INCEPTION REPORT

Predicting Responses between Ocean Transport and Ecological Connectivity of Threatened ecosystems in the West Philippine Sea 2 (PROTECT-WPS 2)

Under

Coastal & Marine Ecosystems Management Program (CMEMP)
Biodiversity Management Bureau (BMB)
Department of Environment and Natural Resources (DENR)

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EXECUTIVE SUMMARY

The Predicting Responses between Ocean Transport and Ecological Connectivity of Threatened Ecosystems in the West Philippine Sea 2 (PROTECT-WPS 2) program under the Coastal & Marine Ecosystems Management Program (CMEMP) of the Biodiversity Management Bureau – Department of Environment and Natural Resources (BMB-DENR) was conceptualized with the goal to build on the insights gained in the past West Philippine Sea expeditions, and further expand efforts in understanding the processes in the West Philippine Sea. This will be helpful in framing policy recommendations that may aid in sustainable utilization and conservation of the resources in the West Philippine Sea in the context of increasing threats of human activities and climate change.

For this study, we will continue with analyses of collected data and samples from the West Philippine Sea from the first PROTECT-WPS expedition under the Marine Science Institute and the DENR-BMB. We plan to visit several reef, coastal habitats, and open ocean sites, in the West Philippine Sea and near mainland Palawan during a 12-day scientific expedition targeted on May 7, 2021 to May 19, 2021. During the cruise, we plan to visit reef, open ocean, and coastal ecosystems that have been previously visited (Sabina Shoal, Northeast Investigator Shoal) in previous expeditions in the West Philippine Sea to investigate possible changes. We also plan to visit sites that have not been visited (Ayungin Shoal, Commodore Reef) to establish baseline data. In this expedition, we also plan to include sites near mainland Palawan (Quezon, Balabac, Rasa Island) to better understand the connectivity of ecosystems in the West Philippine Sea and mainland Palawan.

A surface to bottom approach with more emphasis on the interactions between oceanographic processes and biological processes will be followed. This entails investigating pelagic and benthic ecosystems, and how they are affected by or respond to physicochemical conditions in their environment. Biodiversity surveys will be expanded in this expedition; hard corals, soft corals, and several invertebrates will be collected. Moreover, more comprehensive sampling for macrophytes will also be done. Collected specimen will serve as vouchers and model organisms in further investigating the connectivity of populations found in KIG and those in onshore ecosystems of western Palawan. Lastly, more focused research and sampling design will be carried out to investigate the extent and gravity of plastic pollution in the West Philippine Sea. The presence of these plastics, first observed and reported in 2019, will become a significant threat to the understudied and largely unknown ecosystems of WPS. This holistic approach will help us gain better insights in predicting the response of these ecosystems towards emerging global changes.

OBJECTIVES

The study aims to:

a. Undertake a scientific research expedition to the WPS;
b. Revisit and collect data on biodiversity, oceanography, ecosystems, threats, among others, in previously established monitoring sites in the WPS to further contribute to long-term monitoring efforts in the area;
c. Establish new monitoring sites and new baseline data/information in the WPS to support connectivity studies;

d. Conduct initial synthesis of datasets to gain deeper understanding of the ecological processes in the region, for aiding policy making and crafting of appropriate resource management approaches.

Fig. 1 Map of proposed sites and expedition track

PROGRAM COMPONENTS

COMPONENT 1. OCEANOGRAPHY OF WPS

Component 1A. Physical and Chemical Oceanography of WPS
Study Leaders: Cesar L. Villanoy, Ph.D.
Charissa M. Ferrera, Ph.D.

Component 1B: Microbial Oceanography of WPS
Study Leaders: Deo Florence L. Onda, Ph.D.

COMPONENT 2. STRUCTURE OF HABITATS AND COMMUNITIES IN WPS

Component 2A. Diversity assessment of benthic and coral reef communities
Study Leaders: Maria Vanessa Baria-Rodriguez, Ph.D.
Rachel June Ravago-Gotanco, Ph.D.
Patrick C. Cabaitan, Ph.D
Hazel O. Arceo, Ph.D.

