REPORT AND FINDINGS ON CIGUATERA INDONESIA PROJECT 2022-2023

Indonesian Ciguatera Science Team

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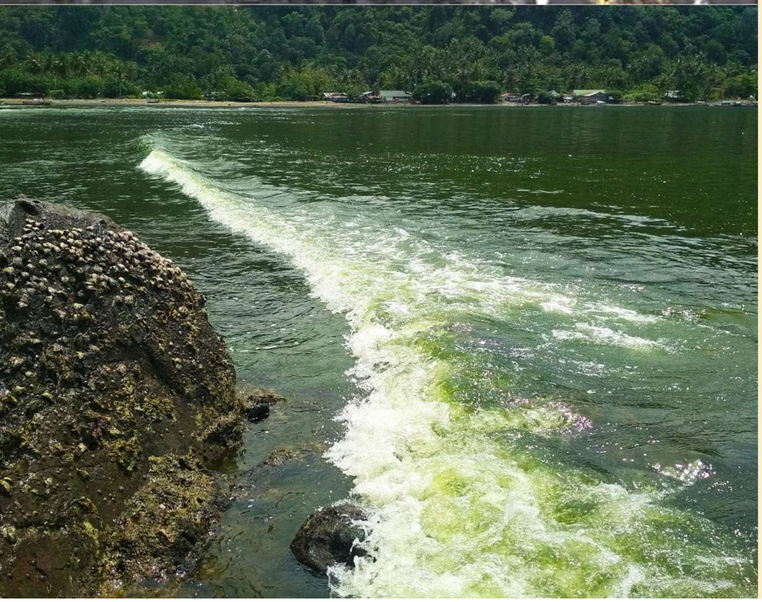
INTRODUCTION



Harmful Algal Blooms (HABs)

- Harmful Algal Blooms (HABs) → one among <u>10 Plagues of the Seas</u> → the occurrence could threaten the ecosystem balance and the life of coastal communities (Duarte et al., 2014)
- Harmful effects (GEOHAB, 2000) →
  - *Ocean discoloration*
  - *Mass fish mortality/fish kill*
  - *Toxin contamination of seafood products*
  - *Altering/disrupting the balance of the ecosystem*
  - *Danger to the health of humans (poisoning cases could lead to death)*
  - *Negatively impacting the economy of coastal communities*

HAB Dashboard

Plankton and Primary Productivity Laboratory

(Mariana D. B. Intan, 2019)

Distribution of reported HABs cases in Indonesia during the periods of 1979 - 2019

Lack of awareness and research on HABs in Indonesia → low report or publications → HABs cases in Indonesia is ***underreported***

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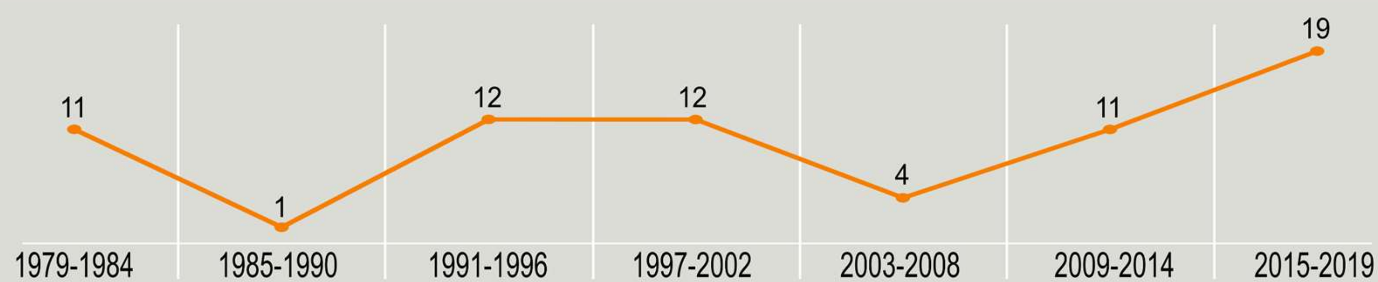
*angka menunjukkan jumlah kejadian



Total Kejadian HAB dalam 5 Tahun Periode

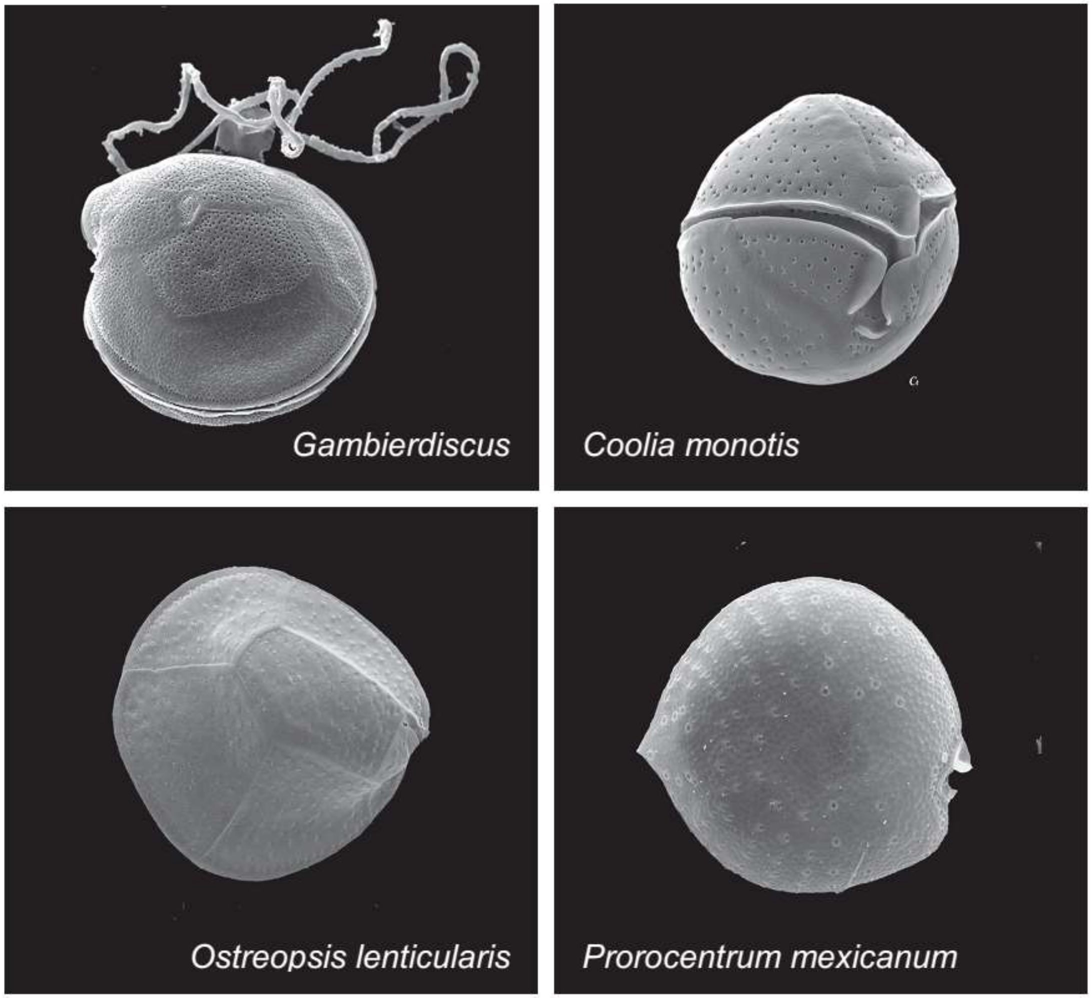


Figure 4. Morphology of *Gambierdiscus*, *Coolia*, *Ostreopsis* and *Prorocentrum* is illustrated in scanning electron micrographs. Cell dimension are estimated by the length and width of the species: *Gambierdiscus* 53-85 µm x 44-58 µm; *Coolia monotis* 23-49 µm x 23-38 µm; *Ostreopsis lenticularis 65-75* µm x 57-63 µm; and *Prorocentrum mexicanum* 32-40 um x 26-30 µm.

(Faust et al. 2009)

Ciguatera Fish Poisoning

- Ciguatera Fish Poisoning → poisoning disease in human or marine mammals due to consumption of reef fishes that are contaminated by ciguateoxin (CTX) produced by several species of benthic dinoflagellates → *Gambierdiscus toxicus* and other associated species → *Ostreopsis ovata, Prorocentrum lima, P. concavum, P. mexicanum (rhathymum)*, and *Amphidinium carterae* (Burkholder 1998; Lehane and Lewis 2000)
- Known symptoms of CFP (deSylva 1994; Lehane dan Lewis 2000) :
  - *diarrhea*
  - *nausea*
  - *vomitting*
  - *stomachache*
  - *reversal of cold-hot sensation*
  - *muscles and joints pain*
  - *tingling (often painful)*
  - *numbness on lips and tongue*
  - *itch*
  - *hypotension (low blood pressure)*

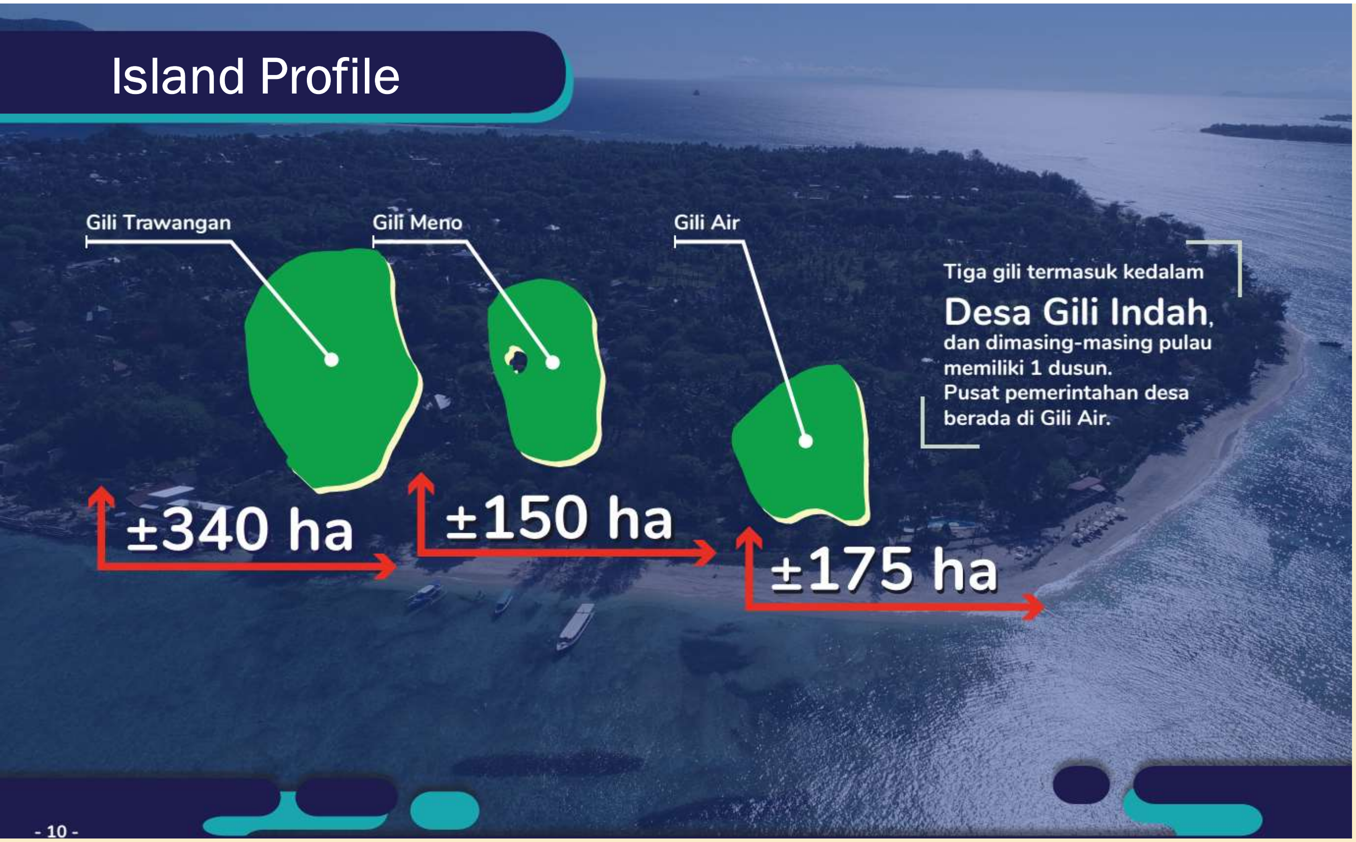
Records of benthic dinoflagellate species associated with CFP

Benthic dinoflagellates which could potentially caused CFP → *Amphidinium* sp., *G. toxicus*, *O. ovata*, *O. siamensis*, *P. lima*, *P. concavum*, dan *P. rhathymum, Gambierdiscus* sp., *Ostreopsis sp* → have been reported and studied from several places in Indonesia:

- Seribu Island
- Belitung Island
- Bali coastal waters
- West coast of South Sumatera
- Bintan Island
- Padang coastal waters
- Lampung Bay
- Weh Island coastal waters
- Gili Matra

Widiarti 2002, Widiarti 2010, Skinner et al. 2011, Widiarti 2011, Thamrin 2014, Dwivayana 2015, Eboni et al. 2015, Oktavian et al. 2015, Seygita et al. 2015, Widiarti & Pudjiarto 2015, Widiarti et al. 2016a, Widiarti et al. 2016b, Widiarti & Adi 2016, Widiarti et al. 2019

bHABs and CFP → not yet considered as a major threat to Indonesian coastal communities or ecosystems (no formal report or huge cases) → lack of awareness and studies



Source: Balai Kawasan Konservasi Perairan Nasional Kupang Wilker TWP Gili Matra, 2019

Sampling Site

- Gili Matra Marine Tourism Park (Taman Wisata Perairan/TWP) → Gili Trawangan, Gili Meno, Gili Air
- An important conservation and tourism area to the local people and marine biota in the coastal area of West Lombok
- Conservation area → 2.273,56 ha
- Important coastal ecosystems:
  - *Mangrove*
  - *Coral Reef*
  - *Seagrass*
- Ecologically vital to some protected and charismatic rare species, such as :
  - *Hiu Sirip Hitam (Blacktip reef shark)*
  - *Hiu Sirip Putih (Whitetip reef shark)*
  - *Penyu (Sea turtle)*
  - *Kima (Giant clam)*
  - *Pari Manta (Manta rays)*

ESTIMATED ECONOMIC VALUE OF GILI MATRA

The estimated value of natural resources and environmental services of Gili Matra National Park was approximately IDR 26,86 trillion in 2019

Coral Reefs
IDR 25,97 trillion

Seagrass
IDR 10,25 billion

Mangrove
IDR 101,54 billion

Coastal waters
IDR 777,38 billion

Sumber: Balai Kawasan Konservasi Perairan Nasional Kupang Wilker TWP Gili Matra, 2019

Joint Scientific Collaboration

- "Ciguatera Indonesia" → research collaboration beyond institutional and country borders
- Involving researchers, academics, experts, and university students from many different disciplines
- The current funding support are come from:
  - PICES-MAFF Ciguatera Project 2020-2023
  - Program House of Biological Research Organization, BRIN (Rumah Program Organisasi Riset Ilmu Pengetahuan Hayati – BRIN)
  - Expedition and Exploration Fund, BRIN (Pendanaan Ekspedisi dan Eksplorasi – BRIN)

Funding and Scientific Support

Program House of Biological Research Organization, BRIN
(Rumah Program OR IPH BRIN)

PICES-MAFF Ciguatera Project
(International Joint Collaboration)

Expedition and Exploration Fund, BRIN
(Pendanaan Ekspedisi dan Eksplorasi BRIN)

Ciguatera Indonesia

BRIN-IRD SELAMAT
(Indonesia & Prancis)

(Supported; term under negotiation)

Integrated Multidisciplinary Research

- Ciguatera Indonesia → integrated multidisciplinary research
- Including several topics (but not limited to):
  - *Biological Oceanography → planktonology dan benthic micoralgal ecology and taxonomy*
  - *Chemical Oceanography → nutrient level and water column chemical properties*
  - *Physical Oceanography → water column's physical properties*
  - *Coastal Ecology → ecology of important coastal ecosystems, such as seagrass, coral reefs, and macroalgal beds*
  - *Information Technology → the use of smartphone application, real-time monitoring via satellite imageries, and machine learning and Artificial Intelligence*
  - *Social-Economic → anthropogenic activities, ecosystem economic valuation*

Research Aims

Ciguatera Indonesia

To study the benthic dinoflagellate communities which could potentially cause CFP and their relationship with habitat condition

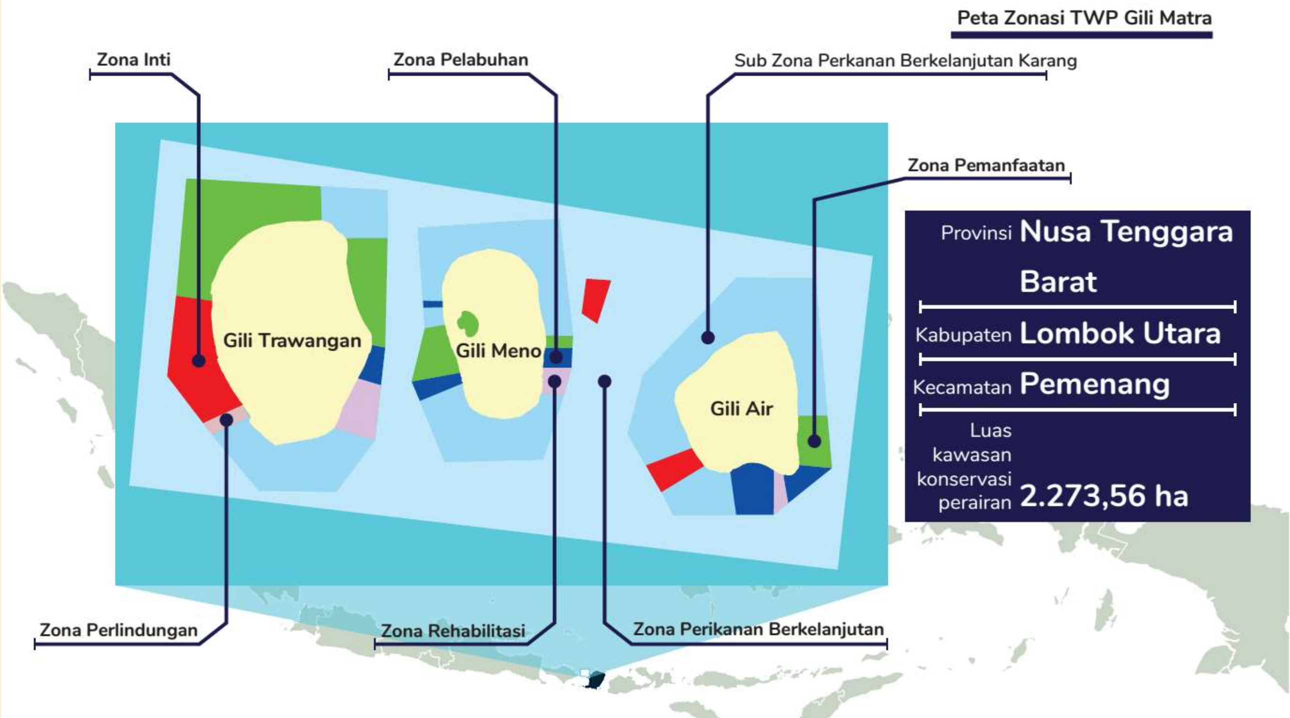
To study the level of anthropogenic pressures and potential economic loss that might be caused by HABs and/or CFP

To disseminate information and increase the local public awareness on the potential danger of HABs and CFP

SAMPLING METHODS

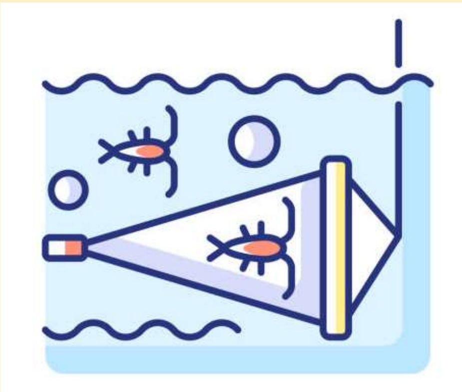
Sampling Area

- Sampling and data collection → will be conducted within selected zones around the Gili Trawangan, Gili Meno, dan Gili Air
- There would be 5 fieldwork within the timeframe of 2022-2023 → May (Transition I), August (Dry Season), October (Transition II), November/Desember (Rainy Season), Januari/Februari (End of Rainy Season)
- Fieldwork → 5-6 days → 3-4 days effective working days
- Microalgal sampling →
  - *Water column,*
  - *Seagrass,*
  - *Macroalgae (on coral reefs)*



Sumber: Balai Kawasan Konservasi Perairan Nasional Kupang Wilker TWP Gili Matra, 2019

