E., & Halpern, B. S. (2008). Biological responses in marine no-take reserves versus partially protected areas. *Marine Ecology Progress Series*, *367*, 49–56. https://doi.org/10.3354/meps07599

Lester, S. E., Halpern, B. S., Grorud-Colvert, K., Lubchenco, J., Ruttenberg, B. I., Gaines, S. D., … Warner, R. R. (2009). Biological effects within no-take marine reserves: A global synthesis. *Marine Ecology Progress Series*, *384*, 33–46. https://doi.org/10.3354/meps08029

Likumahua, S., de Boer, M. K., Krock, B., Hehakaya, S., Imu, L., Müller, A., … Buma, A. G. J. (2020). Variability of dinoflagellates and their associated toxins in relation with environmental drivers in Ambon Bay, eastern Indonesia. *Marine Pollution Bulletin*, *150*(November 2019), 110778. https://doi.org/10.1016/j.marpolbul.2019.110778

Lipps, J. H., & Valentine, J. W. (1970). the Role of Foraminifera in the Trophic

Structure of Marine Communities. *Lethaia*, *3*(3), 279–286. https://doi.org/10.1111/j.1502-3931.1970.tb01271.x

Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. *Ecology Letters*, *10*(12), 1170–1181. https://doi.org/10.1111/j.1461-0248.2007.01117.x

Liu, H., Chen, M., Zhu, F., & Harrison, P. J. (2016). Effect of diatom silica content on copepod grazing, growth and reproduction. *Frontiers in Marine Science*, *3*(JUN), 1–7. https://doi.org/10.3389/fmars.2016.00089

López-García, P., Rodriguez-Valera, F., Pedrós-Alló, C., & Moreira, D. (2001). Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Letters to Nature*, *409*(February), 603–607.

Lourie, S. A., & Vincent, A. C. J. (2004). A marine fish follows Wallace's Line: the phylogeography of the three-spot seahorse (Hippocampus trimaculatus, Syngnathidae, Teleostei) in Southeast Asia. *Journal of Biogeography*, *31*, 1975–1985.

Lundholm, N., & Moestrup, Ø. (2006). The Biogeography of Harmful Algae. In *Ecology of Harmful Algae* (Vol. 189, pp. 23–35). https://doi.org/10.1007/978-3-540-32210-8_3

MacArthur, R. H., & Wilson, E. O. (1963). An equilibrium theory of insular zoogeography. *International Journal of Organic Evolution*, *17*(4), 373–387.

Mahé, F., Vargas, C. De, Bass, D., Czech, L., Stamatakis, A., Lara, E., … Dunthorn, M. (2017a). Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nature Publishing Group*, *1*(March), 1–8. https://doi.org/10.1038/s41559-017-0091

Mahé, F., Vargas, C. De, Bass, D., Czech, L., Stamatakis, A., Lara, E., … Dunthorn, M. (2017b). Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. *Nature Publishing Group*, *1*(March), 1–8. https://doi.org/10.1038/s41559-017-0091

Mahmudi, M., Lusiana, E. D., Herawati, E. Y., & Serihollo, L. G. (2020). Environmental factors and seasonal effect on the potential harmful algae presence at Ambon Bay, Indonesia. *Biodiversitas*, *21*(7), 3101–3107. https://doi.org/10.13057/biodiv/d210730

Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., … Bowler, C. (2016). Insights into global diatom distribution and diversity in the world’s ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(11), E1516–E1525. https://doi.org/10.1073/pnas.1509523113

Mann, D. G., & Droop, S. J. M. (1996). Biodiversity, biogeography and conservation of diatoms. *Hydrobiologia*, *336*(1–3), 19–32. https://doi.org/10.1007/BF00010816

Mao-Jones, J., Ritchie, K. B., Jones, L. E., & Ellner, S. P. (2010). How microbial community composition regulates coral disease development. *PLoS Biology*, *8*(3), 22–26. https://doi.org/10.1371/journal.pbio.1000345

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 5–7.

Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., … Staley, J. T. (2006). Microbial biogeography: Putting microorganisms on the map. *Nature Reviews Microbiology*, *4*(2), 102–112. https://doi.org/10.1038/nrmicro1341

Massana, R., Gobet, A., Audic, S., Bass, D., Bittner, L., Boutte, C., … de Vargas, C. (2015). Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing. *Environmental Microbiology*, *17*(10), 4035–4049. https://doi.org/10.1111/1462-2920.12955

Mayr, E. (1944). Wallace's Line in the Light of Recent Zoogeographic Studies. *The Quarterly Review of Biology*, *19*(1), 1–14.

Mayr, E. (1954a). Change of Genetic Environment and Evolution. In *Evolution as a Process* (pp. 157–180).

Mayr, E. (1954b). Geographic speciation of tropical echinoids. *Evolution*, *8*(1), 1–18.

McManus, J. W., Nanola, C. L., Reyes, R. B., Kesner, K. N., & Mcmanus, J. W. (1997). Resource Ecology of the Bolinao Coral Reef System\rEffects of some destructive fishing methods on coral cover and potential rates of recovery. *Environmental Management*, *21*(1), 69–78.

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4).

https://doi.org/10.1371/journal.pone.0061217

Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwälder, B., Schlötterer, C., & Boenigk, J. (2010). Diversity in a hidden world: Potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Molecular Ecology*, *19*(SUPPL. 1), 32–40. https://doi.org/10.1111/j.1365-294X.2009.04478.x

Moffett, J. W., & Landry, M. R. (2019). Grazing control and iron limitation of primary production in the Arabian Sea: Implications for anticipated shifts in Southwest Monsoon intensity. *Deep-Sea Research Part II: Topical Studies in Oceanography*, (November), 104687. https://doi.org/10.1016/j.dsr2.2019.104687

Moon-Van Der Staay, S. Y., De Wachter, R., & Vaulot, D. (2001). Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. *Nature*, *409*(6820), 607–610. https://doi.org/10.1038/35054541

Moreira, D., & López-García, P. (2002). The molecular ecology of microbial eukaryotes unveils a hidden world. *Trends in Microbiology*, *10*(1), 31–38. Retrieved from http://dx.doi.org/10.1186/s13059-016-0960-x%0Ahttp://dx.doi.org/10.1038/s41587-019-0191-2%0Ahttps://doi.org/10.1016/j.tree.2019.08.008%0Ahttp://dx.doi.org/10.1016/j.protis.2010.01.003%0Ahttp://dx.doi.org/10.1038/ismej.2012.36%0Ahttp://dx.doi.org/10.1038/s

Mous, P. J., Pet, J. S., Arifin, Z., Djohani, R., Erdmann, M. V., Halim, A., … Wiadnya, G. (2005). Policy needs to improve marine capture fisheries management and to define a role for marine protected areas in Indonesia. *Fisheries Management and Ecology*, *12*(4), 259–268. https://doi.org/10.1111/j.1365-2400.2005.00448.x

Muñiz, O., Rodríguez, J. G., Revilla, M., Laza-Martínez, A., Seoane, S., & Franco, J. (2018). Seasonal variations of phytoplankton community in relation to environmental factors in an oligotrophic area of the European Atlantic coast (southeastern Bay of Biscay). *Regional Studies in Marine Science*, *17*, 59–72. https://doi.org/10.1016/j.rsma.2017.11.011

Nasution, A. K., Dian Takarina, N., & Thoha, H. (2021). The presence and abundance of harmful dinoflagellate algae related to water quality in Jakarta Bay, Indonesia. *Biodiversitas Journal of Biological Diversity*, *22*(5), 2909–2917. https://doi.org/10.13057/biodiv/d220556

Nuryanto, A., & Kochzius, M. (2009). Highly restricted gene flow and deep evolutionary lineages in the giant clam Tridacna maxima. *Coral Reefs*, *28*(3), 607–619. https://doi.org/10.1007/s00338-009-0483-y

Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., … Szoecs, E. (2020). *Package ‘ vegan ’: Community ecology package*.