Component 2B. Seagrass and seaweed communities
Study Leader: Wilfred John E. Santiañez, Ph.D.

COMPONENT 1. OCEANOGRAPHY OF WPS

Component 1A. Physical and Chemical Oceanography of WPS

Study Leaders: Cesar L. Villanoy, Ph.D.
Charissa M. Ferrera, Ph.D.

Background

Water mass properties and circulation patterns influence the biodiversity and connectivity of species and ecosystems (Puerta et al. 2020). Climate change, possibly exacerbated by anthropogenic activity, is already causing changes in circulation patterns of some oceans and the physical and chemical properties of different water masses. Consequently, this may cause changes in the community composition, species distribution, functioning, and connectivity of ecosystems. This shows the need for better monitoring of the properties of different water masses, especially in areas most

vulnerable to the effects of climate change. The Coral Triangle is one such area, with the Philippines predicted to be one of the countries in the region that will be most affected by climate change (McLeod et al. 2010). Being one of the most biodiverse areas in the world and one of the most productive areas in terms of fisheries in the Philippines, the West Philippine Sea is of particular interest.

One distinct feature of the West Philippine Sea that does not occur anywhere else is the presence of a complete sequence of atolls, present in the continental shelf, the continental slope, and oceanic atolls (Guozhong 1998). This presents an opportunity to assess coral reef community structures in different reef systems, i.e. shelf and oceanic reefs. Monitoring the properties of water masses in these reefs is of particular importance as they are classified as vulnerable marine ecosystems with high associated biodiversity. This would also allow for possible prediction of the responses of the reefs in the West Philippine Sea to the changing climate.

Objectives

- Study physico-chemical factors which can help understand the distribution and observed patterns in the structure of ecosystems in the project sites, including general circulation patterns (currents, eddies, etc.) around reefs and wave exposure, seawater nutrients and carbonate chemistry, and primary productivity
- Investigate the physico-chemical properties of the target sites to gain insights on habitat connectivity between reefs in the West Philippine Sea and mainland Palawan and the adaptation of marine organisms to varying oceanographic and environmental conditions
- Determine the physico-chemical connectivity between offshore reefs in the West Philippine Sea with mainland Palawan through ocean transport

Methodology

Underway measurements and observations will be collected throughout the cruise. The underway systems include the anemometer, the side-mounted Acoustic Doppler Current Profiler (ADCP) and surface water properties such as temperature, salinity, chlorophyll, turbidity and CDOM from sensors (Thermosalinograph and Fluorometer) installed on the ship's seawater intake system. CTD stations and casts will be made at depths of up to 500m, respectively along transects contingent on schedules of reef survey and available transit time between reef sites.

Sampling for chemical analysis will be carried out onboard and while in a rubber boat using Niskin Bottles deployed in at least 3 depths per sampling point, namely in the surface, deep chlorophyll maxima (DCM) based on Chl a fluorescence, mesopelagic depth in the aphotic zone, and/or the bottom depth. Data acquired by the CTD will be calibrated with the chemical analysis results of the water samples to generate continuous vertical profiles of chlorophyll in the sampling and casting stations.

A pCO2 instrument will be attached to the underway seawater source drawn from about 5m below the water line to measure the partial pressure of CO2 along the cruise track and complement the salinity, temperature, and fluorescence data obtained by the ship's underway seawater system. Dissolved inorganic carbon (DIC) will also be measured using a colourimetric method (Coulometrics Model 5011, VINDTA, Germany). Nutrient analyses (nitrate, nitrite, ammonium, phosphate, silicate) will also be measured. All samples will be stored in liquid nitrogen tanks or ice chests for transport until they reach the laboratory.

Primary productivity measurements will be done using the light and dark bottle method. Water samples at these sites will be obtained from two depths that included the DCM (typically 60-70m at the sites that were occupied) and drawn into 3 replicates of initial, light and dark BOD bottles. Incubation will be done on deck by immersing the bottles into large plastic bins filled with flowing seawater from the ship's underway system. Replicate bottles will also be placed in black netting material to simulate light attenuation at various depths. Light (PAR) and temperature was monitored periodically during the incubation period.