Water column sampling



Plankton (zooplankton, phytoplankton)

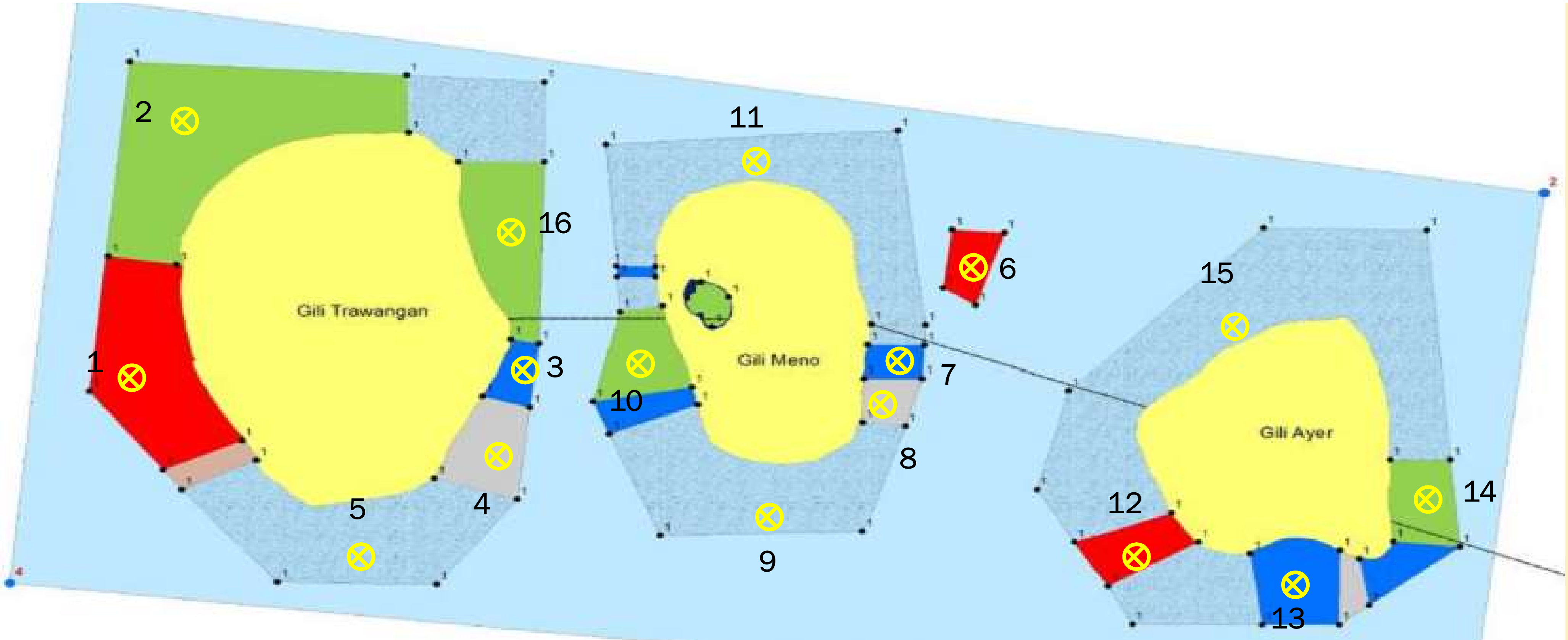
- *Vertical towing with plankton net (zooplankton net, mesh 125 um; phytoplankton net, mesh 20 um)*

Water (nutrient, chlorophyll-a, eDNA?)

- *Van Dorn / Nansen bottle → at minimum, 1 sample at surface layer (0.5 – 1 m depth); if possible, 2 sample (surface + near bottom)*

Water quality

- *Water multiparameter tester or separate measurement devices :*
  - pH meter (pH),
  - hand refractometer (salinity),
  - DO meter (DO and oxygen saturation),
  - TDS meter (turbidity),
  - Digital thermometer (temperature),
  - secchi disk (light penetration depth),

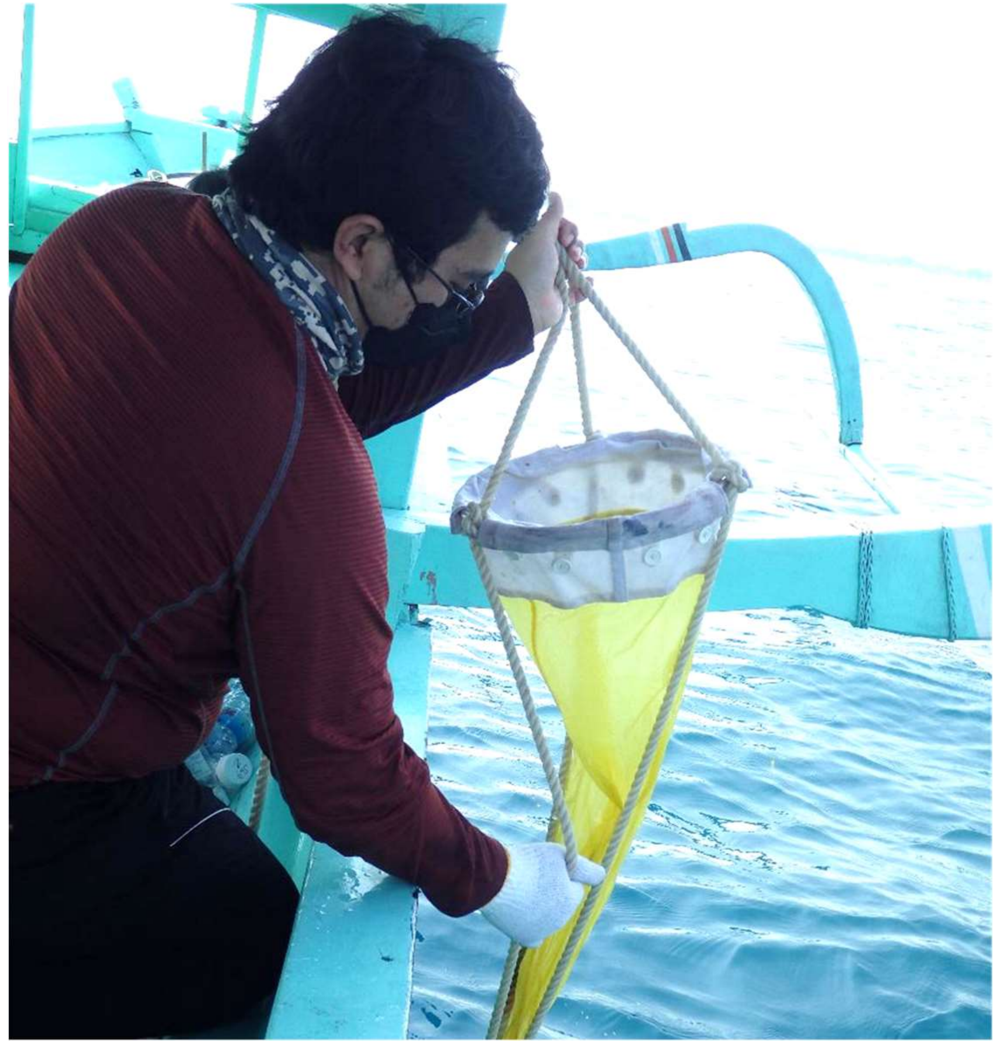


⊗ Water column sampling sites → 16 Sites

- Plankton (phytoplankton + zooplankton) → 32 sample
- Water quality (Temperature, pH, Salinity, DO, TDS) → 16 dataset
- Nutrient concentration → 16 sampel
- Chlorophyll-a
- e-DNA (?)

Sampling sites for water and plankton samples (blue→ prioritized sites if the weather condition is not good)

Station	Long (E)	Lat (S)	Colour Code	Zone	Island
1	116.0236	-8.35352	Red	Core	Gili Trawangan
2	116.0265	-8.33868	Green	Utilization	Gili Trawangan
3	116.0455	-8.3535	Blue	Harbour	Gili Trawangan
4	116.0443	-8.36012	Grey	Rehabilitation	Gili Trawangan
5	116.0358	-8.36574	Light-blue	Sustainable Fisheries	Gili Trawangan
6	116.0681	-8.34595	Red	Core	Gili Meno
7	116.0649	-8.35293	Blue	Harbour	Gili Meno
8	116.065	-8.35575	Grey	Rehabilitation	Gili Meno
9	116.0572	-8.36373	Light-blue	Sustainable Fisheries	Gili Meno
10	116.0502	-8.35152	Green	Utilization	Gili Meno
11	116.0532	-8.33727	Light-blue	Sustainable Fisheries	Gili Meno
12	116.0722	-8.36524	Red	Core	Gili Ayer
13	116.0832	-8.36693	Blue	Harbour	Gili Ayer
14	116.0909	-8.36069	Green	Utilization	Gili Ayer
15	116.0845	-8.34567	Light-blue	Sustainable Fisheries	Gili Ayer
16	116.0448	-8.34519	Green	Utilization	Gili Trawangan

Benthic microalgal sampling

Benthic microalgae

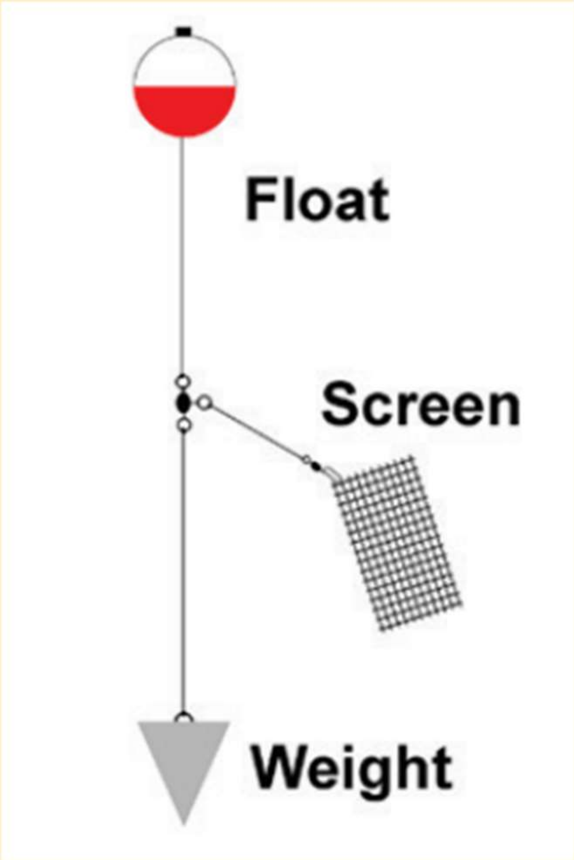
- *Natural substrat (free dive dengan snorkeling)*
- *Artificial substrat (free dive dengan snorkeling)*

Water

- *Van Dorn / Nansen bottle → minimal 1 sampel di lapisan tengah (0.5 – 1m dari dasar/habitat/substrat)*

Water quality

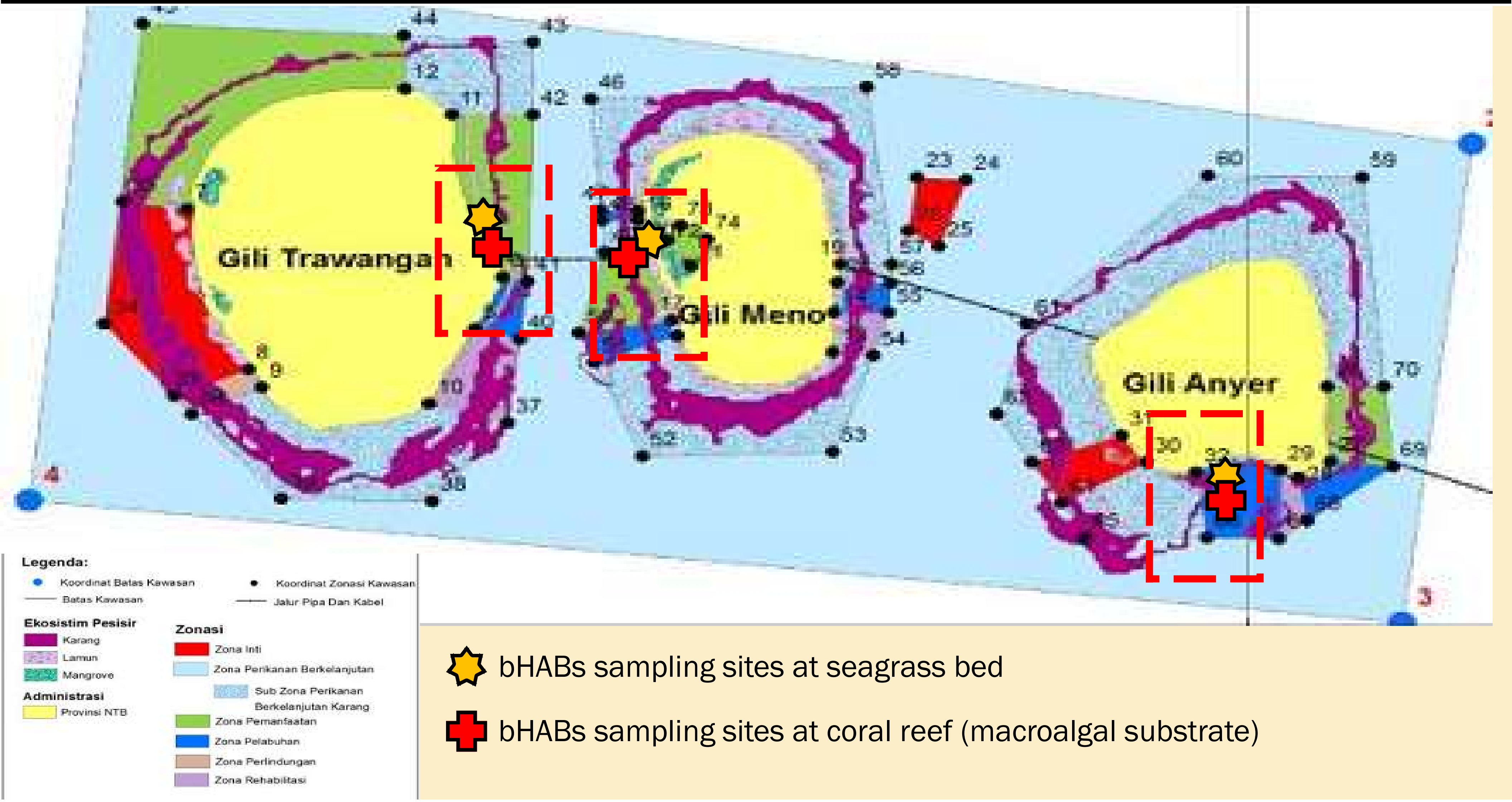
- *Water multiparameter tester or separate measurement devices :*
  - pH meter (pH),
  - hand refractometer (salinity),
  - DO meter (DO and oxygen saturation),
  - TDS meter (turbidity),
  - Digital thermometer (temperature),
  - secchi disk (light penetration depth),



Artificial substrate (IOC-UNESCO Standard 2016)

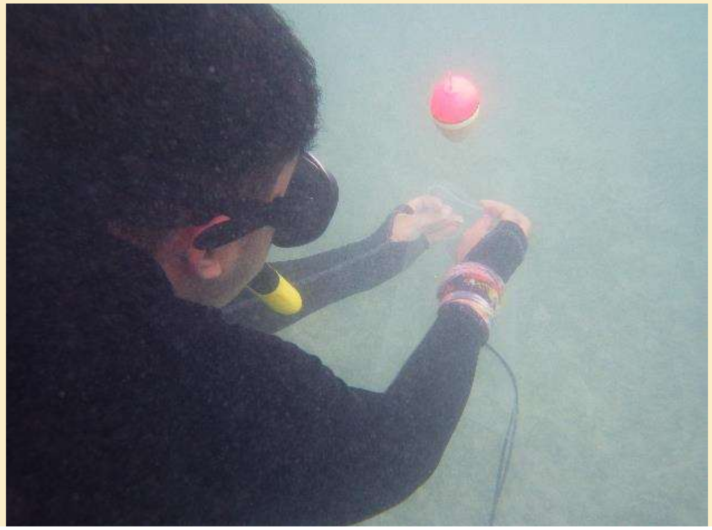
- Deployed close or at the middle of habitat/substrates (macroalgae/seagrass)
- Will be collected after a minimum of 24 hours

Sampling sites based on Mei 2022 sampling → only 3 permanent sites were established due to on-field situation

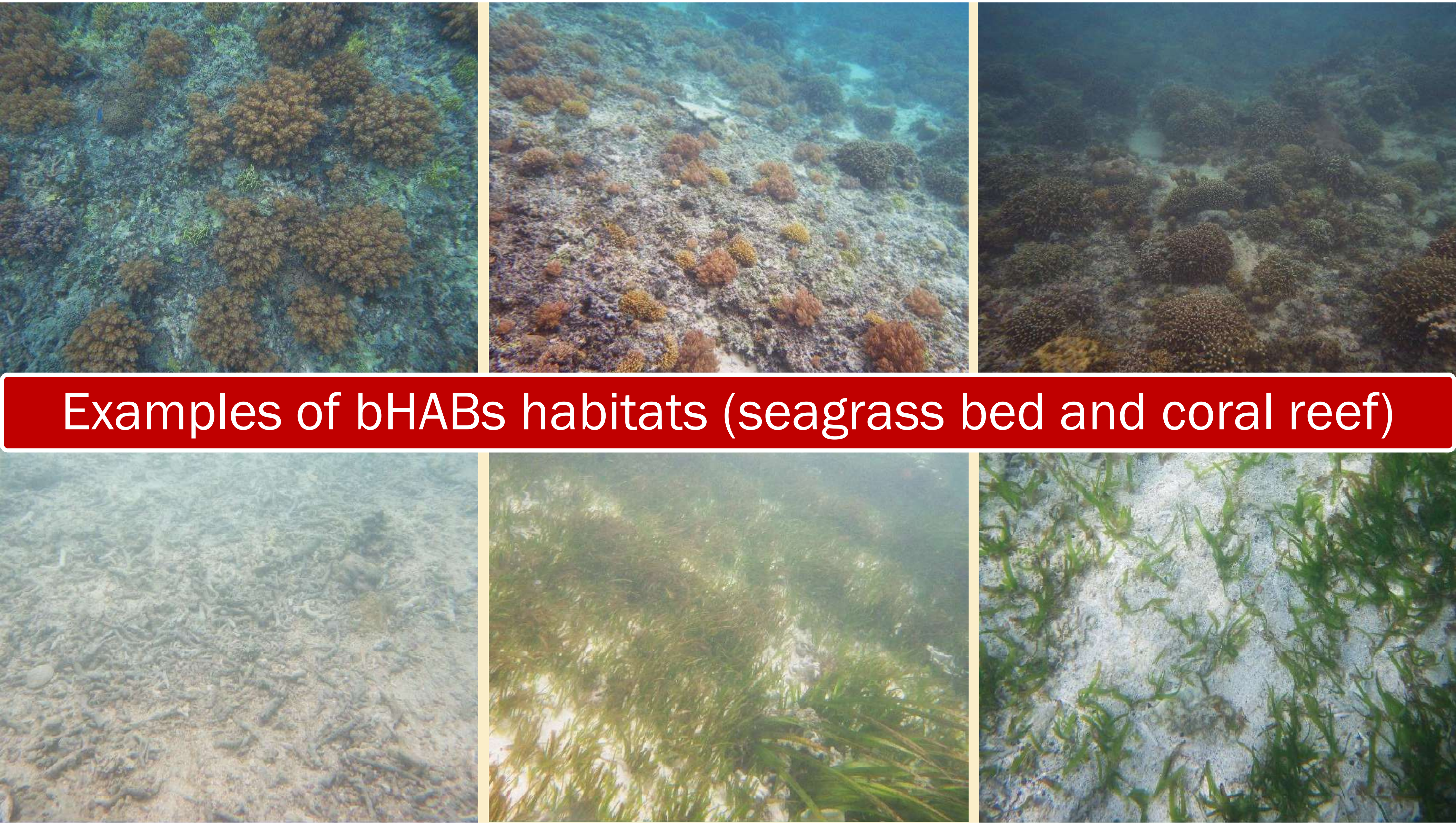


bHABs sampling sites at seagrass bed

bHABs sampling sites at coral reef (macroalgal substrate)



bHABs sampling with *Artificial Substrate*



Examples of bHABs habitats (seagrass bed and coral reef)

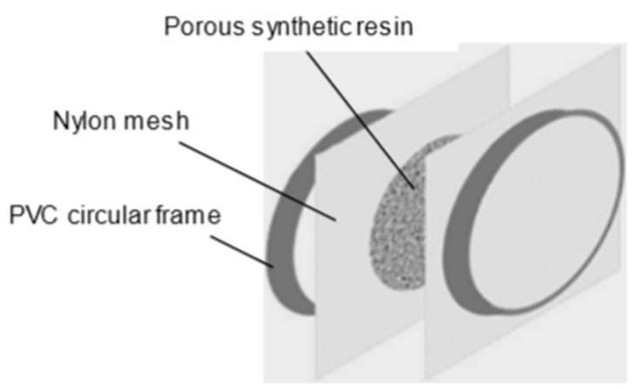
SPATT for ciguatoxin collection from water column