Palumbi, S. R. (2003). Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, *13*(1 SUPPL.), 146–158. https://doi.org/10.1890/1051-0761(2003)013[0146:pgdcat]2.0.co;2

Pansiot, N. (2017). Change of desitination: Tara will not go to Indonesia.

Pesant, S., Not, F., Picheral, M., Kandels-Lewis, S., Le Bescot, N., Gorsky, G., … Searson, S. (2015). Open science resources for the discovery and analysis of Tara Oceans data. *Scientific Data*, *2*(Lmd), 1–16. https://doi.org/10.1038/sdata.2015.23

Pet-Soede, C., Cesar, H. S. ., & Pet, J. S. (2012). An economic analysis of blast fishing on Indonesian coral reefs An economic analysis of blast fishing on Indonesian coral reefs. *Environmental Conservation*, *26*(May 2002), 83–93.

Pet-Soede, C., Van Densen, W. L. T., Pet, J. S., & Machiels, M. A. M. (2001). Impact of Indonesian coral reef fisheries on fish community structure and the resultant catch composition. *Fisheries Research*, *51*(1), 35–51. https://doi.org/10.1016/S0165-7836(00)00236-8

Pet-Soede, L., & Erdmann, M. V. (1998). Blast fishing in Southwest Sulawesi, Indonesia. *Naga, The ICLARM Quarterly*, *21*(2), 4–9.

Peterson, T. D., Crawford, D. W., & Harrison, P. J. (2011). Evolution of the phytoplankton assemblage in a long-lived mesoscale eddy in the eastern Gulf of Alaska. *Marine Ecology Progress Series*, *424*, 53–73. https://doi.org/10.3354/meps08943

Pierce, R. W., & Turner, J. T. (1992). Ecology of Planktonic Ciliates in Marine Food Webs. *Reviews in Aquatic Sciences*, *6*(January), 139–181.

Pitman, N. C. A. (2010). Research in biodiversity hotspots should be free. *Trends in Ecology and Evolution*, *25*(7), 381. https://doi.org/10.1016/j.tree.2010.04.002

Prowe, A. E. F., Pahlow, M., Dutkiewicz, S., Follows, M., & Oschlies, A. (2012). Top-down control of marine phytoplankton diversity in a global ecosystem model. *Progress in Oceanography*, *101*(1), 1–13. https://doi.org/10.1016/j.pocean.2011.11.016

Rahmadyani, R. F., Widiarti, R., & Hendrayanti, D. (2017). Distribution analysis on cyst abundance of HAB causing dinoflagellates at green mussel (Perna viridis) culture area in Kamal Muara , North Jakarta. *Proceeding – ICM-MBT*, (November), 014–021. Retrieved from http://pksplipb.or.id/; email: journal@pksplipb.or.id

Richter, D., Watteaux, R., Vannier, T., Leconte, J., Reygondeau, G., Maillet, N., … Frémont, P. (2020). Genomic evidence for global ocean plankton biogeography shaped by large-scale current systems. *HAL Archives-Ouvertes*.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *2016*(10), 1–22. https://doi.org/10.7717/peerj.2584

Romero, O. E., Fischer, G., Karstensen, J., & Cermeño, P. (2016). Eddies as trigger for diatom productivity in the open-ocean Northeast Atlantic. *Progress in Oceanography*, *147*, 38–48. https://doi.org/10.1016/j.pocean.2016.07.011

Roriris, E. T., Agung, M. U. K., Astuty, S., & Mulyani, Y. (2017). Molecular Identification of Thermally-Tolerant Symbiotic Dinoflagellates from Hard Coral (Scleractinia) in Biawak Island, Indonesia. *Microbiology Indonesia*, *11*(4), 123–128. https://doi.org/10.5454/mi.11.4.3