Component 1B: Microbial Oceanography of WPS
Study Leaders: Deo Florence L. Onda, Ph.D.

Background

Marine microbes are at the base of the whole marine food web and thus, support the functioning of marine ecosystems. Microbial communities are also considered masters of biogeochemical processes in all ecosystems. Therefore, they are important indicators of the changing environment. Marine microbes are the key organisms in two processes that contribute largely to carbon sequestration, namely, the biological pump and the microbial carbon pump. The biological pump starts with primary production, or the synthesis of organic carbon from inorganic carbon (Legendre et al. 2015). Particulate and dissolved organic carbon then sink along the water column, with a fraction respired in the upper column, and another fraction transferred downward into the ocean's interiors (Dang 2020). The mechanism of the microbial carbon pump, on the other hand, is still relatively unclear. However, Jiao and Zheng (2011) suggest that the microbial carbon pump is responsible for transforming labile dissolved organic matter (DOM) with lifetimes of hours to days to recalcitrant dissolved organic matter (RDOM) with lifetimes of thousands of years. Microbial communities are not only vital in supporting productivity of oceanic environments but are also pivotal in the operation of biogeochemical cycles. Aside from the economic and ecological importance of microbes, these organisms also have pharmaceutical, medical, and industrial potential as they are untapped sources of novel bioactive compounds.

Microbial communities are very dynamic and shift in response to a changing climate. Biology-driven processes such as the viral shunt, the microbial loop, host-pathogen interactions, will also be affected by the changing climate (Williams et al. 2014). The response of microbial

communities to a changing ocean needs to be further understood because of its consequences on the marine food web, and consequently, fisheries.

The status of fisheries in the South China Sea has been deemed overexploited, as evidenced by declining fish catch over the past decade (Teh et al. 2017). The ecological functions and services conferred by marine biodiversity are put to risk due to the declining fish populations and marine biodiversity in this region. This may be further exacerbated by changes in the environment due to climate change. One way to study the effects of changing oceans on the function of marine ecosystems is by looking at the microbial community. As compared to organisms at the higher trophic level, photosynthetic and heterotrophic microbes respond more quickly to environmental changes since they are at the base of the marine food web. In this regard they act as "sentinels" and indicators of possible changes in trophic interactions (Bode et al. 2015; Kaur-Kahlon et al. 2016).

Objectives

- Survey and assess the diversity (functional and taxonomic) of the planktonic microorganisms (viruses, archaea, protists) and zooplankton found in the West Philippine Sea and KIG;
- Initial synthesis of historical datasets to understand physico-chemical factors driving microbial community structuring and connectivity and infer insights on their potential responses to emerging and future human (i.e., coastal eutrophication, climate-related changes) and natural changes (i.e., environmental gradients across ecological provinces).
- Assess microplastic abundance and possibly detect microplastic spatio-temporal trends in WPS

Methodology

Sampling protocols for pelagic microbial communities will mainly follow that of the standardized pipeline of the Tara Oceans consortium (see Karsenti et al. 2011; Guidi et al. 2016). Sampling will target the microbial communities (viruses, archaea, bacteria and protists) and zooplankton. For the microbes and zooplankton, nucleic acid samples and ancillary data will be collected.

Collection of DNA Samples

Sampling will be carried out using 10-L type Niskin Bottles deployed alongside a conductivity, temperature and depth (CTD) profiler (Sea-Bird Electronics, Inc., California, USA) coupled with dissolved oxygen (DO), coloured dissolved organic matter (CDOM), chlorophyll a and (Chl a) fluorescence. Collected seawater samples will be transferred to 10-L collapsible carboys prior to filtration.

DNA Filtration

For microbes, at least 5L of the water samples will be serially filtered through a 100-µm mesh, and material collected onto 3 µm pore size 47–mm polycarbonate (PC) filters (AMD Manufacturing) and then a 0.2 µm Sterivex™ filter unit (Millipore;) using a peristaltic pump (Cole-Parmer, USA). Sample filters will be directly immersed and stored in liquid nitrogen until they reach the UP MSI laboratory for downstream processing. For the zooplankton, a series of

plankton nets with different mesh sizes (>3 mm, >200 um) will be also used to collect waters at the same depths as the Niskin. Zooplankton samples will be preserved with 95% ethanol in jars.