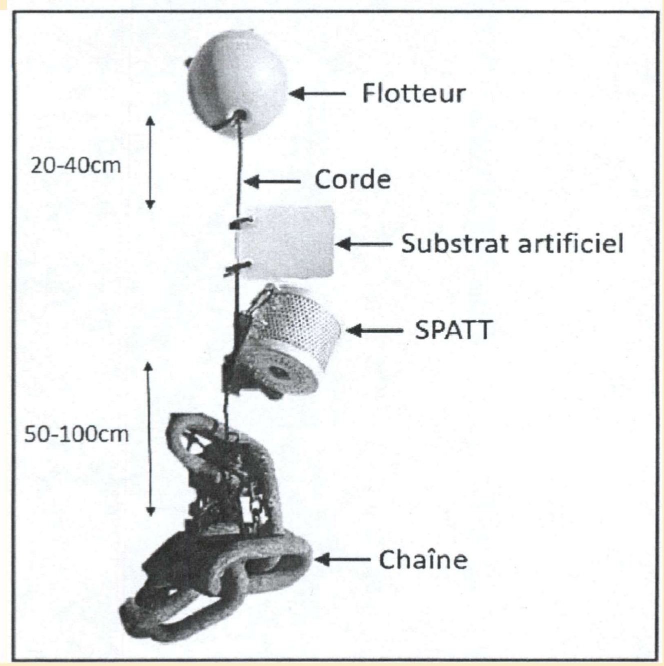
- **Solid Phase Adsorption Toxin Tracking (SPATT)** → new method to collect the phytotoxin substances directly from the water column → SPATT using in this study have been treated/prepared specifically to collect ciguatoxin (CTX)
- **SPATT components** → *synthetic resin* in a 'sandwich' of two layers of nybolt mesh → the resin was activated with absolute methanol (99%) → arranged in a rig along side several *artificial substrates* → *deployment time* 7-10 hari (standard protocol) → in Ciguatera Indonesia → **SPATT was deployed for 5 days in December 2022**
- **Retrieved SPATT** → stored frozen in temperature <10ºC → CTX analysis will be conducted in November 2023 in France (IRD / University of Montpelier)

BRIN
BADAN RISET DAN INOVASI NASIONAL

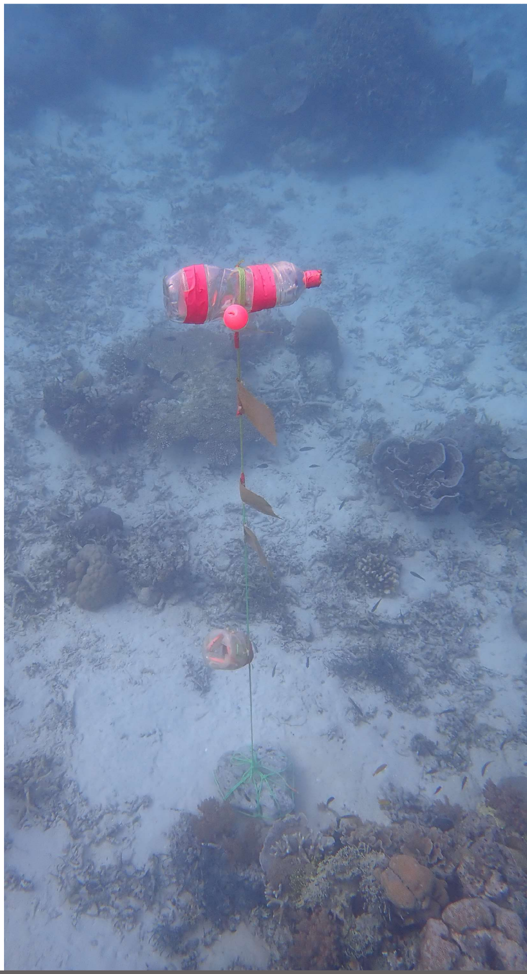
French National Research Institute for Sustainable Development
IRD
Institut de Recherche pour le Développement
FRANCE

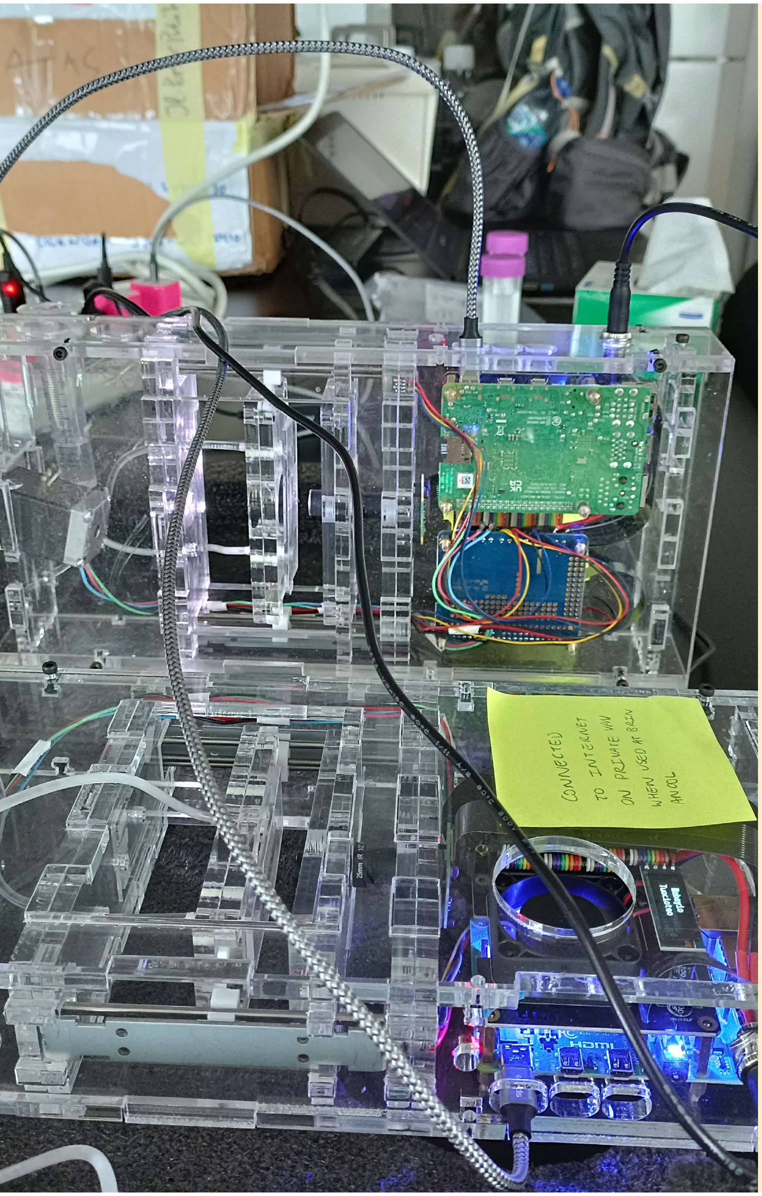
SEntinel LAboratory of the Indonesian MArine biodiversiTy
IJL Selamat





SPATT Deployment in one selected site in Gili Meno

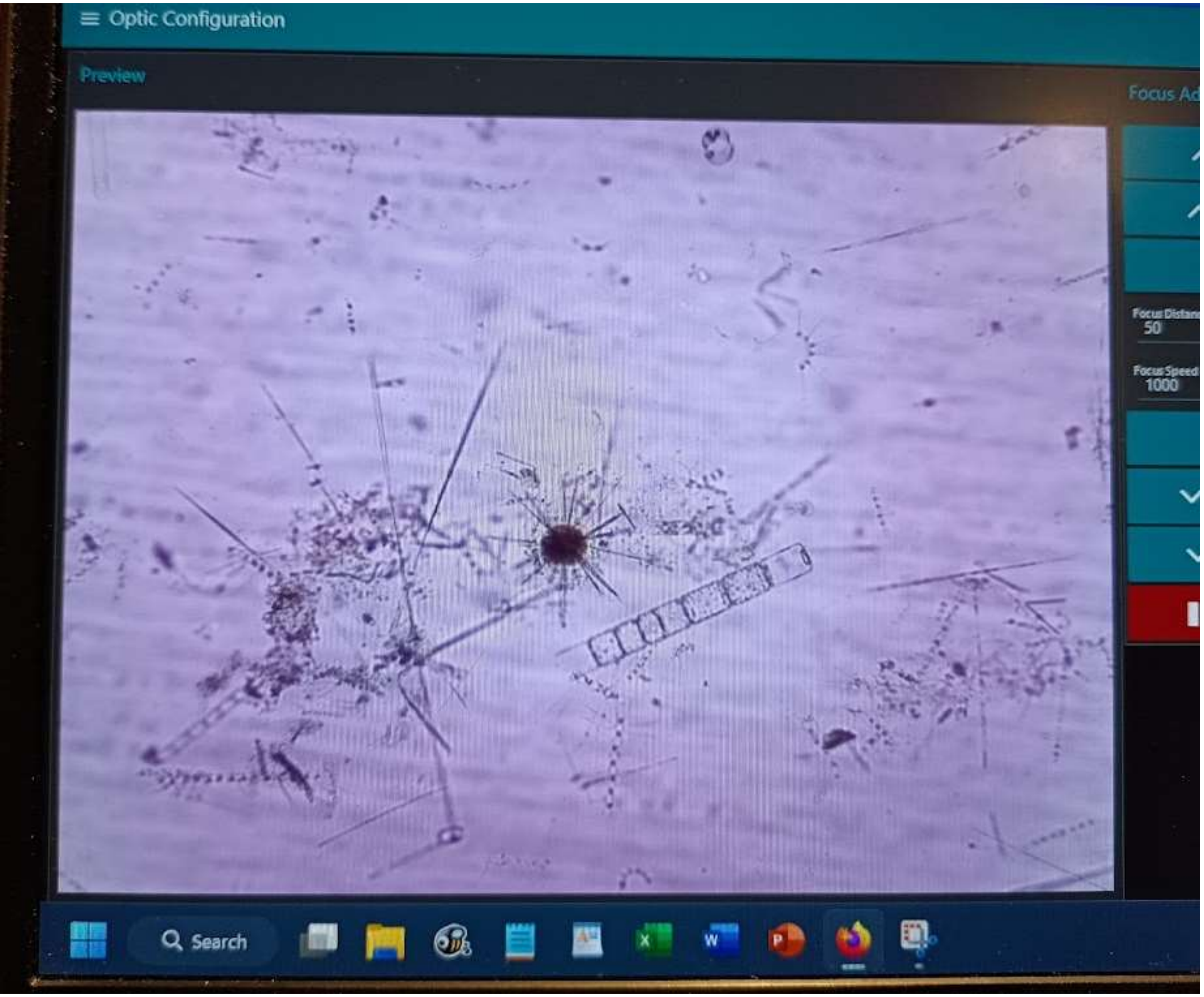


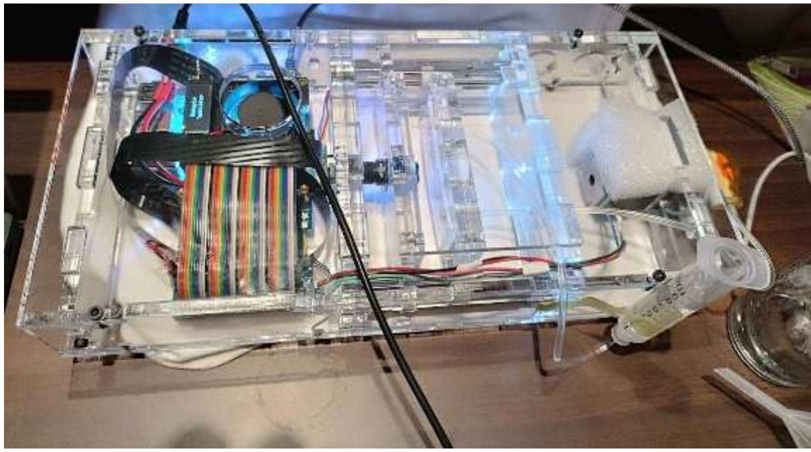


Planktoscope analysis

- Plankton samples for analysis will be collected from
  - *Plankton Net samples*
  - *Water samples*
  - *Macrophyte natural samples*
- Samples will not be preserved → to avoid staining in the microfluidic column
- Fresh sample will be analyzed with Planktoscope at hotel
- If the analysis can't be carried out directly at the same day → sample can be stored in low temperature (4ºC) to reduce the rate of decomposition
- Samples that have been analyzed → will be preserved with Alcohol 70%

Analysing live/fresh phytoplankton sample with Planktoscope

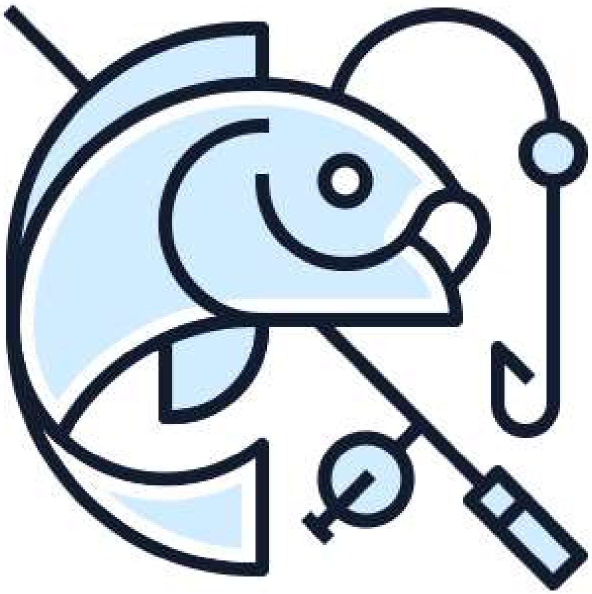




Social-economy sampling/data collection

On-site survey	Questionnaire	Focus Group Discussion	Secondary data
• Random sampling by finding respondents at the study area (Gili Matra and coastal area of West Lombok) • On-site interview with the help of questionnaire	• Spreading paper questionnaire to the respondents or selected groups of respondents (purpose sampling) • Spreading digital questionnaire via Google Form to gather information at wider scale to random respondents	• Discussion with local community or other important/relevant stakeholders (local government, academics, NGO, fisheries department, conservation department, etc)	• Collecting secondary data from related institutions or local government

Socio-economics data sampling via direct interview with local people in Northern Lombok and Gili Matra



Fish Sampling for Ciguatoxin Analysis

Fish Sampling

Fish sampling → conducted by buying fish from local market in Lombok or in Gili (possibly, in Gili Trawangan) OR by the aid of fisherman who catch coral reef fishes

Targeted fish → Coral reef fishes that was sold and (most likely) catch locally around Gili Matra or Lombok

Sample Handling

Fish tissue → viscera, gill, body flesh (min. 500gr) will be collected from each species → will be frozen until analysis

Toxin analysis → LC50 via mouse bioassay in the laboratory of the Fish Quarantine and Inspection Agency, Ministry of Marine Affairs and Fisheries, Indonesia

Fish tissue (ciguatoxin analysis)

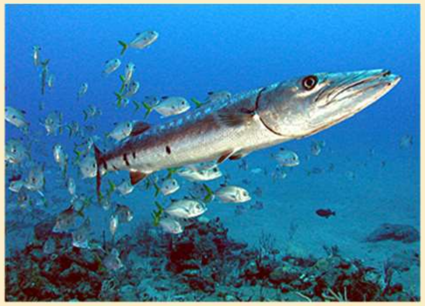
- Fish from local market in Lombok or in Gili Matra (Gili Trawangan & Air)
- Viscera, gill, body flesh (min. 500gr) → collected from each targeted species
- Ciguatoxin analysis → mouse bioassay (the laboratory of the Fish Quarantine and Inspection Agency, Ministry of Marine Affairs and Fisheries, Indonesia)

Targeted Ciguatera Fishes

(common fishes that have been reported to cause CFP in humans)

Sources: Todd 1990, Legrand 1998, Lehane & Lewis 2000

- Moray eel (*Lycodontis* or *Gymnothorax* sp.) – Ikan Kerondong
- Barracuda (*Sphyraena* spp.) – Ikan Barakuda
- Grouper (*Epinephelus* spp.)– Ikan Kerapu
- Snapper (*Lutjanus* spp.) – Ikan Kakap
- Mackerel (*Scomberomorus* spp.) – Ikan Kembung
- Parrotfish (*Scarus* spp.) – Ikan Kakatua
- Maori wrasse (*Chelinus* sp.) – Ikan Napoleon
- Trevally (*Caranx* spp.) – Ikan Kuwe
- Kingfish/ Amberjack (*Seriola* spp.) – Ikan Aji-aji
- Frigate tuna (*Auxis thazard*) – Ikan Tongkol
- Surgeonfish (Acanthuridae) – Ikan Botana



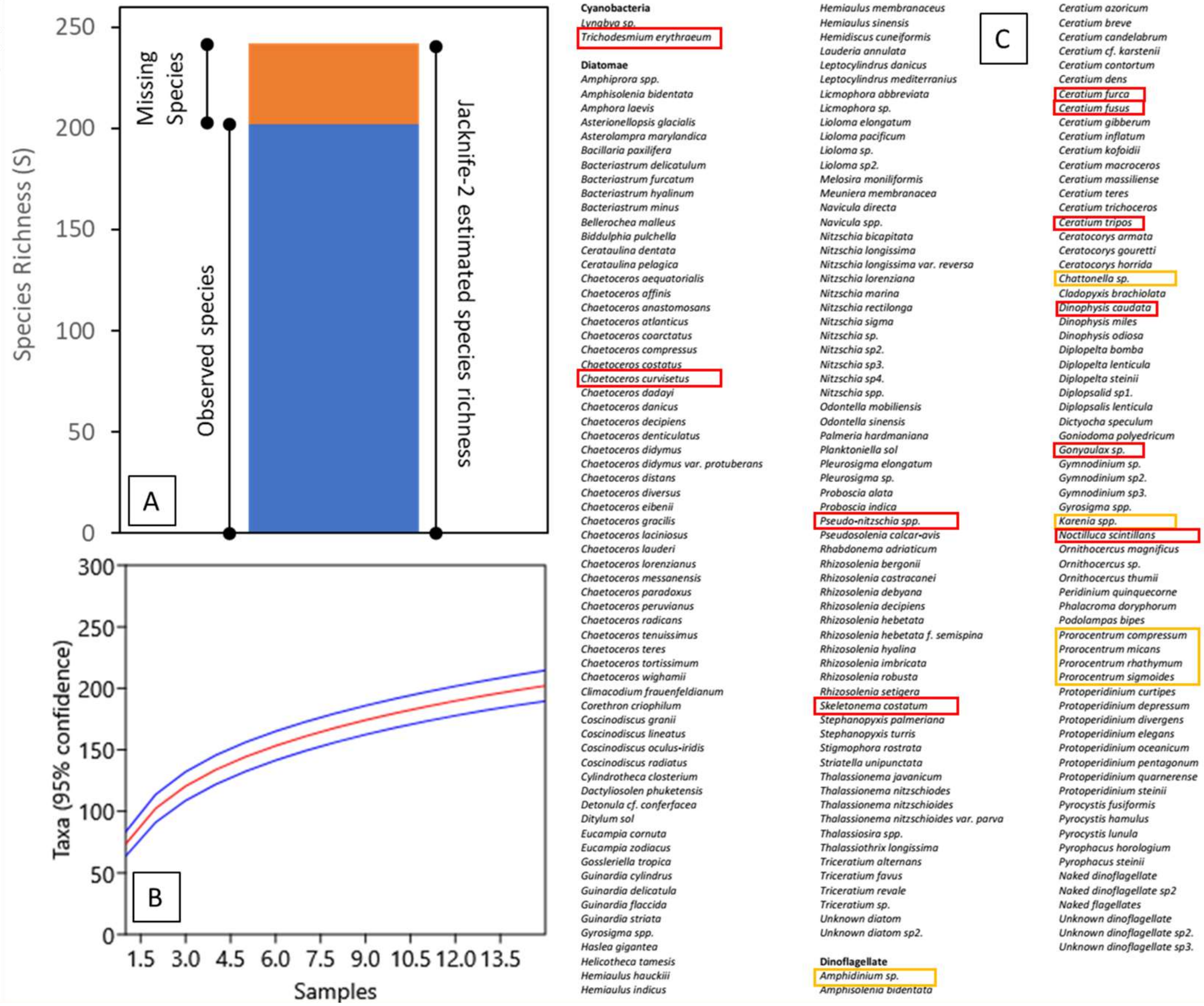
Fish Sampling at Local Fish Landing/Port in Gili Island, Western Lombok, and Northern Lombok