Rosindell, J., Hubbell, S. P., & Etienne, R. S. (2011). The Unified Neutral Theory of Biodiversity and Biogeography at Age Ten. *Trends in Ecology and Evolution*, *26*(7), 340–348. https://doi.org/10.1016/j.tree.2011.03.024

Sadovy, Y. (2005). Trouble on the reef: The imperative for managing vulnerable and valuable fisheries. *Fish and Fisheries*, *6*(3), 167–185. https://doi.org/10.1111/j.1467-2979.2005.00186.x

Saila, S. B., Kocic, V. L., & McManus, J. W. (1993). Modelling the effects practices on tropical coral reefs. *Marine Ecology Progress Series*, *94*(1), 51–60.

Sale, P. F., Cowen, R. K., Danilowicz, B. S., Jones, G. P., Kritzer, J. P., Lindeman, K. C., … Steneck, R. S. (2005). Critical science gaps impede use of no-take fishery reserves. *Trends in Ecology and Evolution*, *20*(2), 74–80. https://doi.org/10.1016/j.tree.2004.11.007

Santoferrara, L., Burki, F., Filker, S., Logares, R., Dunthorn, M., & McManus, G. B. (2020). Perspectives from Ten Years of Protist Studies by High-Throughput Metabarcoding. *Journal of Eukaryotic Microbiology*, *67*(5), 612–622. https://doi.org/10.1111/jeu.12813

Satria, A., & Matsuda, Y. (2004). Decentralization of fisheries management in Indonesia. *Marine Policy*, *28*(5), 437–450. https://doi.org/10.1016/j.marpol.2003.11.001

Satta, C. T., Anglès, S., Garcés, E., Lugliè, A., Padedda, B. M., & Sechi, N. (2010). Dinoflagellate cysts in recent sediments from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact. *Deep-Sea Research*

Part II: Topical Studies in Oceanography, *57*(3–4), 256–267. https://doi.org/10.1016/j.dsr2.2009.09.013

Savin, M. C., Martin, J. L., LeGresley, M., Giewat, M., & Rooney-Varga, J. (2004). Plankton diversity in the bay of fundy as measured by morphological and molecular methods. *Microbial Ecology*, *48*(1), 51–65. https://doi.org/10.1007/s00248-003-1033-8

Sawall, Y., Jompa, J., Litaay, M., Maddusila, A., & Richter, C. (2013). Coral recruitment and potential recovery of eutrophied and blast fishing impacted reefs in Spermonde Archipelago, Indonesia. *Marine Pollution Bulletin*, *74*(1), 374–382. https://doi.org/10.1016/j.marpolbul.2013.06.022

Schaum, C. E., Buckling, A., Smirnoff, N., Studholme, D. J., & Yvon-Durocher, G. (2018). Environmental fluctuations accelerate molecular evolution of thermal tolerance in a marine diatom. *Nature Communications*, *9*(1), 1–14. https://doi.org/10.1038/s41467-018-03906-5

Shinoda, T., Han, W., Metzger, E. J., & Hurlburt, H. E. (2012). Seasonal Variation of the Indonesian Throughflow in Makassar Strait. *Journal of Physical Oceanography*, *42*(7), 1099–1123. https://doi.org/10.1175/jpo-d-11-0120.1

Sidabutar, T., Bengen, D. G., Wouthuyzen, S., & Partono, T. (2016). The abundance of phytoplankton and its relationship to the N/P ratio in Jakarta Bay, Indonesia. *Biodiversitas*, *17*(2), 673–678. https://doi.org/10.13057/biodiv/d170241

Sidabutar, T., Thoha, H., Bayu, M. D., Rachman, A., Sianturi, O. R., Fitriya, N., … Masseret, E. (2016). Occurrence of Pyrodinium bahamense blooms related to cyst accumulation in the bottom sediments in the bays at Ambon, Lampung and Jakarta, Indonesia. *Harmful Algae News*, (52), 8–9. Retrieved from www.ioc-unesco.org/hab

Silveira, C. B., Cavalcanti, G. S., Walter, J. M., Silva-Lima, A. W., Dinsdale, E. A., Bourne, D. G., … Thompson, F. L. (2017). Microbial processes driving coral reef organic carbon flow. *FEMS Microbiology Reviews*, *41*(4), 575–595. https://doi.org/10.1093/femsre/fux018

Simpson, G. G. (1977). Too Many Lines ; The Limits of the Oriental and Australian Zoogeographic Regions. *Proceedings of the American Philisophical Society*, *121*(2), 107–120.