Phytoplankton Collection

Phytoplankton samples for microscopy will be collected from the integrated depths via vertical casting of 20 µm-mesh size plankton net (Relox 2002). The plankton net will be washed via rinsing three times at the site, prior to collection. Two hundred milliliters of the sample will be collected. Cells will then be fixed by adding 1.5 mL of 37% formaldehyde, for further analysis. Half of the samples (15 mL) will be fixed with glutaraldehyde (final concentration of 1%) for CLSM and TEM processing.

Plankton samples will then be quantified using 1 mL aliquots transferred to a Sedgewick-rafter counting chamber following Yap et al. (2004). Phytoplankton counts will be carried out using a compound light microscope with 200X magnification with replicate counts for each sample. For the phytoplankton, counting will be limited to the microphytoplankton diatoms and dinoflagellates, and other microzooplankton such as ciliates and rhizaria. Identification will be down to the genus or species level whenever possible, or the lowest possible rank (i.e. phyla) when limited. Smaller phytoplankton such as chlorophytes, haptophytes, and cyanobacteria will also be noted. Species classification will follow Thomas (1997), Omura et al., (2012), Yamaji (1970), and Matsuoka and Fukuyo (2000). For the 3D high resolution imaging, sample preparation and staining will follow that of Onda et al. (2013, 2014, 2015) and Colin et al. (2017). Z-stack images will be obtained using the LSM 710 (Carl Zeiss, Germany), to reconstruct 3D images.

Collection of Microplastic Samples

Two methods will be used to collect microplastic samples: horizontal towing and underway. For horizontal towing, a weighted 20 µm-mesh size plankton net will be horizontally towed, while attached to a rubber boat, for five minutes. The distance will be calculated from the starting and ending coordinates. This will be used to arrive at the flow rate/velocity. Contents of the net will then be drained to glass jars. For underway towing, underway seawater will be passed through a filter. Filtrate will then be drained to glass jars. For both methods, glass petri dishes containing Whatman® GF/F filter papers will be exposed to the air during sampling to account for possible airborne contamination.

COMPONENT 2. STRUCTURE OF HABITATS AND COMMUNITIES IN WPS

Component 2A. Diversity assessment of benthic and coral reef communities

Study Leaders: Maria Vanessa Baria-Rodriguez, Ph.D.
Rachel June Ravago-Gotanco, Ph.D.
Patrick C. Cabaitan, Ph.D
Hazel O. Arceo, Ph.D.

Background

Coral reefs provide several important services for humans, plants, and animals. Coral reefs act as a shelter for hundreds of thousands of plant and animal species (Roberts et al. 2002) while also providing millions of jobs for people. Coral reefs also house various organisms that

have medical potential. Coral reefs are especially economically and ecologically important for archipelagic and island states, one of which is the Philippines. Fishing communities in the Philippines are highly dependent on coral reef fisheries. According to White et al. (2001) coral reefs in the Philippines provide US $1 billion per year in terms of livelihood. Moreover, coral reefs also serve as a buffer for shorelines; the economic value of which is estimated to be at $4.94 billion per year (Cruz-Trinidad et al. 2011). The Philippines is part of the coral triangle, where species richness for marine life peaks, and is said to be the center of the center of marine biodiversity (Carpenter and Singer 2005; Roberts et al. 2002).

The West Philippine Sea area covers a large portion of the total area of Philippine reefs (approximately 26,000 km^2). This is home to 484 out of 721 known reef fish species in the Philippines (Vo et al. 2013; ADB 2014). The annual fishery production in the West Philippine Seas was around 21% of the total annual fishery production in 2012, the second most productive in the country (Teh al. 2017; ADB 2014). In the KIG, more than 4 million people are heavily dependent on fishery resources. Fish stocks, however, are declining possibly due to human activity and the changing environment. Thus, it is important to assess and monitor to be able to examine its state and potential recovery.