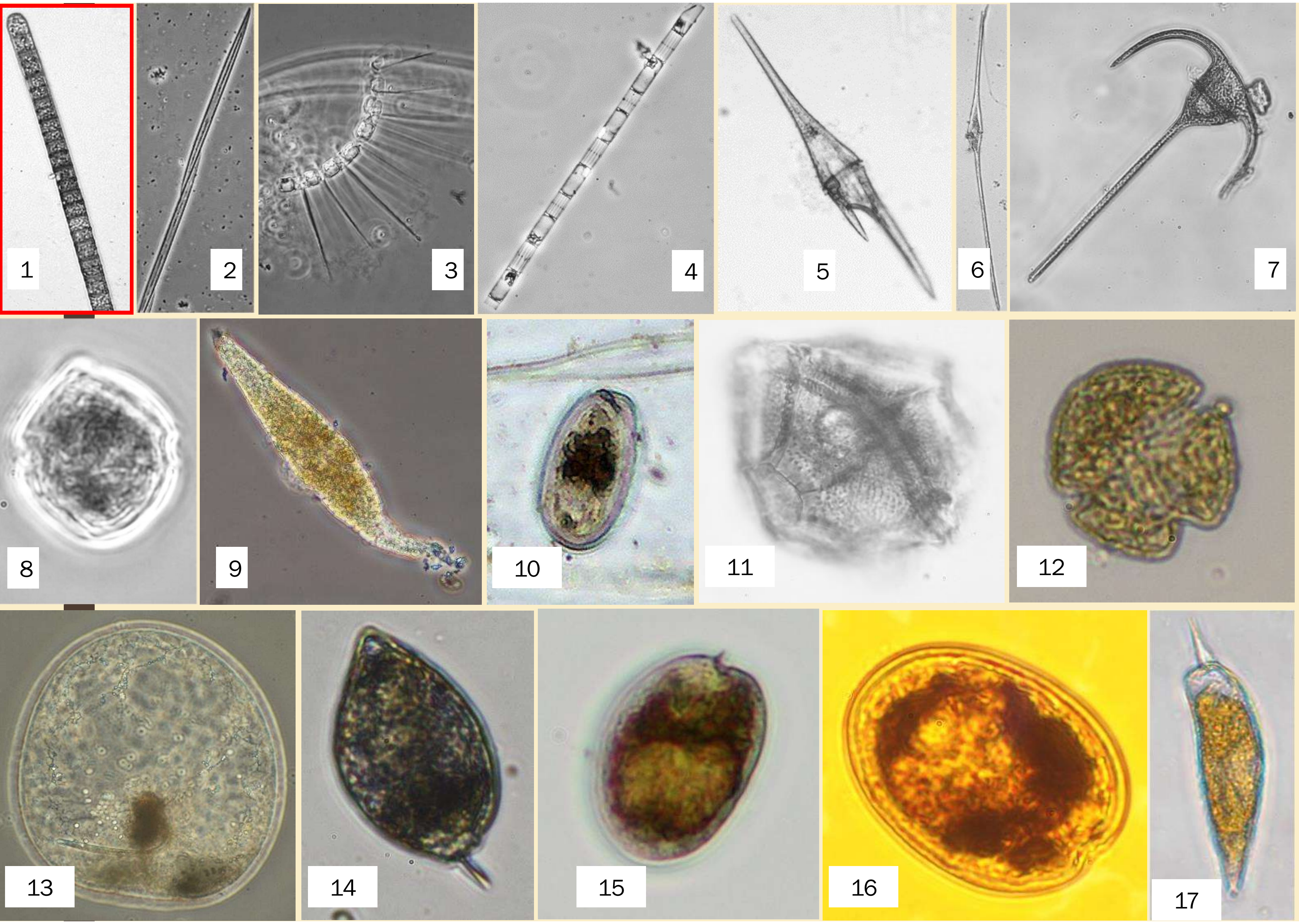
RESULTS

Planktonic Microalgae

Phytoplankton (planktonic micr

- In total → 202 species of phytoplankton has been identified from the Core, Harbour, and Sustainable Fisheries zones of Gili Meno at two seasons (n =15) (ongoing progress) → 15 potentially harmful species
- Jacknife-2 species estimator with 1000 permutation → estimated 215-242 species at the current sampling effort → at least 40 missing species
- Species Accumulation Curve (SAC) → indicating that the number of identified species was representative to the estimated real species assemblages in Gili Meno

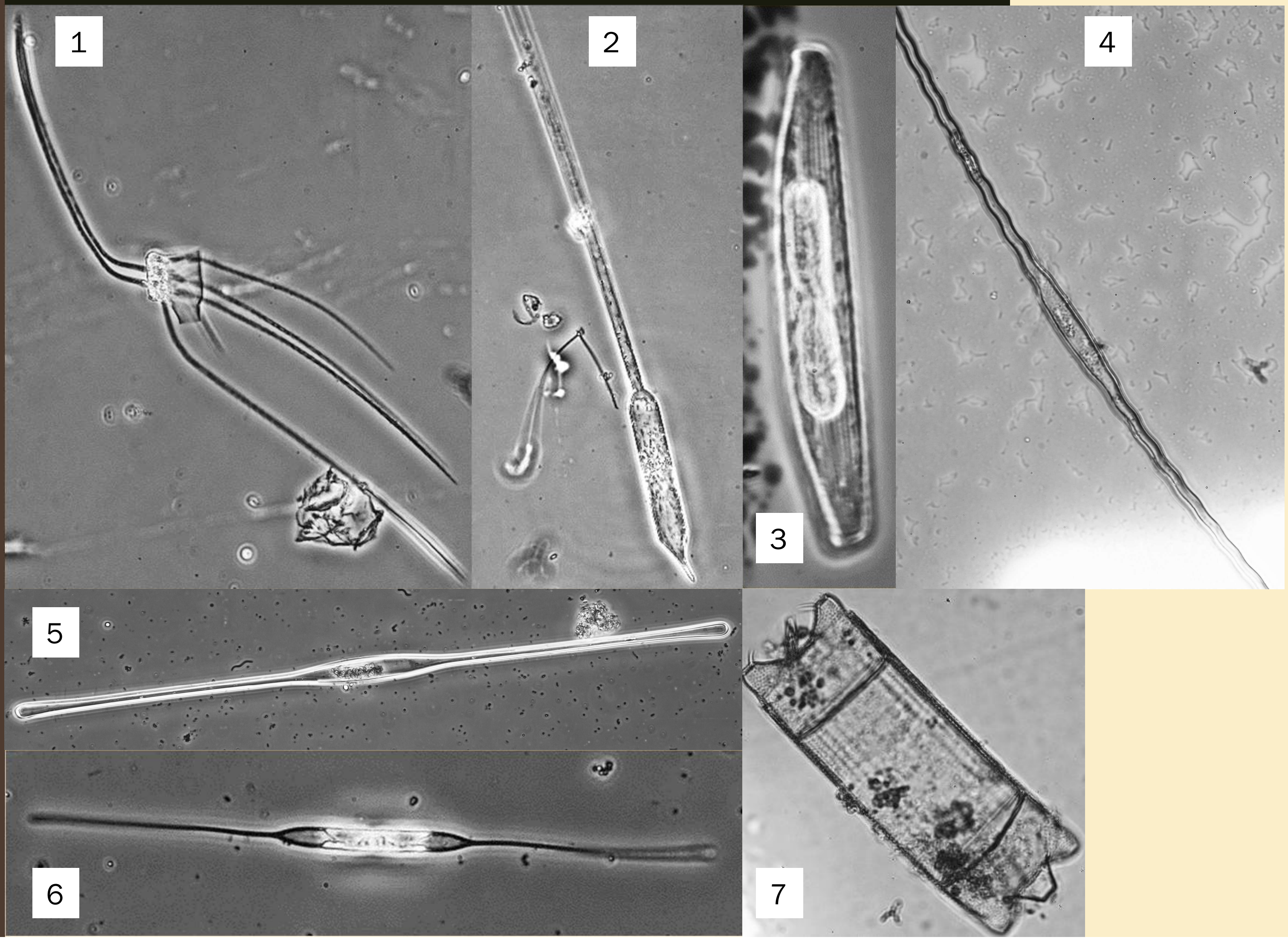




17 potentially harmful species found so far

1. *Trichodesmium erythraeum* → *often blooms in Seribu Islands*
2. *Pseudo-nitzschia spp.* → *Some species produced Domoic Acid, causing Amnesic Shellfish Poisoning (ASP)*
3. *Chaetoceros curvisetus* → *several blooms cases recorded in Jakarta Bay*
4. *Skeletonema costatum* → *blooms in Jakarta Bays, particularly in wet season*
5. *Tripos furca* → *often blooms in Lampung Bay*
6. *Tripos (Ceratium) fusus*
7. *Tripos muelleri (Ceratium tripos)*
8. *Scrippsiella trochoidea*
9. *Chattonella spp.* → *Fish Killer*
10. *Amphidinium spp.*
11. *Gonyaulax spp.* → *some species cause recent blooms in Ambon Bay*
12. *Karenia spp.* → *some species produces Brevetoxin, causing Neirotic Shellfish Poisoning (NSP)*
13. *Noctiluca scintillans* → *often blooms in Jakarta Bay, Lampung Bay, fish killer*
14. *Prorocentrum micans*
15. *Prorocentrum rathymum*
16. *Prorocentrum compressum*
17. *Prorocentrum sigmoides*

Unusual and Rare Diatom Species

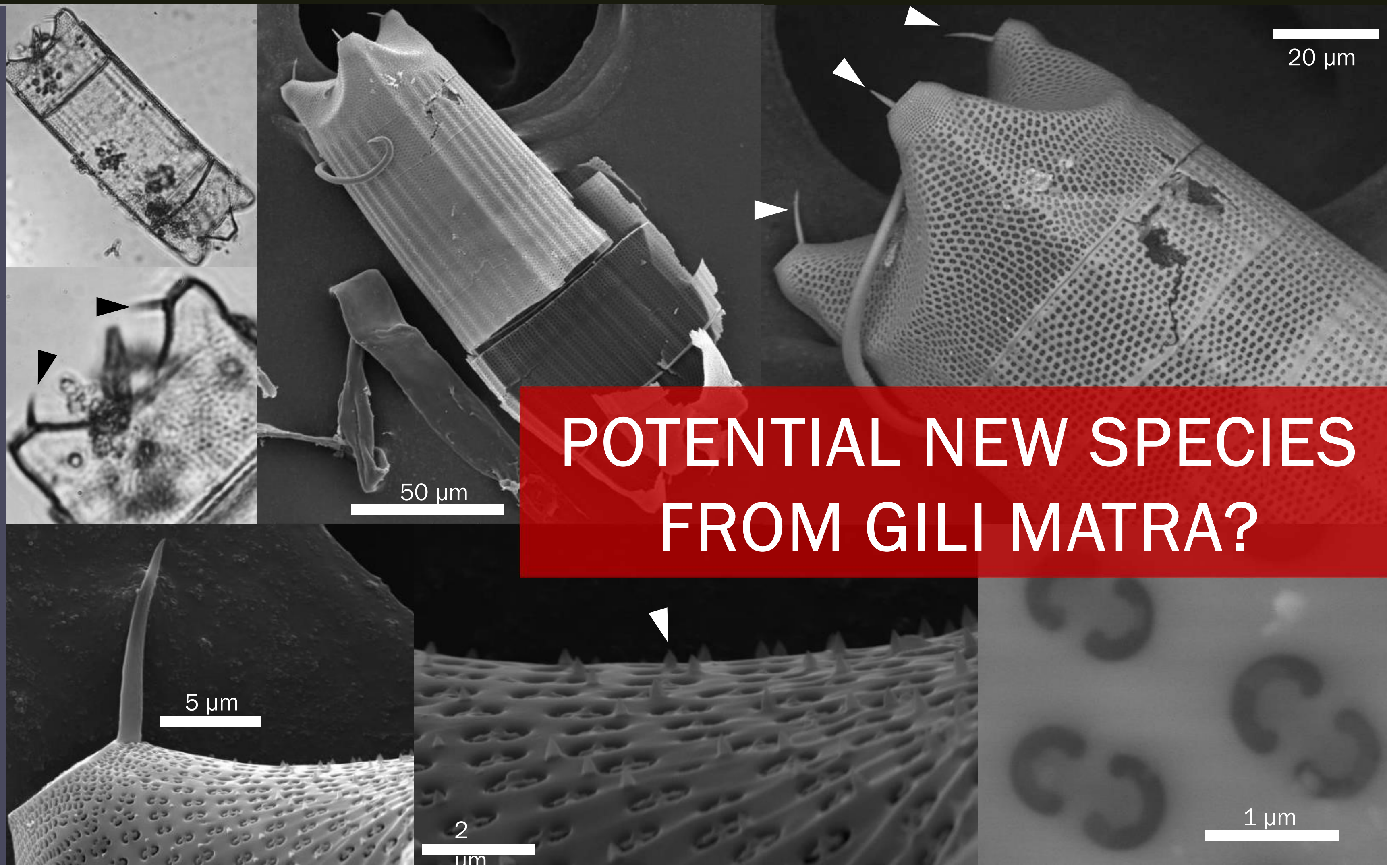


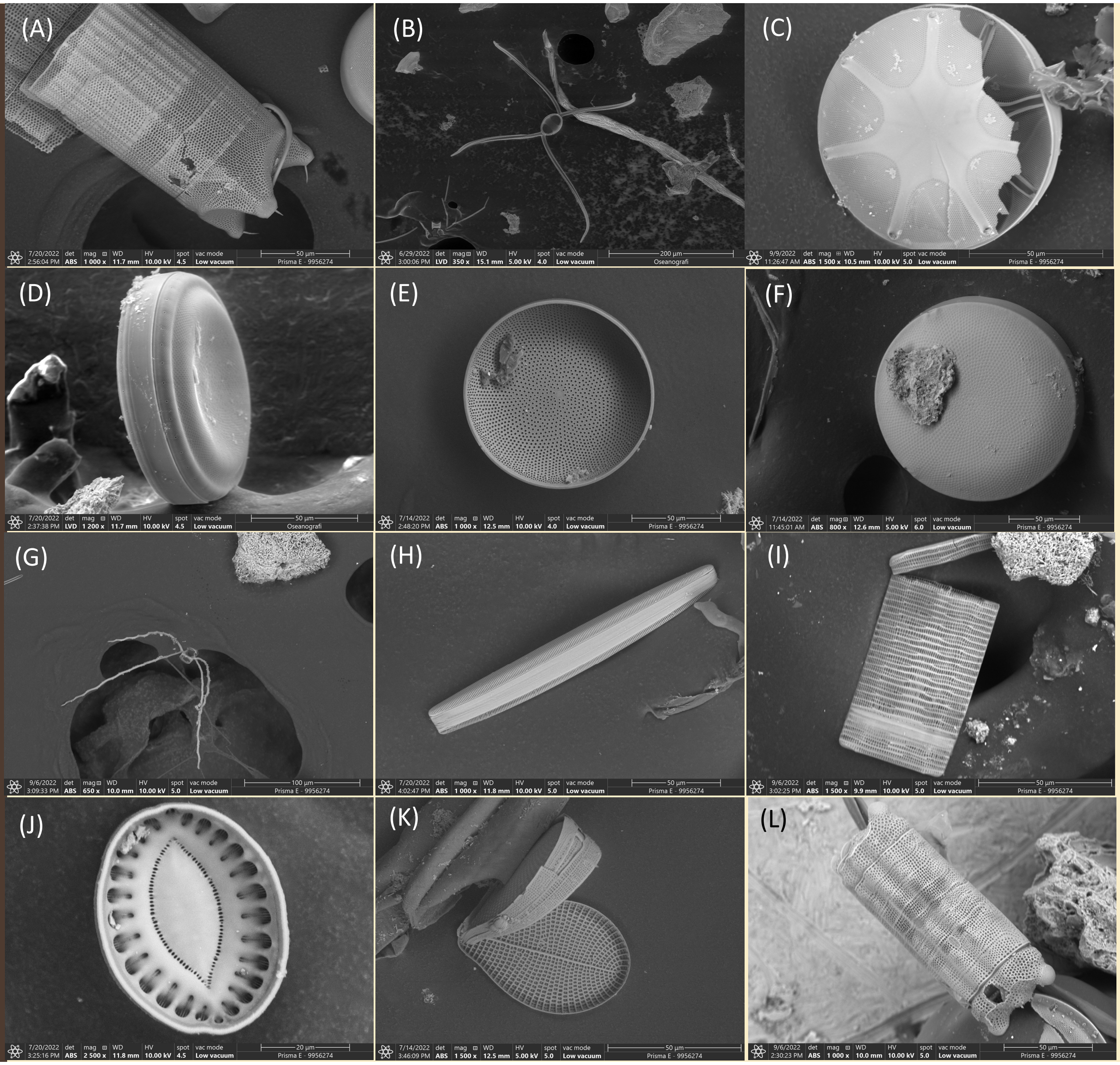
Rare and unusual diatom species:

1. *Chaetoceros dadayi,* rare and never recorded before, was only present during Transitonal Season I (May) (so far)
2. *Proboscia alata,* unusual frustule growth
3. *Trachyneis* sp., rare and never recorded before
4. *Nitzshica sp4.,* wavy frustule
5. *Nitzschia sp5.,* frustule with rounded and bulb ends
6. *Nitzschia rectilonga,* rare and never recorded before
7. *Triceratium sp.,* unusual species due to existence of spine at the end of the frustule → *Lampriscus cf. shadboltianum*

Morphological Details on Unusual Diatom species

Unusual species found from macroalgal extract in Gili Meno during May 2022 sampling → morphological character similar to *Lampriscus,* particularly *Lampriscus shadboltianum,* but with spines at the end of the frustule and small spines on frustules → temporarily named *Lampriscus cf. shadboltianum*





SEM images of some diatoms from the electron microscopy trial with Prisma-E (Thermo Science). SEM images taken under low-vacuum (40-50 Pa) at 5 – 10 kV acceleration using Low Vacuum Detector (LVD) or Angular Back Scatter (ABS) detectors:

(A) *Lampriscus cf. shadboltianum* unusual species due to existence of spine at the end of the frustule

(B) *Chaetoceros coarctatus*

(C) *Asterolampra marylandica*

(D) *Thalassiosira* sp., girdle view

(E) *Thalassiosira* sp., half-frustule at valve view

(F) *Thalassiosira* sp., valve view

(G) *Chaetoceros compressus*

(H) *Nitzschia* sp.

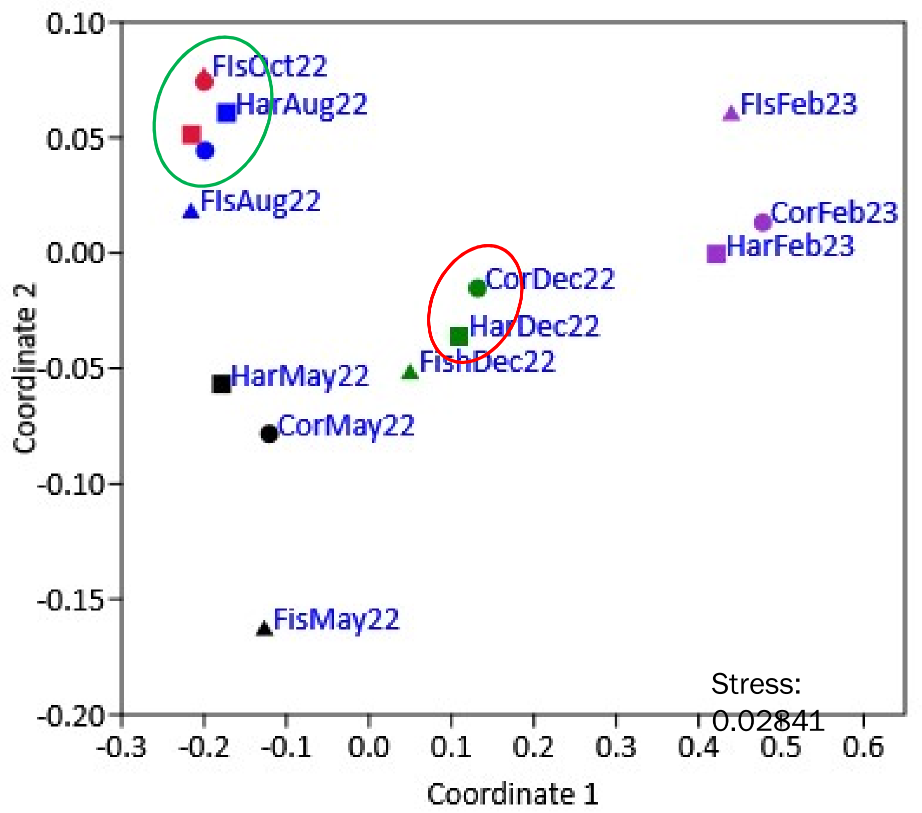
(I) *Fragilariopsis sp.*

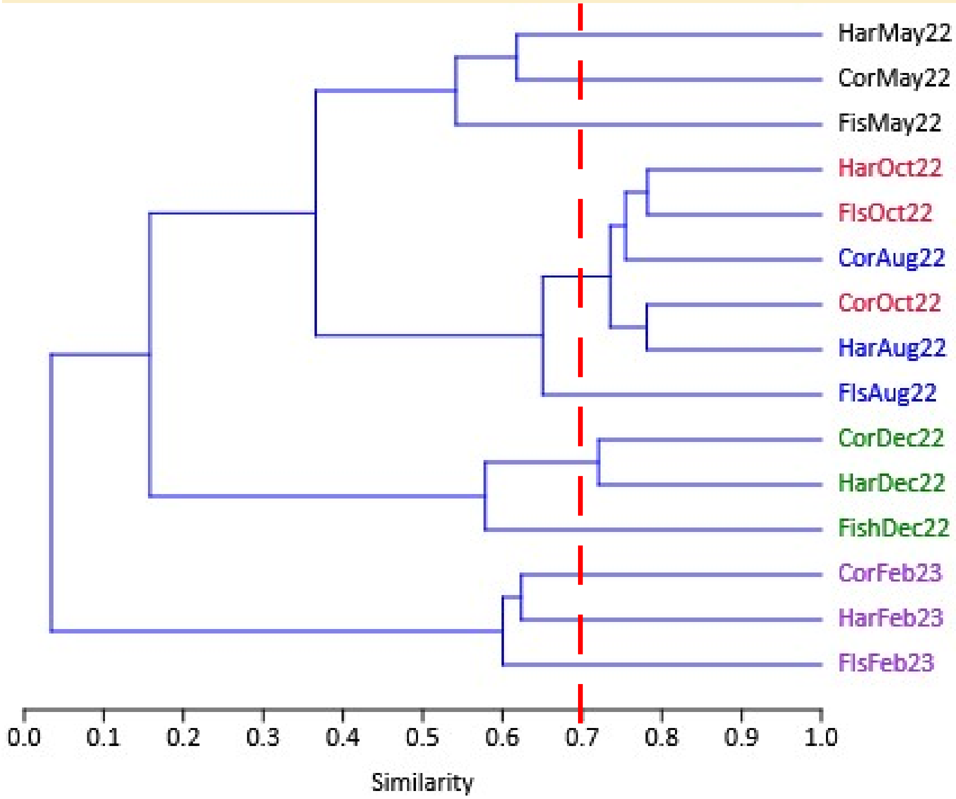
(J) *Campylodiscus* sp.