Siry, H. Y. (2007). Making Decentralized Coastal Zone Management Work for the Southeast Asian Region: Comparative Perspectives. In *Oceans and Law of Sea*.

Šlapeta, J., López-García, P., & Moreira, D. (2006). Global dispersal and ancient cryptic species in the smallest marine eukaryotes. *Molecular Biology and Evolution*, *23*(1), 23–29. https://doi.org/10.1093/molbev/msj001

Smith, S. R., Dupont, C. L., McCarthy, J. K., Broddrick, J. T., Oborník, M., Horák, A., … Allen, A. E. (2019). Evolution and regulation of nitrogen flux through compartmentalized metabolic networks in a marine diatom. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-12407-y

Sommeria-Klein, G., Watteaux, R., Iudicone, D., Bowler, C., & Morlon, H. (2020). Global drivers of eukaryotic plankton biogeography in the sunlit ocean. *BioRxiv*. Retrieved from https://www.biorxiv.org/content/10.1101/2020.09.08.287524v1.abstract

Sprintall, J., & Revelard, A. (2014). The Indonesian Throughflow response to Indo-Pacific climate variability. *Journal of Geophysical Research: Oceans*, *119*, 8410–8421. https://doi.org/10.1002/2013JC009533.Received

Sunagawa, S., Acinas, S. G., Bork, P., Bowler, C., Acinas, S. G., Babin, M., … de Vargas, C. (2020). Tara Oceans: towards global ocean ecosystems biology. *Nature Reviews Microbiology*, *18*(8), 428–445. https://doi.org/10.1038/s41579-020-0364-5

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., … Lepoivre, C. (2015). *Structure and function of the global ocean microbiome*. *348*(6237), 1–10.

Suteja, Y., & Purwiyanto, A. I. S. (2018). Nitrate and phosphate from rivers as mitigation of eutrophication in Benoa bay, Bali-Indonesia. *IOP Conference Series: Earth and Environmental Science*, *162*(1). https://doi.org/10.1088/1755-1315/162/1/012021

Suteja, Yulianto, Dirgayusa, I. G. N. P., Afdal, Cordova, M. R., Rachman, A., Rintaka, W. E., … Purwiyanto, A. I. S. (2021). Identification of potentially harmful microalgal species and eutrophication status update in Benoa Bay, Bali, Indonesia. *Ocean & Coastal Management*, *210*(April), 105698. https://doi.org/10.1016/j.ocecoaman.2021.105698

Suzuki, N., & Not, F. (2015). Biology and Ecology of Radiolaria. In *Marine Protists: Diversity and Dynamics* (pp. 179–222). https://doi.org/10.1007/978-4-431-

55130-0

Swearer, S. E., Shima, J. S., Hellberg, M. E., Thorrold, S. R., Jones, G. P., Robertson, D. R., … Warner, R. R. (2002). Evidence of self-recruitment in demersal marine populations. *Bulletin of Marine Science*, *70*(1 SUPPL.), 251–271.