Marine Protected Areas have been utilized to help the recovery of fish stocks and other marine populations. The efficacy of these MPAs, however, heavily relies on the population dynamics and spatial dynamics, especially larval dispersal (Willis et al. 2003). Population connectivity may be fundamental in local population dynamics and community structure (Cowen et al. 2007). Thus, information on genetic diversity and population connectivity of marine organisms in proximal habitats is pivotal in identifying appropriate spatial management techniques and units. The influences of spatial scales and patterns of dispersal and population connectivity will also be important in the design of spatially-explicit management approaches and in identifying priority populations for management purposes.

The general aim of this study is to monitor and examine the state of coral reefs by assessing coral (including hard and soft corals) and fish assemblages and determining patterns of population genetic structure and gene flow to infer reef connectivity.

Objectives

- Assess and monitor fish community assemblages found in the WPS
- Initial synthesis of historical datasets to determine distribution, abundance and diversity of selected groups of hard and soft corals in reefs of WPS exposed to different environmental and oceanographic conditions
- Collect representative coral species and invertebrates for population genetics studies to infer reef connectivity

Methodology

Coral and other benthic community assessment

The coral and other benthic community assemblage will be documented using the photo-quadrat method. Each site will be subdivided into sampling stations separated by ~100m distance and two replicates of 50 m transects will be laid at each station. Photographs of the benthos will be taken every 1 m interval along the transect using an underwater camera mounted on a tetrapod

(50 photo frames per transect) Images taken will be analyzed using Coral Point Count with excel extensions (CPCe; Kohler and Gill, 2006). In each image, 10 random sampling points will be overlaid and the benthic organisms intercepted by the points will be identified as coral (HC), dead coral (DC), soft coral (SC), algae (e.g., turf and macroalgae; ALG), invertebrates (INV) and abiotic components (e.g., sand and silt; AB). Coral genera richness and abundance will be obtained from the image, along with their respective morphological characteristics (e.g., branching, massive, etc.).

An assessment of soft corals will also be conducted using field and laboratory methods. Using the same photos taken for the benthic community assemblages, a separate image analysis will be done to determine the distribution, abundance, and covers of the different soft coral species. Additional close up photos of soft coral colonies outside the belt transect were also taken. Soft coral tissues of selected species were collected at all sites. These will be used for species identification using sclerites which will be photographed under a compound microscope and/scanning electron microscope. Voucher specimens were collected and will be transferred to the MSI museum and will serve as reference for future use. The detailed photos and the sclerite characteristics of soft corals collected will be used to validate the genera and species identification.

Reef fish community assessment

The fish visual census method (FVC method; English et al. 1997) will be used to assess the fish community assemblages. The survey area covers 10 x 50 m. Divers will survey all the fish encountered and identify them down to species level, count their abundance, and estimate their length (in cm). The biomass of each individual fish will be obtained using the formula $W = aL^b$ where a and b are species specific growth coefficients per fish species, and L is the estimated length (Froese 2006).

BRUVs Deployment

A Baited Remote Underwater Video System (BRUVs) will also be deployed to complement visual fish census surveys. Each BRUVs will consist of two GoPro cameras in custom-made housings, mounted on a base bar. One kilogram of sardines will be utilized as bait per deployment. Video footage will then be analyzed using EventMeasure v. 4.11. Fish will be identified up to species level. The software will be used to generate data on species composition, relative density, and fish size.

Collection of select corals and invertebrates

Samples of select corals (soft corals, *Heliopora coerulea,* and *Pocillopora acuta*) and invertebrates starfish (*Linckia laevigata*) will also be collected for diversity and genetic studies. For soft corals, samples will be characterized for sclerites in the laboratory and processed for voucher specimens in the MSI museum. For other corals and invertebrates, these will be used for genetic studies. A RAD-sequencing approach (Baird and Maynard 2008) will be used to interrogate the genome for single nucleotide polymorphisms (SNPs) useful for inferences regarding population demographic history, gene flow, and population structure (Reitzel et al. 2013), and the identification of neutral versus adaptive differentiation (Gagnaire et al. 2015). Whole genomic DNA will be extracted, followed by library preparation and high-throughput sequencing. Short-read sequences from analyzed individuals will be subjected to bioinformatic analysis for SNP discovery and population genomic analysis.