DATA COLLECTION PROTOCOL

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Claudia Campanini

PROJECT AIMS

This thesis project would have four main objectives:

1) characterize differences in community composition and functions among impacted and pristine sites;
2) observe the differences in community composition and impacts at different depths;
3) assess whether the eDNA signal is localized and how it changes with distance among different sites;
4) compare the efficiency of a metazoans metabarcoding approach from eDNA against diver-operated underwater visual census/video for an eventual implementation of current monitoring protocols.

LOCATIONS

site	abbr	island	coord	log_scale	pressure
Sakibaru 1	S1	Aka	26.182134, 127.275936	0	low
Sakibaru 2	S2	Aka	26.183636, 127.276719	0.1	low
Hizushi Beach	HI	Aka	26.190828, 127.272513	1	low
Cape Zanpa	CZ	Okinawa	26.441218, 127.712427	10	medium
Zatsun	ZA	Okinawa	26.837648, 128.248738	100	medium
Ginowan Port 1	G1	Okinawa	26.288120, 127.744761	0	high
Ginowan Port 2	G2	Okinawa	26.288536, 127.744863	0.1	high
Sunabe South Steps	SN	Okinawa	26.321623, 127.746061	1	high
Mizugama	MI	Okinawa	26.359467, 127.738471	10	medium
Sukuta	SK	Okinawa	26.569222, 127.979799	100	high
Yonama	YO	Okinawa	26.852150, 128.250316	100	medium
Awa	AW	Okinawa	26.600749, 127.913589	100	high

BEFORE THE DIVE

ENVIRONMENTAL DATA

REMOTE

- SST (from MODIS Aqua Data, GIOVANNI)
- ChlA (turbidity proxy, from MODIS Aqua Data, GIOVANNI)
- Degree Heating Week from Coral Reef Watch
- Impact scoring system per each site (DiBattista et al., 2020)
- Reef type
- Exposure
- Tide
- Wave

ON-SITE:

- *In situ* coord
- Shore distance
- Date
- Weather condition
- Visibility
- Relevant photos of disturbances (trash/nets/diseases/…)

FROM DIVE COMPUTER

- Transect depth intervals
- Time of the dive
- Bottom T
- Surface temperature

DIVE PROTOCOL (following GUE EDGE structure of Global Underwater Explorers)

GOALS

OBJECTIVES

OBJECTIVE (DATASET)	METHOD	RESPONSE VARIABLES
Fish diversity	Video transect	→ fish richness → fish abundance
Relative benthic cover	Photo Line Intercept Transect (PLIT)	→ total hard coral cover (%) by growth form → total Algal cover (%) by functional group → total non-living substrate cover (%) → total cover of other benthic organisms (soft coral, zoantharians, etc.)
Echinoderm diversity	Belt transect (4m*25m=100m^2) + eventual photos for id	→ echinoderms abundance by class/genus
Mollusks diversity	Belt transect (2m*25m=50m^2) + photos for id	→ mollusks abundance by class/genus
Metazoans community composition	eDNA water sampling	→ MOTUs richness → …

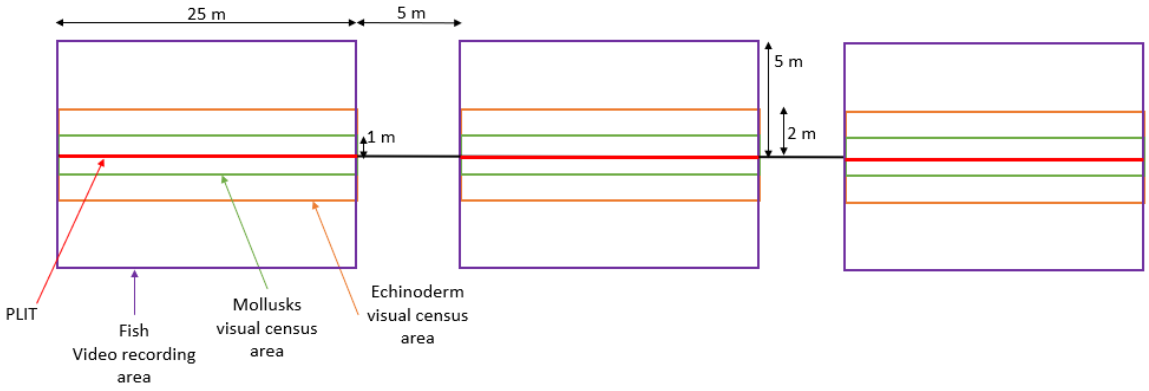
For each site, the sampling will be carried out at each location at two different depths, approximately 3-5 and 13-15 m, which correspond to reef flat/crest and reef slope respectively, depending on the site. The divers will perform 3 25-m-

long line transects at each depth level set generally parallel to shore; consequently, 6 spatially structured replicates will be obtained for each location.