(K) *Podocystis spathulate*, valve split open

(L) *Biddulphia pulchella*

Phytoplankton (planktonic microalgae) density and diversity

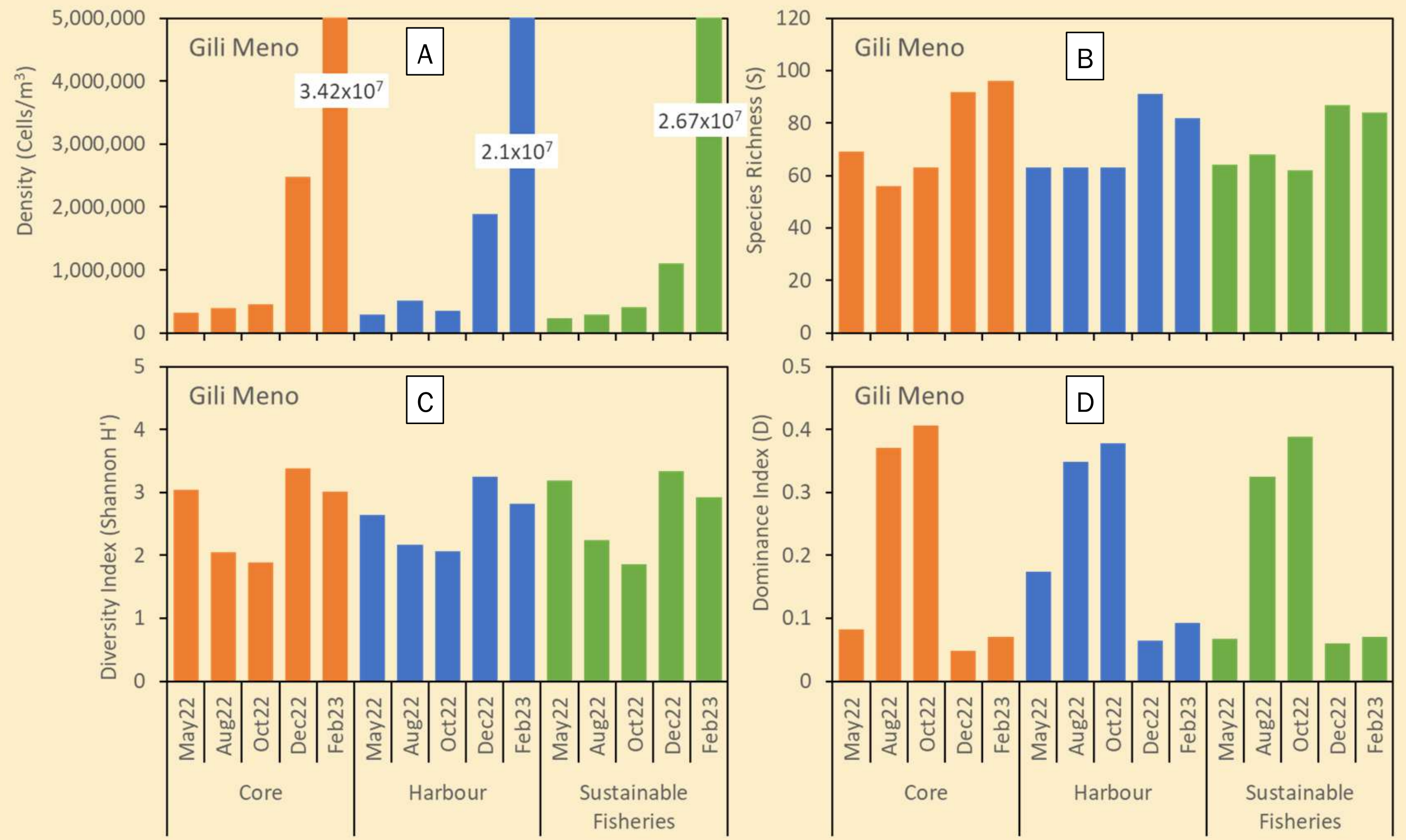




- nMDS analysis (bray-curtis) → most zones in Gili Meno in each seasons → have significantly different phytoplankton species composition
- Grouping based on nMDS and UPGMA Clustering → Sustainable fisheries zone in Gili Meno was always have different and distinct phytoplankton community in all seasons

Phytoplankton (planktonic microalgae) density and diversity

- Phytoplankton cell density → 2.33 x 10^5 – 3.42 x 10^7 cells/m^3 → higher mesotrophic water (according to Spatharis & Tsirtsis, 2010)
- Low diversity and high dominance index value in Dry season (August) and transitional season II (October) → indicating a domination of certain species → *Trichodesmium erythraeum* (Cyanobacteria)

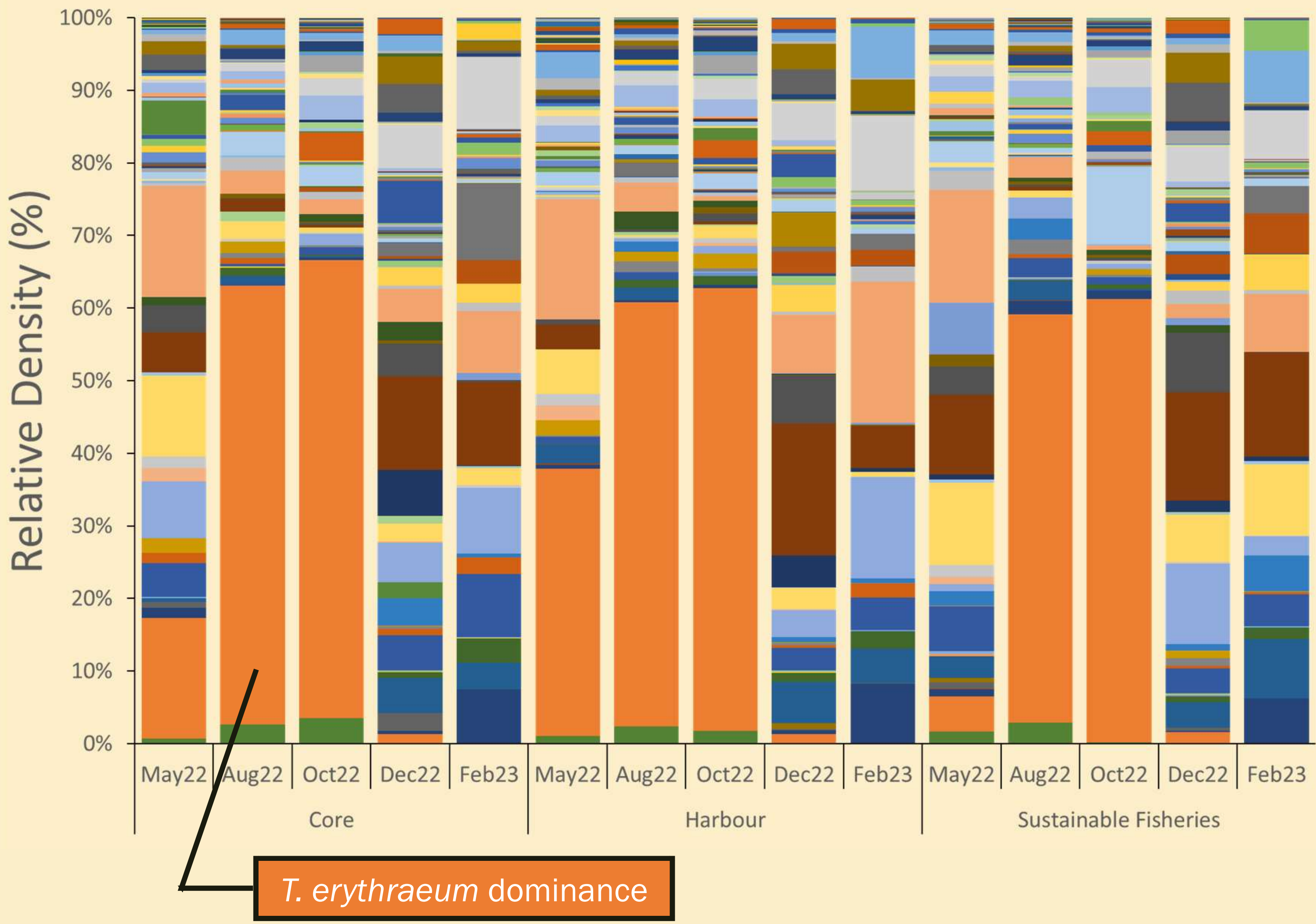


Significant increase in phytoplankton cell density → was observed during the wet season in December 2022 and reached its maximum at February 2023

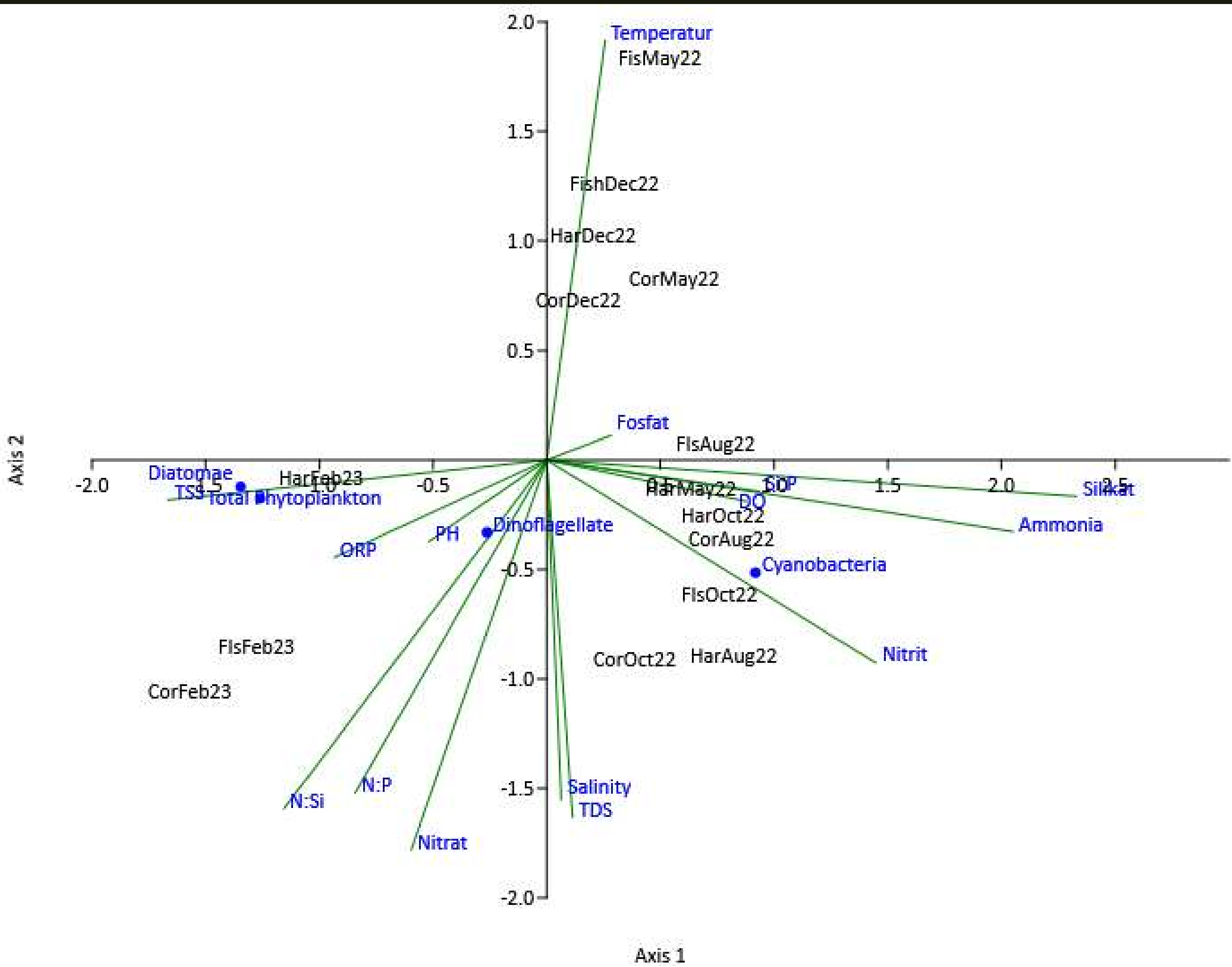
Trophic level → change from oligotrophic (May – October 2022) to higher mesotrophic (December 2022 – February 2023)

Phytoplankton (planktonic microalgae) density and diversity

- Diatoms → generally dominate the phytoplankton community in Wet Season (Feb 2023) and Transitional Season I (May 2022)
- *T. erythraeum* (Cyanobacteria) → overtake the diatom dominance in the phytoplankton community of Gili Meno during Dry Season (Aug 2022) and Transitional Season II (Oct 2022)
- High variation in the species composition between zones in Gili Meno during the same season → indicating different water condition which favour higher growth rate of certain species



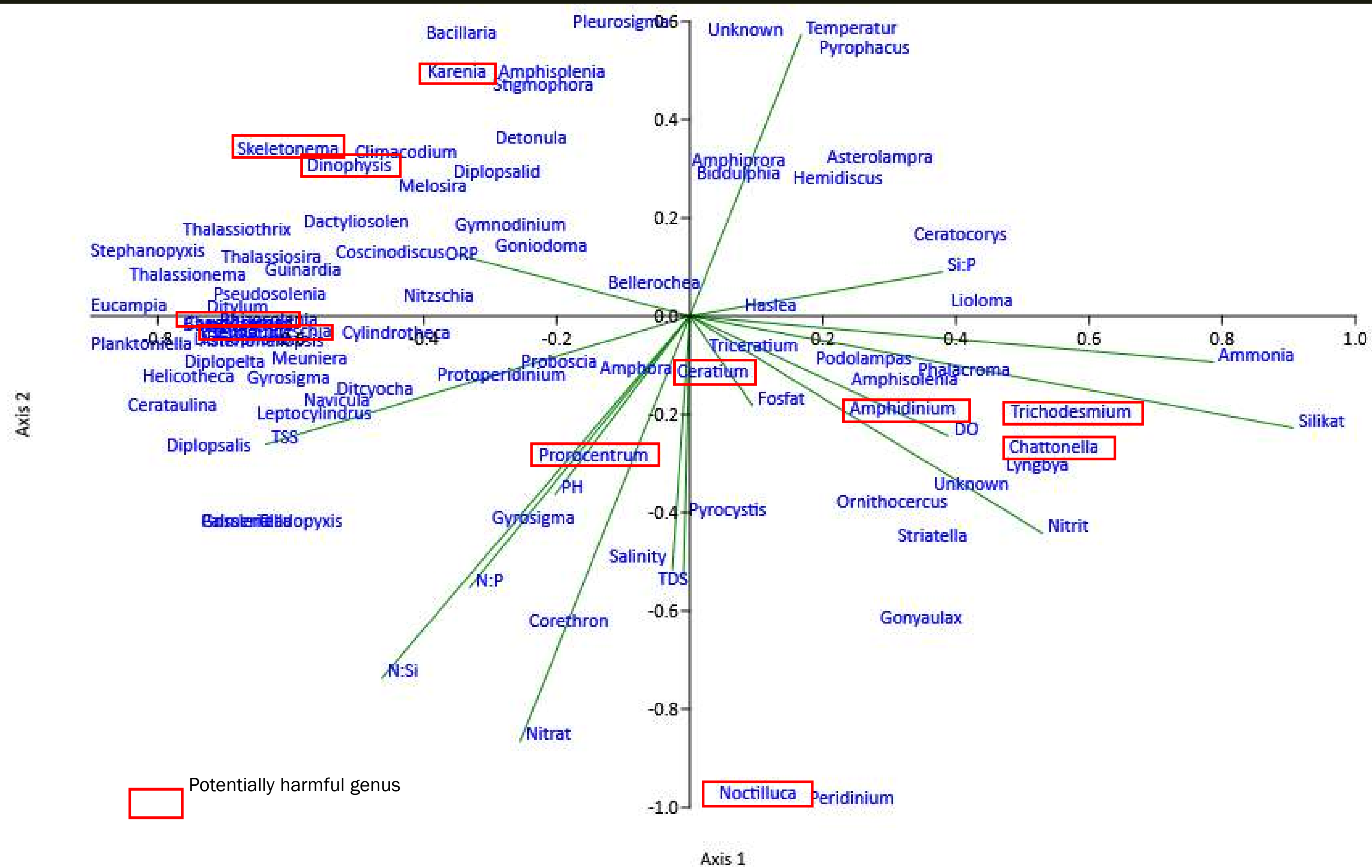
Parameters affecting the density of phytoplankton genera in Gili Matra



- In general → phytoplankton groups (diatoms, dinoflagellates, cyanobacteria) → more abundant in colder sites and months/seasons
- Diatoms abundance → might be regulated by nitrate and nutrient balance between nitrogen, phosphate, and silicate
- Dinoflagellates abundance → also regulated mainly by nitrogen and its ratio towards silicate and phosphate
- Cyanobacteria abundance → might be regulated by nitrite, ammonia, and Si;P ratio

Parameters affecting the density of phytoplankton genera in Gili Matra

- Most dominant genus → *Trichodesium* → abundant at waters with higher salinity and nitrates concentration
- Common and abundant diatoms → *Chaetoceros* → more abundant at turbid water, which rich in phosphate
- Some potentially harmful genus
  - *Ceratium/Tripos* → more abundant at higher temperature and pH and did not strongly affected by any nutrient concentration
  - *Chattonella* → prefer nitrate-rich high salinity water, similar with *Trichodesmium*
  - *Pseudo-nitzschia* → more abundant at ammonia-rich waters
  - *Prorocentrum* → more abundant at turbid waters, which also rich with phosphate