Sweet, M., & Bythell, J. (2012). Ciliate and bacterial communities associated with White Syndrome and Brown Band Disease in reef-building corals. *Environmental Microbiology*, *14*(8), 2184–2199. https://doi.org/10.1111/j.1462-2920.2012.02746.x

Sweet, M. J., Croquer, A., & Bythell, J. C. (2014). Experimental antibiotic treatment identifies potential pathogens of white band disease in the endangered Caribbean coral Acropora cervicornis. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1788). https://doi.org/10.1098/rspb.2014.0094

Sweet, M. J., & Séré, M. G. (2016). Ciliate communities consistently associated with coral diseases. *Journal of Sea Research*, *113*, 119–131. https://doi.org/10.1016/j.seares.2015.06.008

Syakti, A. D., Idris, F., Koenawan, C. J., Asyhar, R., & Apriadi, T. (2019). Biological pollution potential in the water of Bintan-Riau Islands Province, Indonesia: First appearance of harmful algal bloom species. *Egyptian Journal of Aquatic Research*, *45*(2), 117–122. https://doi.org/10.1016/j.ejar.2019.04.002

Tang, S., Rachman, A., Fitria, N., Thoha, H., & Chen, B. (2018). Phytoplankton changes during SE monsoonal period in the Lembeh Strait of North Sulawesi, Indonesia, from 2012 to 2015. *Acta Oceanologica Sinica*, *37*(12), 9–17.

https://doi.org/10.1007/s13131-018-1283-4

Teh, L. S. L., Teh, L. C. L., & Sumaila, U. R. (2013). A Global Estimate of the Number of Coral Reef Fishers. *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0065397

Teske, P. R., Papadopoulos, I., Newman, B. K., Dworschak, P. C., McQuaid, C. D., & Barker, N. P. (2008). Oceanic dispersal barriers, adaptation and larval retention: An interdisciplinary assessment of potential factors maintaining a phylogeographic break between sister lineages of an African prawn. *BMC Evolutionary Biology*, *8*(1), 1–14. https://doi.org/10.1186/1471-2148-8-341

Thoha, H., & Rachman, A. (2018). Marine Phytoplankton in Port and Ship's Ballast Water at Tanjung Priok Harbour, North Jakarta, Indonesia. *ASEAN Journal on Science & Technology for Development*, *35*(1–2), 71–77.

Thoha, H., Rachman, A., Sidabutar, T., Fitriya, N., Bayu, M. D., Iwataki, M., … Masseret, E. (2015). First record of the harmful dinoflagellate Cochlodinium polykrikoides in Lampung Bay, Indonesia. *Harmful Algae News*, (50), 14–15. Retrieved from www.ioc-unesco.org/hab

Thompson, D. M., Kleypas, J., Castruccio, F., Curchitser, E. N., Pinsky, M. L., Jönsson, B., & Watson, J. R. (2018). Variability in oceanographic barriers to coral larval dispersal: Do currents shape biodiversity? *Progress in Oceanography*, *165*(February), 110–122. https://doi.org/10.1016/j.pocean.2018.05.007

Thorrold, S. R., Jones, G. P., Hellberg, M. E., Burton, R. S., Swearer, S. E., Neigel, J.

E., … Warner, R. R. (2002). Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bulletin of Marine Science*, *70*(1 SUPPL.), 291–308.

Tittensor, D. P., Mora, C., Jetz, W., Lotze, H. K., Ricard, D., Berghe, E. Vanden, & Worm, B. (2010). Global patterns and predictors of marine biodiversity across taxa. *Nature Letters*, *466*(7310), 1098–1101. https://doi.org/10.1038/nature09329

Tornabene, L., Valdez, S., Erdmann, M., & Pezold, F. (2015). Support for a "Center of Origin" in the Coral Triangle: Cryptic diversity, recent speciation, and local endemism in a diverse lineage of reef fishes (Gobiidae: Eviota). *Molecular Phylogenetics and Evolution*, *82*(PA), 200–210. https://doi.org/10.1016/j.ympev.2014.09.012

Toullec, J., Vincent, D., Frohn, L., Miner, P., Le Goff, M., Devesa, J., & Moriceau, B. (2019). Copepod Grazing Influences Diatom Aggregation and Particle Dynamics. *Frontiers in Marine Science*, *6*(December). https://doi.org/10.3389/fmars.2019.00751

Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., … Pondaven, P. (2018). Influence of diatom diversity on the ocean biological carbon pump. *Nature Geoscience*, *11*(1), 27–37. https://doi.org/10.1038/s41561-017-0028-x

Treml, E. A., Roberts, J., Halpin, P. N., Possingham, H. P., & Riginos, C. (2015). The emergent geography of biophysical dispersal barriers across the Indo-West Pacific. *Diversity and Distributions*, *21*(4), 465–476.

https://doi.org/10.1111/ddi.12307

Treml, E. A., Roberts, J. J., Chao, Y., Halpin, P. N., Possingham, H. P., & Riginos, C. (2012). Reproductive output and duration of the pelagic larval stage determine seascape-wide connectivity of marine populations. *Integrative and Comparative Biology*, *52*(4), 525–537. https://doi.org/10.1093/icb/ics101

Ujianti, R. M. D., Anggoro, S., Bambang, A. N., Purwanti, F., & Androva, A. (2019). Environmental Study on Phytoplankton in Garang Watershed, Central Java, Indonesia and Its Water Quality. *IOP Conference Series: Earth and Environmental Science*, *246*(1). https://doi.org/10.1088/1755-1315/246/1/012070

Veron, J. E. N., Turak, E., Stafford-Smith, M., Stuart, K., Devantier, L. M., Green, A. L., & Peterson, N. (2009). Delineating the Coral Triangle. *Galaxea, Journal of Coral Reef Studies*, *11*(2), 91–100. https://doi.org/10.3755/galaxea.11.91

Voris, H. K. (2000). Maps of Pleistocene sea levels in Southeast Asia : shorelines , river systems and time durations. *Journal of Biogeography*, *27*, 1153–1167.

Ward, B. A., Dutkiewicz, S., & Follows, M. J. (2014). Modelling spatial and temporal patterns in size-structured marine plankton communities: Top-down and bottom-up controls. *Journal of Plankton Research*, *36*(1), 31–47. https://doi.org/10.1093/plankt/fbt097

Williams, S. L., Sur, C., Janetski, N., Hollarsmith, J. A., Rapi, S., Barron, L., … Mars, F. (2019). Large-scale coral reef rehabilitation after blast fishing in Indonesia. *Restoration Ecology*, *27*(2), 447–456. https://doi.org/10.1111/rec.12866

Williamson, D. H., Harrison, H. B., Almany, G. R., Berumen, M. L., Bode, M., Bonin, M. C., … Jones, G. P. (2016). Large-scale, multidirectional larval connectivity among coral reef fish populations in the Great Barrier Reef Marine Park. *Molecular Ecology*, *25*(24), 6039–6054. https://doi.org/10.1111/mec.13908

Woodland, D. J. (1983). Zoogeography of the Siganidae (Pisces): An interpretation of distribution and richness patterns. *Bulletin of Marine Science*, *33*(3), 713–717.

Woolsey, E. S., Byrne, M., & Baird, A. H. (2013). The effects of temperature on embryonic development and larval survival in two scleractinian corals. *Marine Ecology Progress Series*, *493*, 179–184. https://doi.org/10.3354/meps10499

Yeager, L. A., Marchand, P., Gill, D. A., Baum, J. K., & McPherson, J. M. (2017). Marine Socio-Environmental Covariates: queryable global layers of environmental and anthropogenic variables for marine ecosystem studies. *Ecology*, *98*(7), 1976. https://doi.org/10.1002/ecy.1884

Zhu, F., Massana, R., Not, F., Marie, D., & Vaulot, D. (2005). Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS Microbiology Ecology*, *52*(1), 79–92. https://doi.org/10.1016/j.femsec.2004.10.006

Zimmermann, J., Glöckner, G., Jahn, R., Enke, N., & Gemeinholzer, B. (2015). Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. *Molecular Ecology Resources*, *15*(3), 526–542. https://doi.org/10.1111/1755-0998.12336