SINGLE DATASET METHODS

METHOD	DATASET	PROTOCOL
PLIT	Benthic cover	1) Take a picture of the slate with depth and transect number or hand before starting with a transect. 2) Take a picture every 0.5 m (20 pic/transect) at a constant vertical distance (around 1 m). SEE (Nakajima et al., 2010)
Video	Fish diversity	The fish video recorder will swim 1.5 meters from the bottom at a slow constant pace (~3 s/m). The camera will be placed in an underwater housing, kept steady, and perpendicular to the bottom. Surveyed transect area = transect length * camera lens angle *distance to the seabed (camera altitude) <u>N.B. Use always the same camera or cameras with the same lens angle</u>
Visual census + photos for ID	Mollusks Echinoderm	1) Take a picture of the slate with depth and replicate number before starting with a transect. 2) Visual census performed by two divers along the line transect. Focus on all visible macro-mollusks / echinoderms (>1 cm)
eDNA water sampling	Metazoans community composition	3 replicates of eDNA will be sampled for each depth at the end of the dive. 1 field control will be taken at the diving site. 2 L bottle of mineral water will be kept open outside for about 5 min before pouring the water in the sampling bag. Samples will be kept in a cooler box until filtering at the lab.

3 × 25-long transects



N.B. This scheme is theoretical, distance between transects can be adjusted according to site conditions.

Tasks:

- T1 - Deployment measuring tape + Video transect (fish)
- T2 - PLIT
- T3 - Visual census for mollusks
- T4 - Visual census for echinoderms
- T5 - Line retrieval
- T6 - eDNA sampling
- T7 – Transect cleaning

UNIFIED TEAM

Each diver will focus on a target dataset and will be assigned different tasks prior to the dive. The timeline is just theoretical.

Timeline:

D1	T1- T6-
D2	T2- T7
D3	T3-
D4	T4-

EQUIPMENT

DIVE

- 50m-long measuring tape) x2
- 5x wet notes
- 4 cameras*
- 5 rulers (as a reference) for visual census
- Sampling bags for eDNA

POST-DIVE:

- Cooler boxes for eDNA
- Marker to tag the sampling bags
- 2 L water for field control
- Gloves
- 10% bleach

*Ideally with a minimum quality of 1080 with 60fps for video transects

EXPOSURE

DIVE FOR SHALLOW TRANSECTS

60 min / average depth around 3-8 m

DIVE FOR DEEP TRANSECTS

60 min / average depth around 10-15 m

DECOMPRESSION

minimum decompression strategy

GAS

From MISE diving rules:

- 100 (PSI 1500): start to return to shore, surface, entry point, and inform your buddy of your air level. You should have no less than 50 (PSI 500) when you leave the water.
- Inform your buddy @ 50 as well.

Maximum distance from the entry point: ~85 m

ENVIRONMENT

site-specific recommendations

SAVING FILES/FOLDERS

Upload data in the shared cloud folder.
Name folders/files as follows:

"Nested labelling"	
SITE ABBREVIATION	See location table
DATE	DD_MM
DEPTH	S (shallow), D (deep)
REPLICATES	1,2,3
DATASET	PLIT, ech, mol, fish, eDNA
DIVER	write your initials

Example: folder for coral cover of the PLIT from the shallow transect, replicate 2, collected by Claudia Campanini from Sunabe (05/05/22)

SN_05_05_S2_PLIT_CC

REFERENCES

Nakajima, R., Nakayama, A., Yoshida, T., Kushairi, M., Othman, B., Toda, T., 2010. An evaluation of photo line-intercept transect (PLIT) method for coral reef monitoring. https://doi.org/10.3755/GALAXEA.12.37