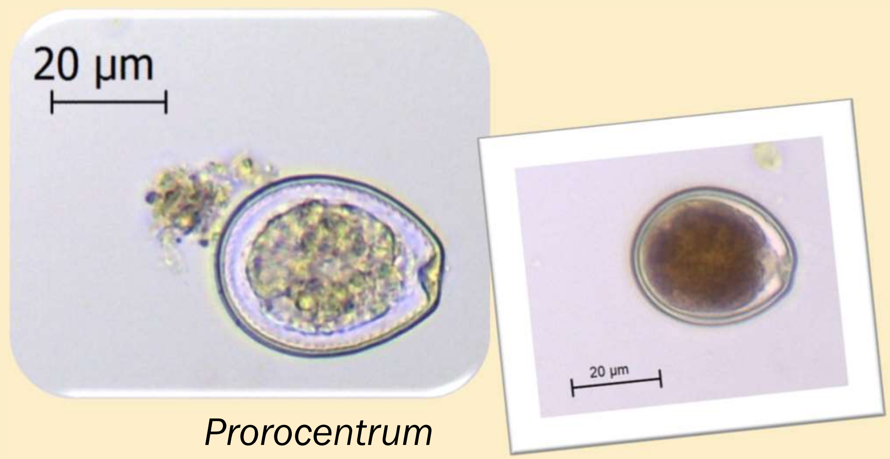
RESULTS

Benthic Microalgae

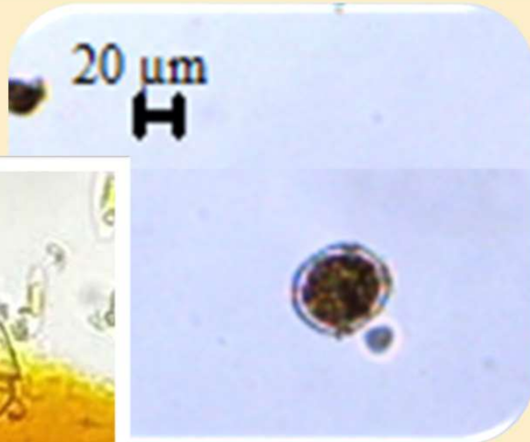
Six Genera of Benthic Dinoflagellates

Gambierdiscus

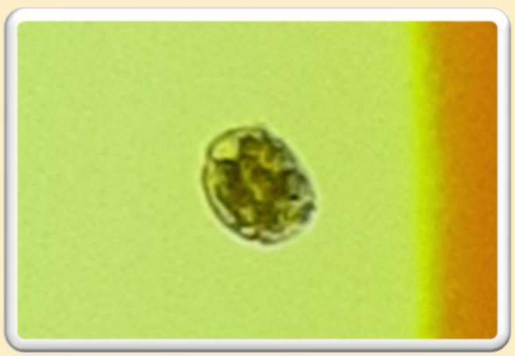
Ostreopsis



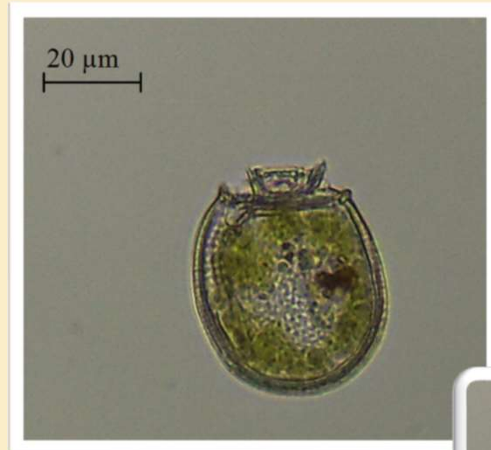
Prorocentrum



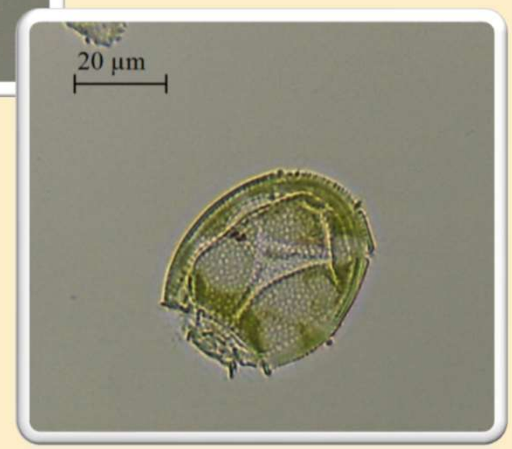
Coolia



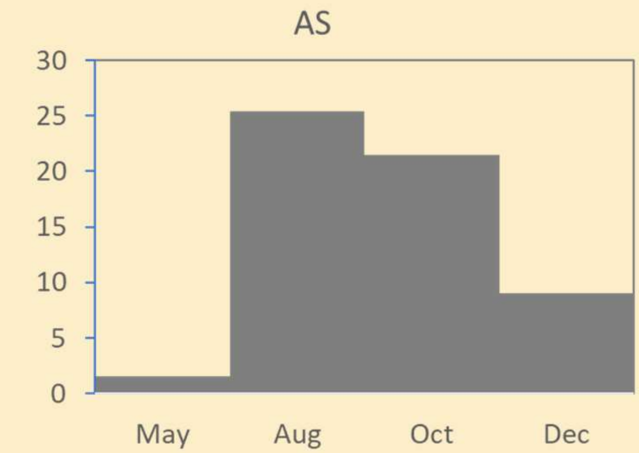
Amphidinium

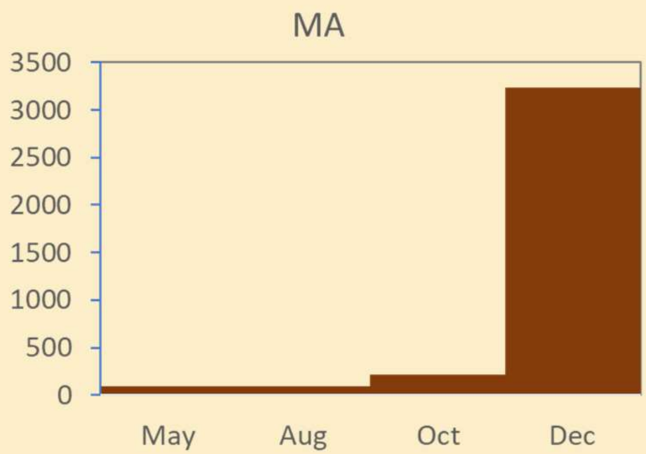


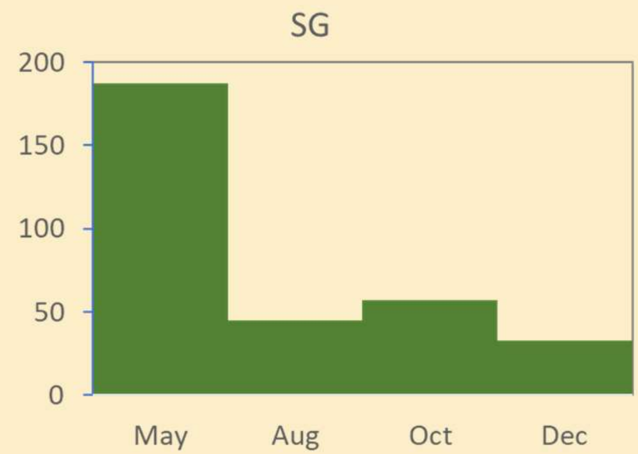
Sinophysis



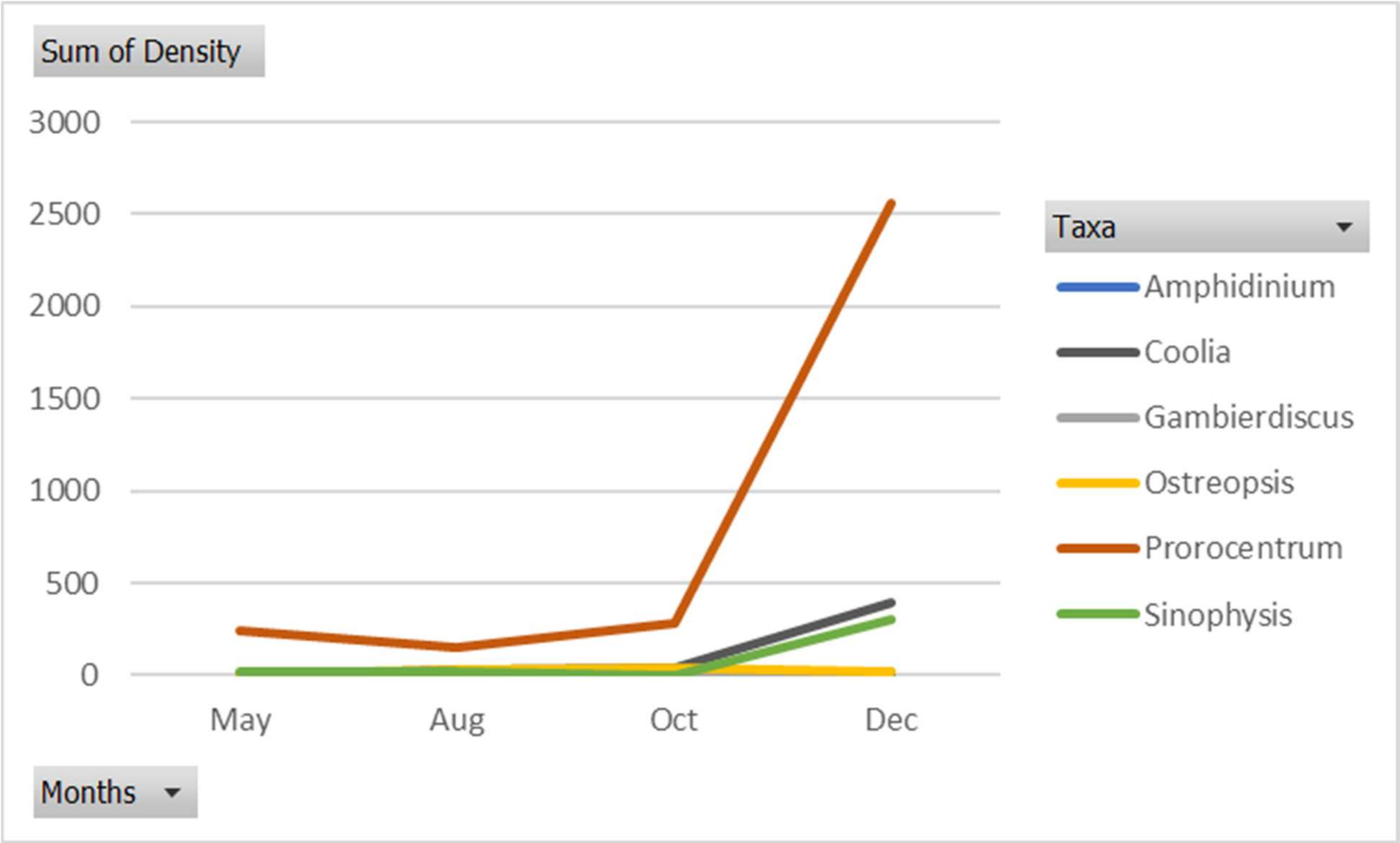
NON TOXIC





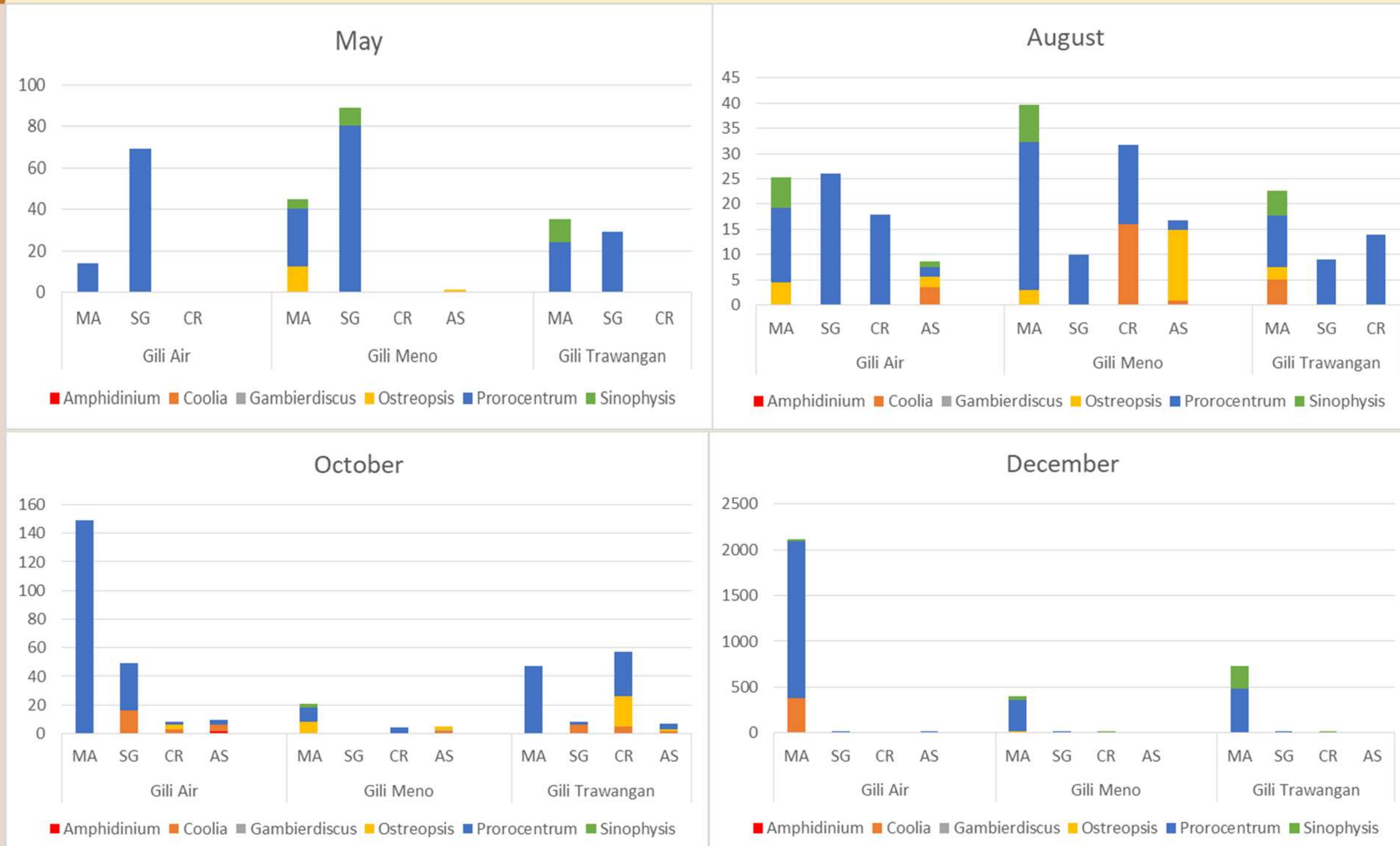


- In general → we found a significant increase in cell density of targeted genera in wet season (December 2022) → due to high increase of cells found in macroalgae
- In seagrass → dinoflagellate cells are mor abundant in transitional season I (May 2022)


Sum of Density
3000
2500
2000
1500
1000
500
0
May
Aug
Oct
Dec
Months
Taxa
Amphidinium
Coolia
Gambierdiscus
Ostreopsis
Prorocentrum
Sinophysis

Important Trend:

- Benthic dinoflagellates were found in any kind of substrates
- *Prorocentrum* is the most abundance dinoflagellate found, especially on macroalgae and sea grass substrates, and usually together with *Sinophysis*.
- *Ostreopsis* and *Coolia* were often found on non living susbtrates, such as coral rubble and artificial substrate.
- *Gambierdiscus* and *Amphidinium* were found in the lowest number.



MA: Macro Algae, SG: Sea grass, CR: Coral Rubble, AS: Artificial Substrate

DNA Analysis

Electrophoresis Result

BLAST Result

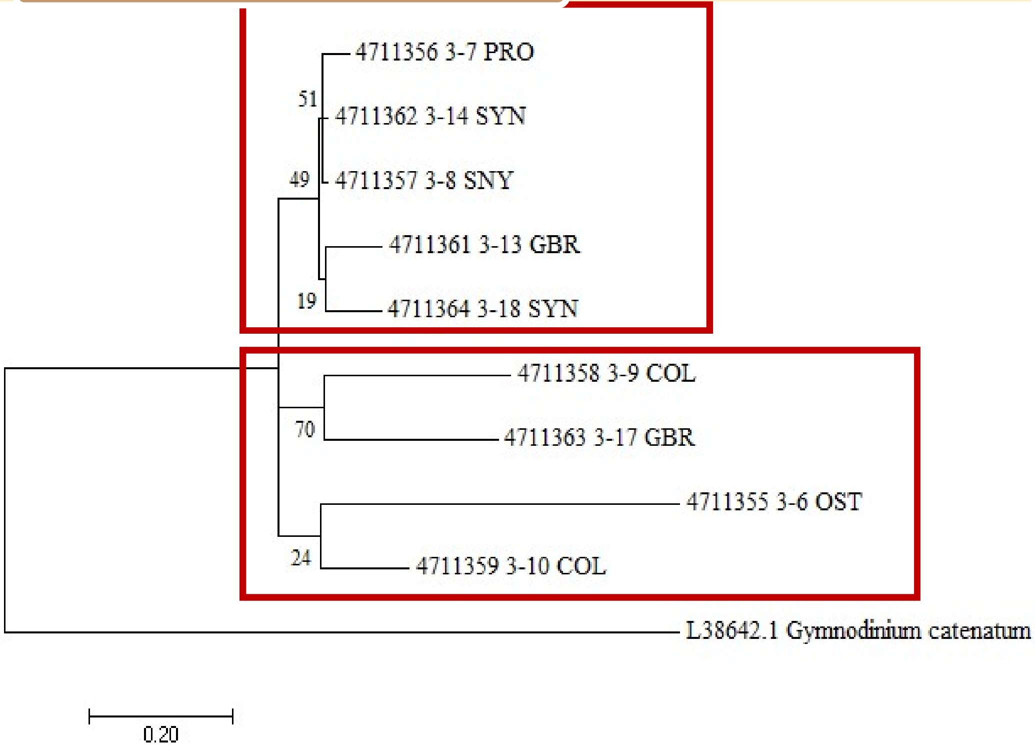
No.	Names	Query (%)	Similarity (%)
1	4711355_3-6_OST	5	88.46
2	4711356_3-7_PR[illegible]	62	67.28
3	4711357[illegible]	74	71.05
4	[illegible]_COL	27	74.07
[illegible]	[illegible]1359_3-10_COL	18	72.00
6	4711361_3-13_GBR	31	77.70
7	4711362_3-14_SYN	33	82.02
8	4711363_3-17_GBR	25	74.24
9	4711364_3-18_SYN	26	76.90

Query covers are low

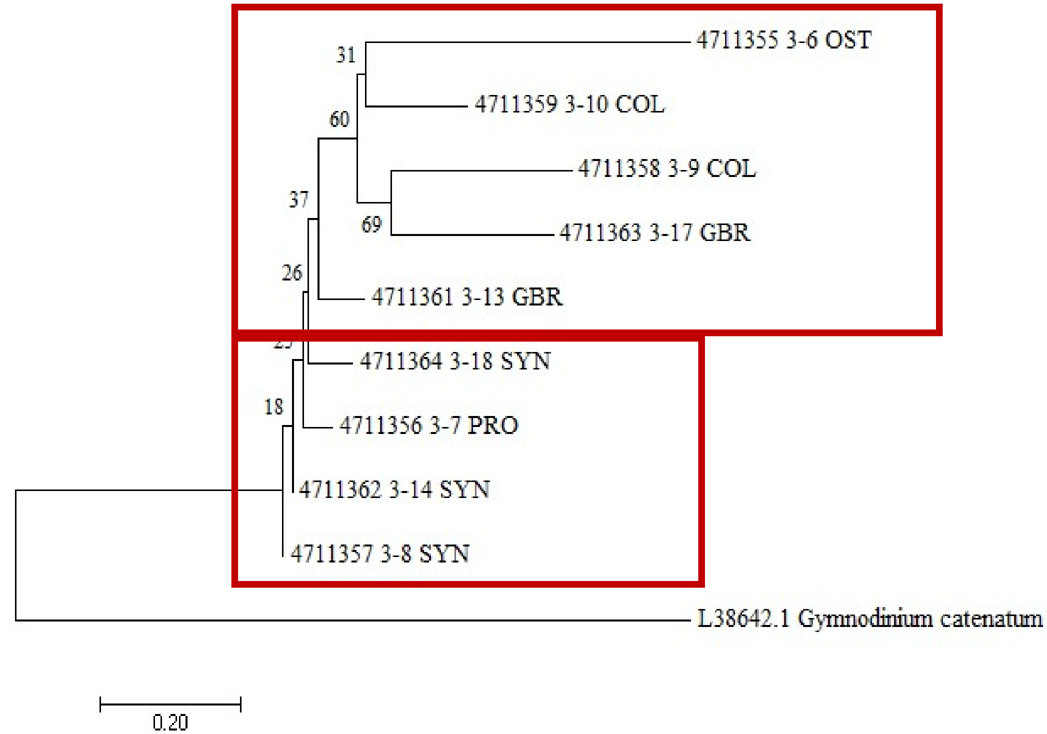
- Samples from the environment, usually contain fungal contamination.
- Sequens from benthic dinoflagellates in Indonesian waters, including from Lombok waters, has not recorded yet in gene bank, such as the NCBI.

Phylogenetic Trees

Maximum Likelihood (ML)



Neighbor Joining (NJ)



- Group 1, the Gonyaulacoid : *Ostreopsis*, *Coolia* and *Gambierdiscus*
- Group 2, the Prorocentroid and Dinophysoid : *Prorocentrum* and *Sinophysis*

eDNA Results (on progress)

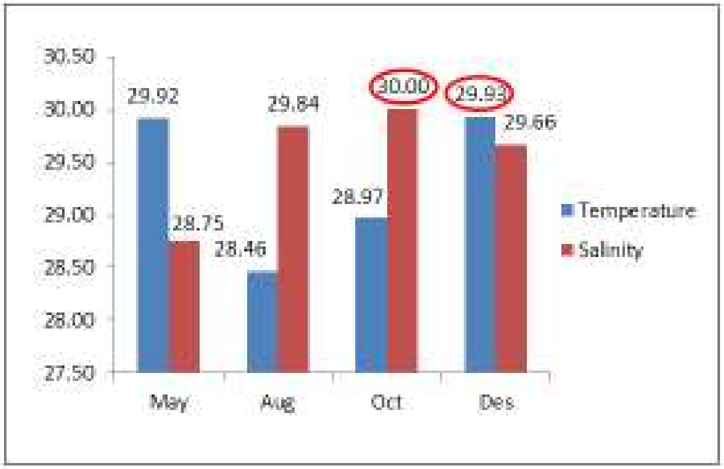
No	Substrat	DNA Concentration (ng/µl)
1	Artificial Substrate	3.16
2	Macro Algae	3.56
3	Coral Rubble	0.84

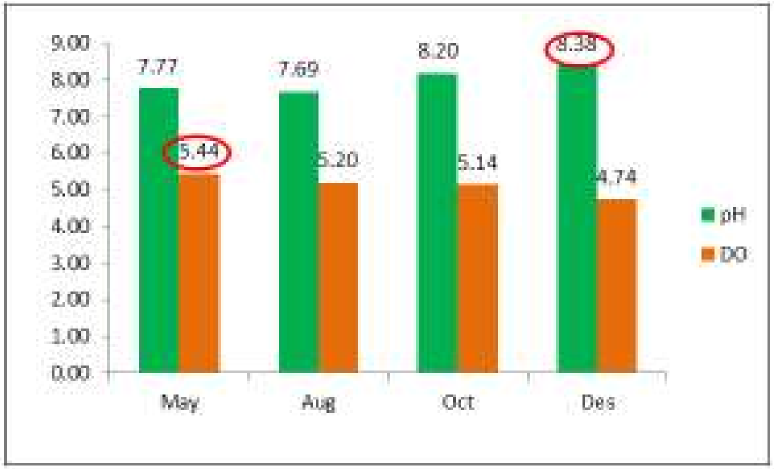
- There are sufficient amount of DNA on the macroalgae and artificial substrate
- We do hope it is from the benthic microalgae community, including the targeted toxic dinoflagellates

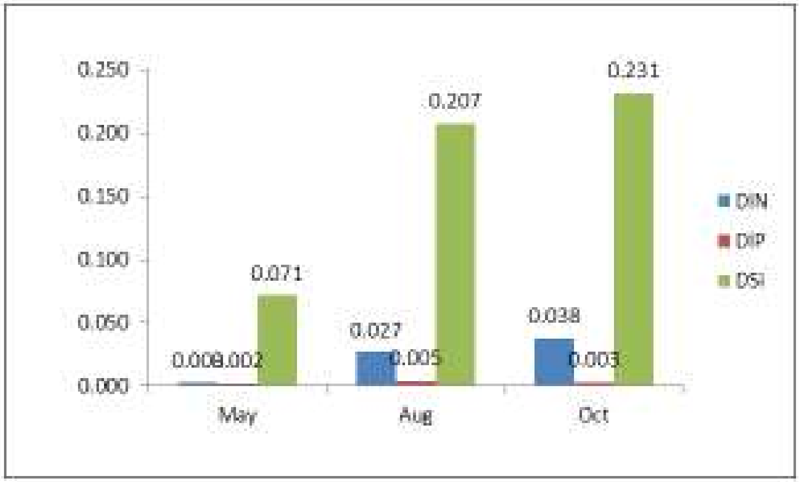
RESULTS

Nutrient Dynamic

Variation of some parameters of water quality







- Most dominant genus → *Trichodesium* → abundant at waters with higher salinity and nitrates concentration
- Common and abundant diatoms → *Chaetoceros* → more abundant at turbid water, which rich in phosphate
- Some potentially harmful genus
  - *Ceratium/Tripos* → more abundant at higher temperature and pH and did not strongly affected by any nutrient concentration
  - *Chattonella* → prefer nitrate-rich high salinity water, similar with *Trichodesmium*
  - *Pseudo-nitzschia* → more abundant at ammonia-rich waters
  - *Prorocentrum* → more abundant at turbid waters, which also rich with phosphate

Variation of nutrient concentration in some coastal areas

Location	Sampling time	DIN (μM)		DIP (μM)		DSi (μM)		DIN/DIP		Reference
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Jakarta Bay (Annuals)	2001		20.8		5.1		46.8			Damar et al. 2019
	2007		18.1		4.2		48.3			
	2013		10.9		5.4		45.2			
Pagametan Bay	Aug 2014	1.42–17.14	7.85	0–0.97	0.65				12.08	Tammi et al. 2015
Lampung Bay			14.3		2.3		39.3		6.22	Damar et al. 2012
Semangka Bay			3.5		0.4		28.4		8.75	
Tambelan and Serasan waters	Nov 2010	0.78–11.6	2.07	0.02–0.13	0.06	2.03–4.8	3.66	11.2–133.7	40.04	Prayitno and Suherman 2012
Jakarta Bay	Feb 2007		41.46		0.21		7.82		197	Nugrahadi et al. 2010
Coastal waters of Madura Strait			0.3		0.1		55.4		30	Jennerjahn et al. 2004
Gili Matra Islands	May 2022	0.083-0.316	0.182	0.037-0.130	0.075	1.517-3.817	2.545		2.43	Our study
	Aug 2022	0.499-4.508	1.906	0.092-0.189	0.146	3.035-57.052	7.38		13.05	
	Oct 2022	0.526-10.465	2.679	0.092-0.140	0.104	1.030-18.812	8.233		25.76	

RESULTS

Fish, Ciguatoxin Analysis, Traceability

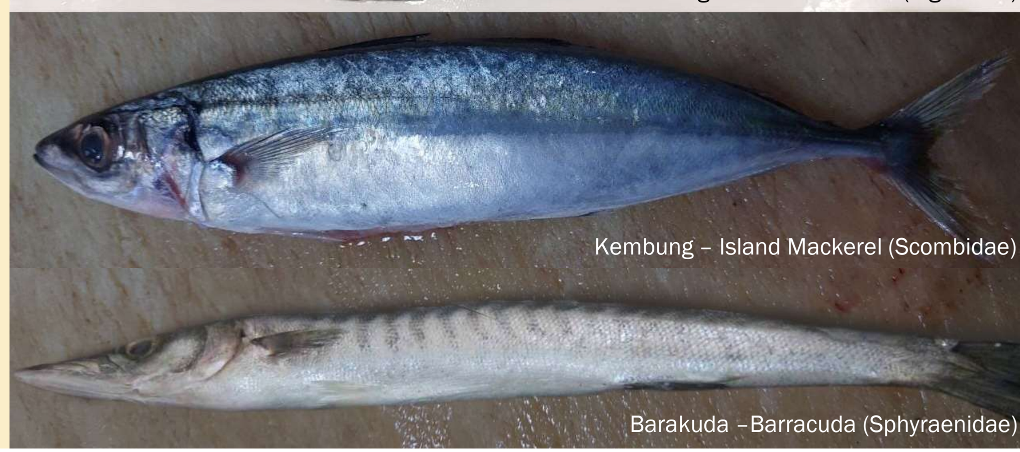
Ciguatoxin analysis (mouse bioassay)

- **Ciguatoxin** → was undetected with mouse bioassay analysis from 19 fish samples → 13 from May and 6 from August sampling
- **Ciguatoxin concentration** → might be <u>below the concentration</u> that required for the laboratory mice to show observable symptoms or lethality
- **However** → we can't rule out the possibility of low (or very low) concentration of ciguatoxin exist in the reef fish tissue → could be accumulate over time in higher trophic level organisms (particularly, fish and later, human)
- **More detailed chemical based analysis** → such as ELISA → needed to quantify the concentration of ciguatoxin (if there are any)

Analyzed fish:
- Rabbitfish (Siganidae)
- Parrotfish (Scaridae)
- Barracuda (Sphyraenidae)
- Island Mackerel (Scombidae)
- Grouper (Serranidae)
- Longtail Tuna (Scombridae)


Kakatua – Parrotfish (Scaridae)


Baronang hitam - Rabbitfish (Siganidae)


Kembung – Island Mackerel (Scombidae)

Barakuda –Barracuda (Sphyraenidae)

Ciguatoxin analysis (mouse bioassay)

Example of the results of Ciguatoxin analysis using Mouse bioassay → "*Tidak Terdeteksi*" = Not Detected

BALAI UJI STANDAR KARANTINA IKAN, PENGENDALIAN MUTU DAN KEAMANAN HASIL PERIKANAN
Jl. Harapan I No. 1A, Kelurahan Setu Kecamatan Cipayung, Jakarta Timur 13880
Telp.(021) 845 1378 / 845 99367, Fax.(021) 844 8523
Email. buski@bkipm.kkp.go.id, buski_jkt@yahoo.com, buskipm@gmail.com

LAPORAN HASIL UJI
REPORT OF ANALISYS
No. 0172/LHU/42.0/06/2022

Nama Pelanggan *Customer* : Riani Widiarti
Alamat *Address* : Haji Ramli No. 31, Menteng Dalam, Tebet
Telp. 08129680996, Email: rianiwid@yahoo.co.id
Tanggal Penerimaan *Receipt Date* : 14 Juni 2022 **Tanggal Pengujian** *Date of analysis* : 14 Juni 2022
Pejabat yang dihubungi *Contact Person* : drh. Insariani
Kode Contoh Uji *Sample Code* : 0172

No	**Laboratorium** *Laboratory*	**Contoh Uji**/ *Sample*			**Pengujian**/ *Analysis*			**Keterangan** *Remark*
		Nama *Name*	**Bentuk** *Form*	**Media** *Media*	**Parameter** *Parameter*	**Metode** *Method*	**Hasil** *Result*	
1	Lab. Kimia	**Ikan Pogot - Tanjung** Tan-1	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
2	Lab. Kimia	**Ikan karang sp.1 - Tanjung** Tan-2	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
3	Lab. Kimia	**Ikan karang sp.2 - Tanjung** Tan-3	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
4	Lab. Kimia	**Ikan Baronang - Tanjung** Tan-4	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
5	Lab. Kimia	**Ikan Karang sp.5 - Tanjung** Tan-5	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
6	Lab. Kimia	**Ikan Karang sp.6 - Tanjung** Tan-6	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
7	Lab. Kimia	**Ikan Tuna - Bintaro** Bin-2	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
8	Lab. Kimia	**Ikan Kakatua - Bintaro** Bin-3	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
9	Lab. Kimia	**Ikan Karang sp.3 - Bintaro** Bin-4	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
10	Lab. Kimia	**Ikan Barakuda** Bin-5	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
11	Lab. Kimia	**Ikan Karang sp.4 - Bintaro** Bin-6	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
12	Lab. Kimia	**Ikan Kerapu - BIntaro** Bin-7	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	

BALAI UJI STANDAR KARANTINA IKAN, PENGENDALIAN MUTU DAN KEAMANAN HASIL PERIKANAN
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Telp.(021) 845 1378 / 845 99367, Fax.(021) 844 8523
Email. buski@bkipm.kkp.go.id, buski_jkt@yahoo.com, buskipm@gmail.com

LAPORAN HASIL UJI
REPORT OF ANALISYS
No. 0305/LHU/42.0/08/2022

Nama Pelanggan *Customer* : Riani Widiarti
Alamat *Address* : Haji Ramli No. 31, Menteng Dalam, Tebet
Telp. 08129680996, Email: rianiwid@yahoo.co.id
Tanggal Penerimaan *Receipt Date* : 29 Agustus 2022 **Tanggal Pengujian** *Date of analysis* : 29 Agustus 2022
Pejabat yang dihubungi *Contact Person* : drh. Insariani
Kode Contoh Uji *Sample Code* : 0305

No	**Laboratorium** *Laboratory*	**Contoh Uji**/ *Sample*			**Pengujian**/ *Analysis*			**Keterangan** *Remark*
		Nama *Name*	**Bentuk** *Form*	**Media** *Media*	**Parameter** *Parameter*	**Metode** *Method*	**Hasil** *Result*	
1	Lab. Kimia	**Ikan Kakaktua 1** Trawangan_2	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
2	Lab. Kimia	**Ikan Kakaktua 2** Trawangan_3	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
3	Lab. Kimia	**Ikan Kakaktua 3** Trawangan_4	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
4	Lab. Kimia	**Ikan kembung** Air_1	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
5	Lab. Kimia	**Ikan Barakuda** Air_2	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	
6	Lab. Kimia	**Ikan Baronang Hitam** Trawangan_1	Beku	-	Ciguatoxin Non Ruang Lingkup	Mouse Bioassay	Tidak Terdeteksi	

Catatan :
Note

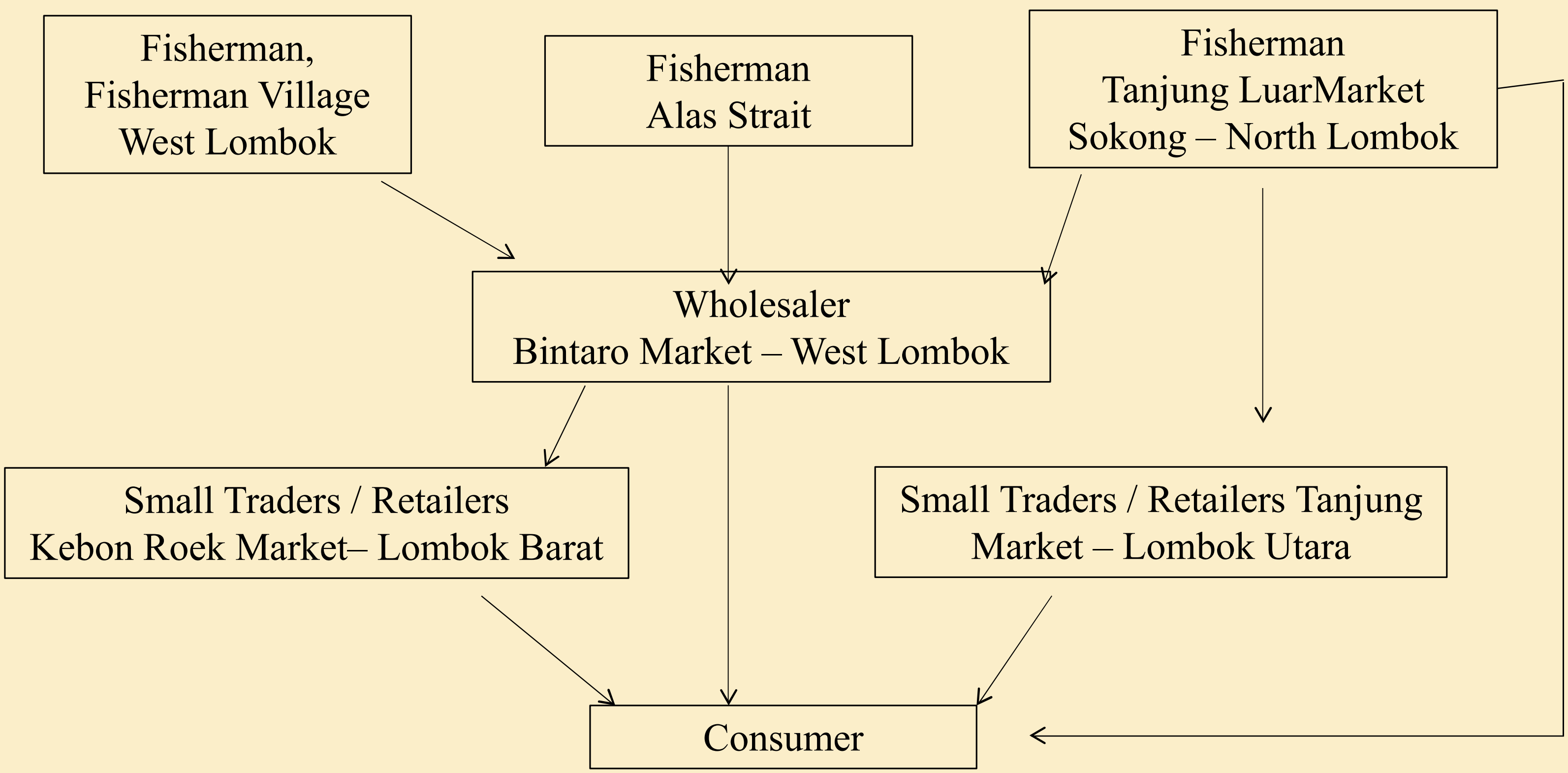
1. Hasil Uji ini hanya berlaku untuk contoh uji yang diuji.
   This analytical result are only valid for the tested sample.
2. Laporan hasil uji ini terdiri dari 1 (satu) lembar asli (stempel asli).
   The Report of Analysis consist of one original page (ORIGINAL SIGN).
3. Laporan hasil uji ini tidak boleh digandakan kecuali secara lengkap dan seizin tertulis dari Manajer Puncak / Kepala BUSKIPM, stempel copy.
   The Report of Analysis shall not be reproduced (copied) except for the completed one and with the written permission on Head of Standard Testing Laboratory of Fisheries Quarantine and Product Quality and Safety (copy sign).
4. Hasil pemeriksaan terlampir.
   The result of examination is attached.

New fish samples from February 2023

Famili	Spesies	Panjang Total (TL)	Jumlah
Carangidae	Decapterus macarellus	24,5 cm	1
	Elagatis bipinnulata	21,5 cm	1
Chaetodontidae	Heniochus diphreutes	16 cm	1
Kyphosidae	Kyphosus cinerascens	22,5 cm	1
Labridae	Oxycheilinus digrammus	25 cm	1
Lutjanidae	Caesio caerulaurea	23 cm	1
Scaridae	Scarus quoyi	33,5 cm	1
	Scarus tricolor	23 cm	1
Scombridae	Gymnosarda unicolor	22 cm	1
	Rastrelliger kanagurta	26,5 cm	1
	Ikan 4	36 cm	1
	Ikan11	30,6 cm	1

Ciguatoxin analysis is ongoing

Traceability of Reef Fish at Survey Sites



No.	Criteria	Wholesalers at Bintaro Market, Ampenan	Retailers Kebon Roek Market, Ampenan	Retailers Tanjung Market, Sokong	Restaurant Nipah Beach, Malaka Village, Pemenang
1.	Appearance				
	a. Eyes	8,7	8,7	7,0	8,0
	a. Gills	9,0	9,0	8,3	8,0
	a. Mucus/slime	8,7	9,0	7,7	8,0
2	Meat	8,3	8,3	7,7	8,0
3	Smell/odor	8,7	8,0	7,3	8,0
4	Textur2	8,7	8,0	7,7	8,0
Average freshness rating		8,7	8,5	7,7	8,0

Average Organoleptic Value of Reef Freshness in Wholesalers, Retailers and Restaurants in North and West Lombok,

West Nusa Tenggara, Based on SNI 2729, 2013

Gap Analysis Results of Conformity Value of Reef Freshness in Wholesalers, Retailers and Restaurants in North and West Lombok, West Nusa Tenggara

No	Those involved	Conformity Percentage	Conformity Level
1.	Wholesalers at Bintaro Market, ampenan	96%	Suitable 81 % - 100%
2	Retailer at Kebon Roek Market, Ampenan	94 %	
3	Retailer at Pasar Tanjung Market, Sokong	86%	
4	Restaurants in Nipah Beach, Malaka Village, Pemenang	89 %	

Gap Analysis Results of Appropriate Value of Reef Fish Handling at Wholesalers, Retailers and Restaurants in North and West Lombok, West Nusa Tenggara

No.	Those involved	Average Handling Value	Conformity Percentage	Conformity Level
1.	Wholesalers at Bintaro Market, ampenan	2,7	68%	Almost suitable (66% - ≤ 80%)
2	Retailer at Kebon Roek Market, Ampenan	1,7	43%	Not suitable (34% - ≤ 50%)
3	Retailer at Pasar Tanjung Market, Sokong	1,5	38%	
4	Restaurants in Nipah Beach, Malaka Village, Pemenang	1,6	39%	

The range of fish handling values based on SNI 2729 of 2013, namely 1 - 4

PRELIMINARY RESULTS

1. eDNA sampling in May, August, October and December 2022

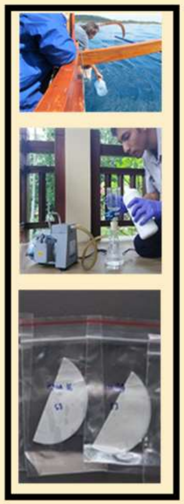
	Gili Trawangan	Gili Meno	Gili Ayer	May 2022	Aug 2022	Oct 2022	Dec 2022
Core Zone	St 1	St 6	St 12	3	3	3	3
Harbour Zone	St 3	St 7	St 13	3	3	3	3
Coral Sustainable Fisheries Sub Zone	St 5	St9,11	St 15	4	4	4	4
Protection Zone	St 4	St 8		2	2	2	2
Utilization Zone	St 2, 16	St 10	St 14	4	4	4	4
Rehabilitation Zone							
				16	16	16	16

2. Extraction of eDNA sampling

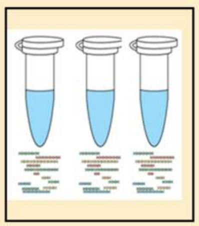
NEXT STEPS

1 → 2 → 3 → 4 → 5 → 6 → 7

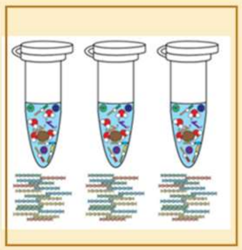
1 Collect and filter eDNA sample



2 Extraction of eDNA sample

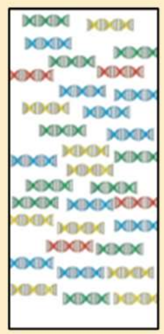


3 Amplify by DNA marker



MiFish primers (Miya et al., 2015)

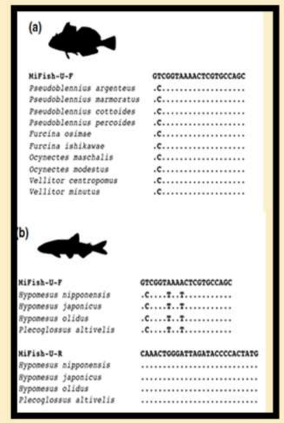
4 High-throughput Sequencing/NGS



5 Bioinformatic processing

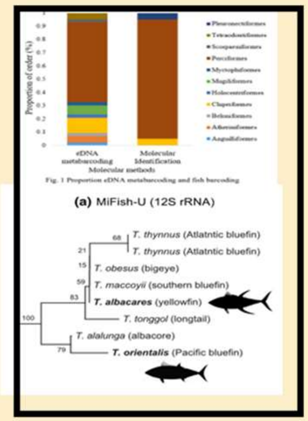
FASTQ File

6 Species identification



MiFish Pipeline (Sato et al., 2018).

7 Ecological Analysis

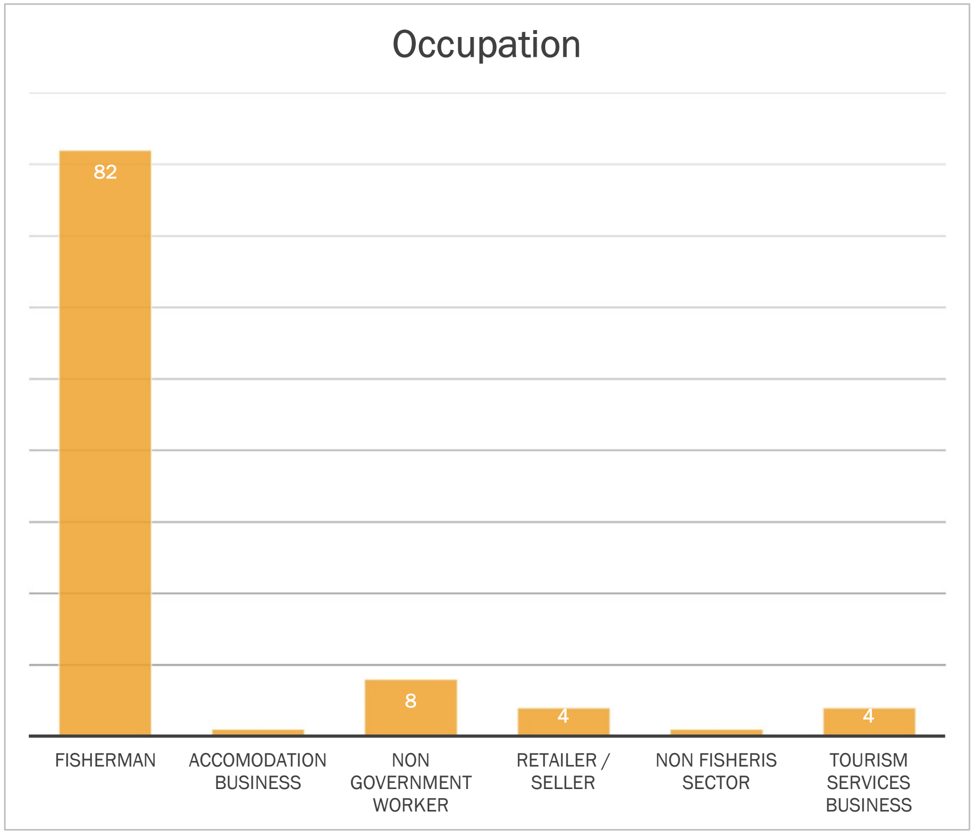


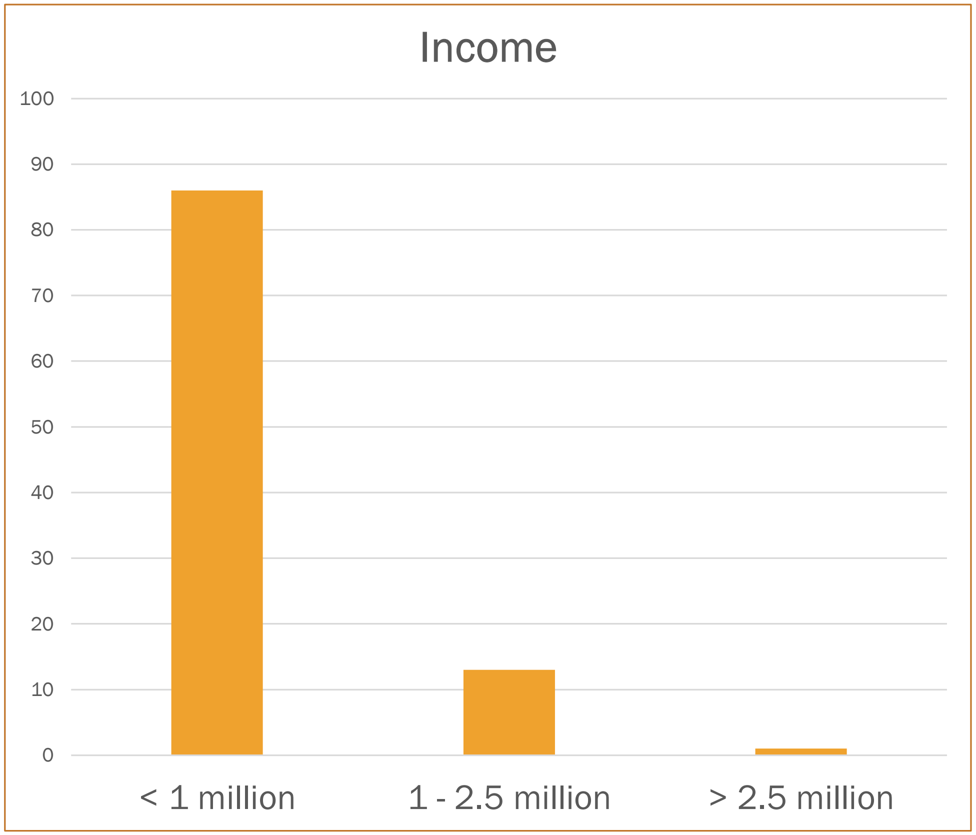
Diversity : Shannon-Wiener Index (H') (Magurran, 2004),

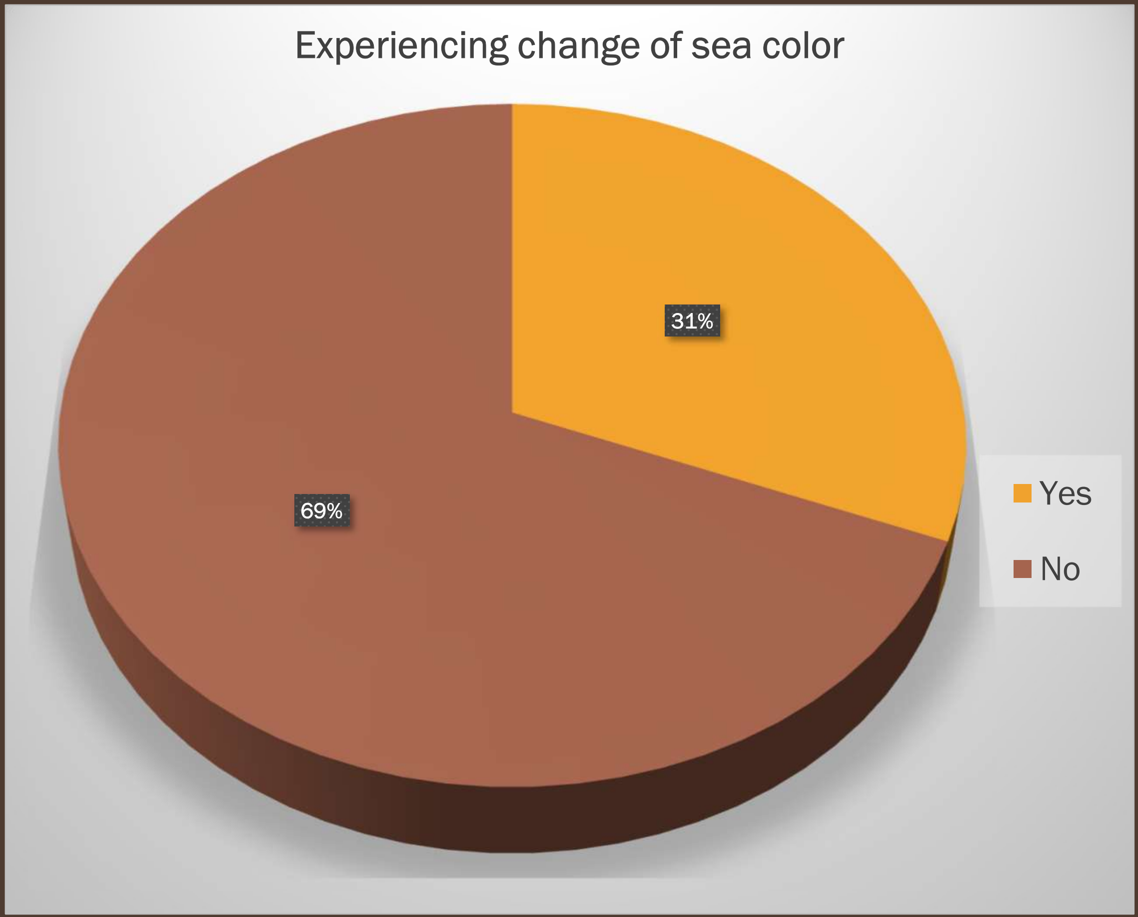
May, 2022 – Jan 2023

RESULTS

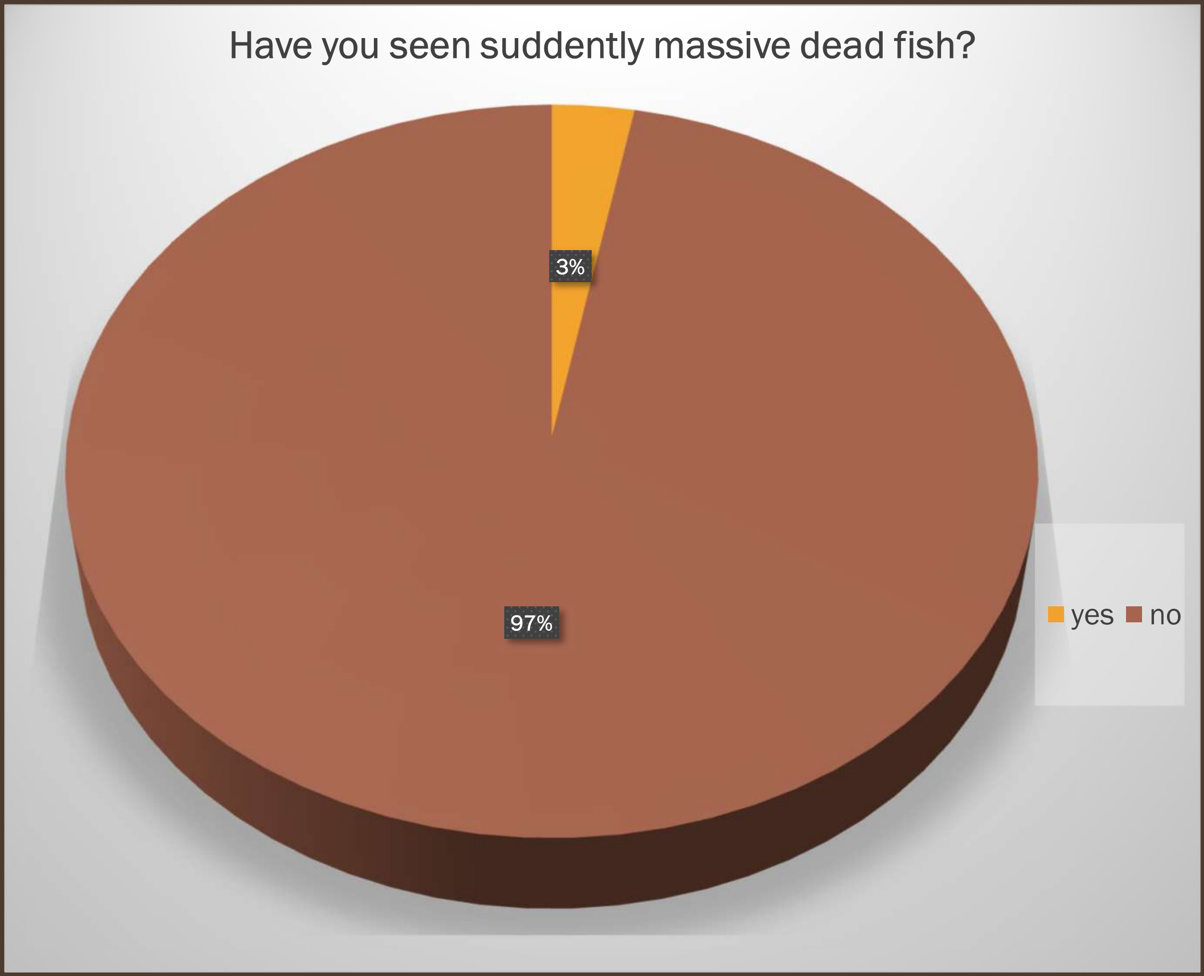
Socio-Economic


Occupation
82
8
4
4
FISHERMAN
ACCOMODATION BUSINESS
NON GOVERNMENT WORKER
RETAILER / SELLER
NON FISHERIS SECTOR
TOURISM SERVICES BUSINESS

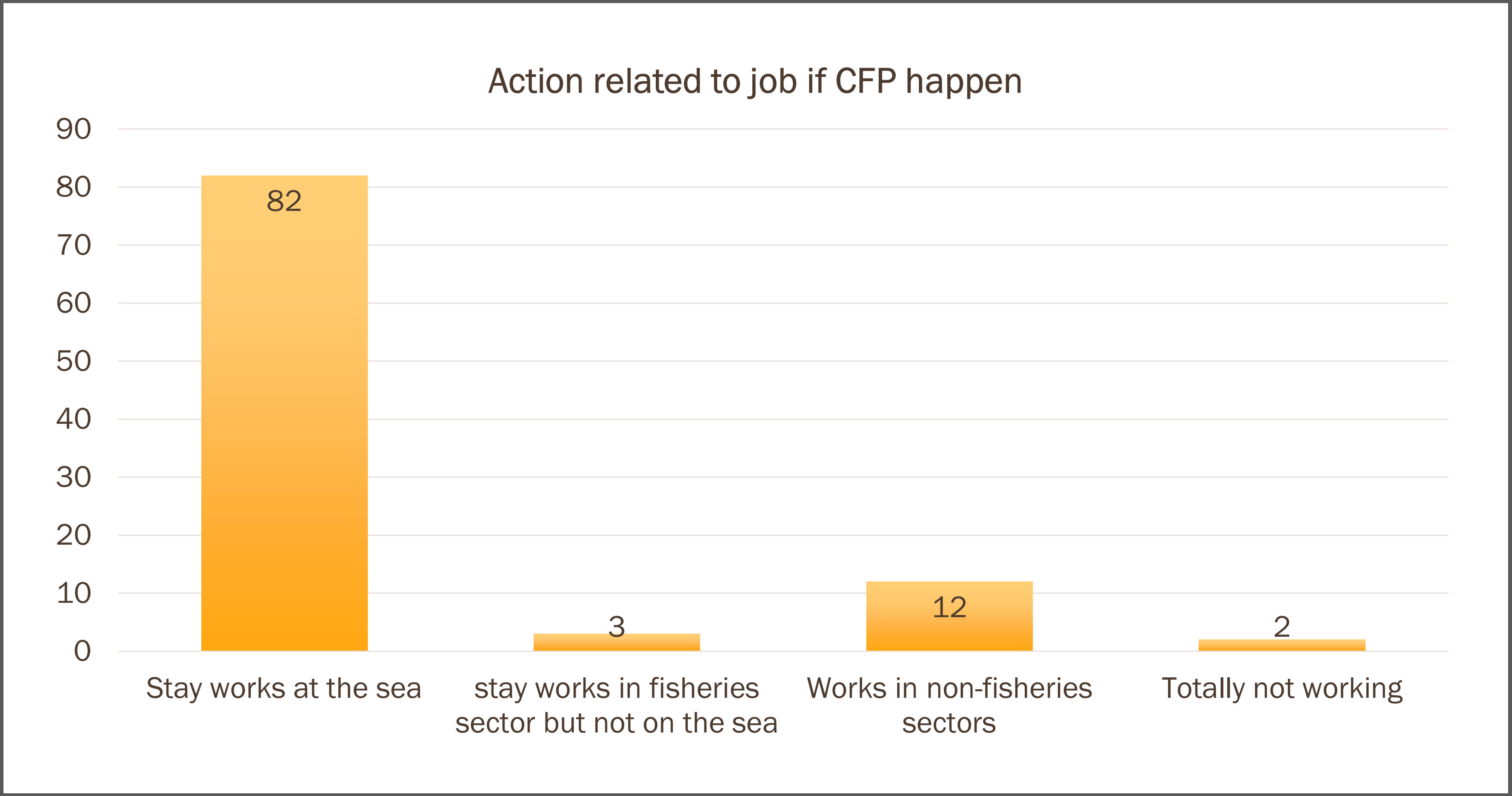

Income
100
90
80
70
60
50
40
30
20
10
0
< 1 million
1 - 2.5 million
> 2.5 million



- ”The sea color change to brown when it is raining. It is mainly close to the area that a river coming to the sea”
- It was last until 24 – 36 hours



- It was around 1990s caused by potassium.
- People use potassium to catch fish

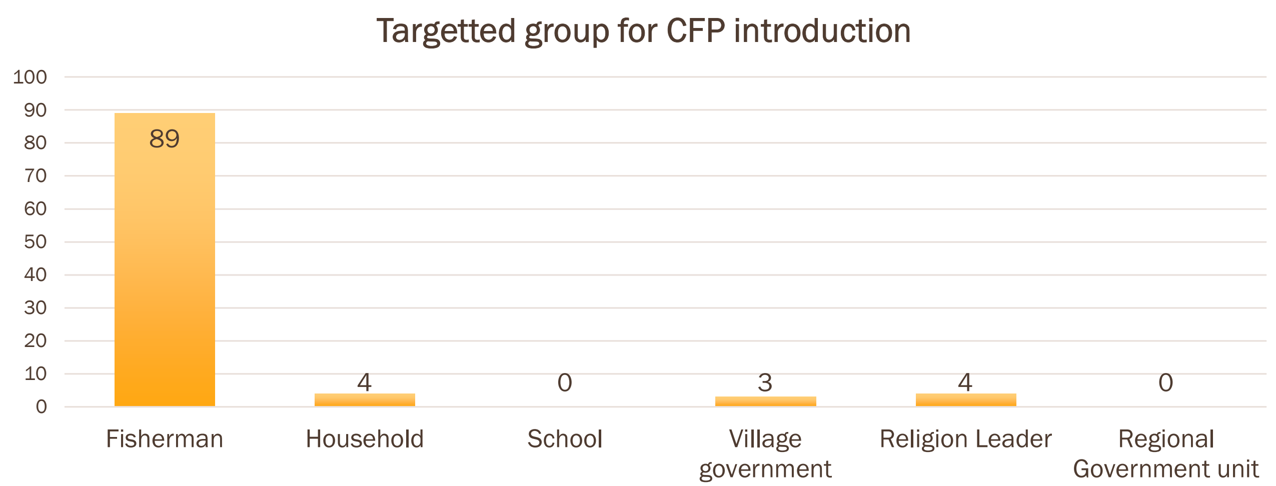

Action related to job if CFP happen
90
80
70
60
50
40
30
20
10
0
82
3
12
2
Stay works at the sea
stay works in fisheries sector but not on the sea
Works in non-fisheries sectors
Totally not working

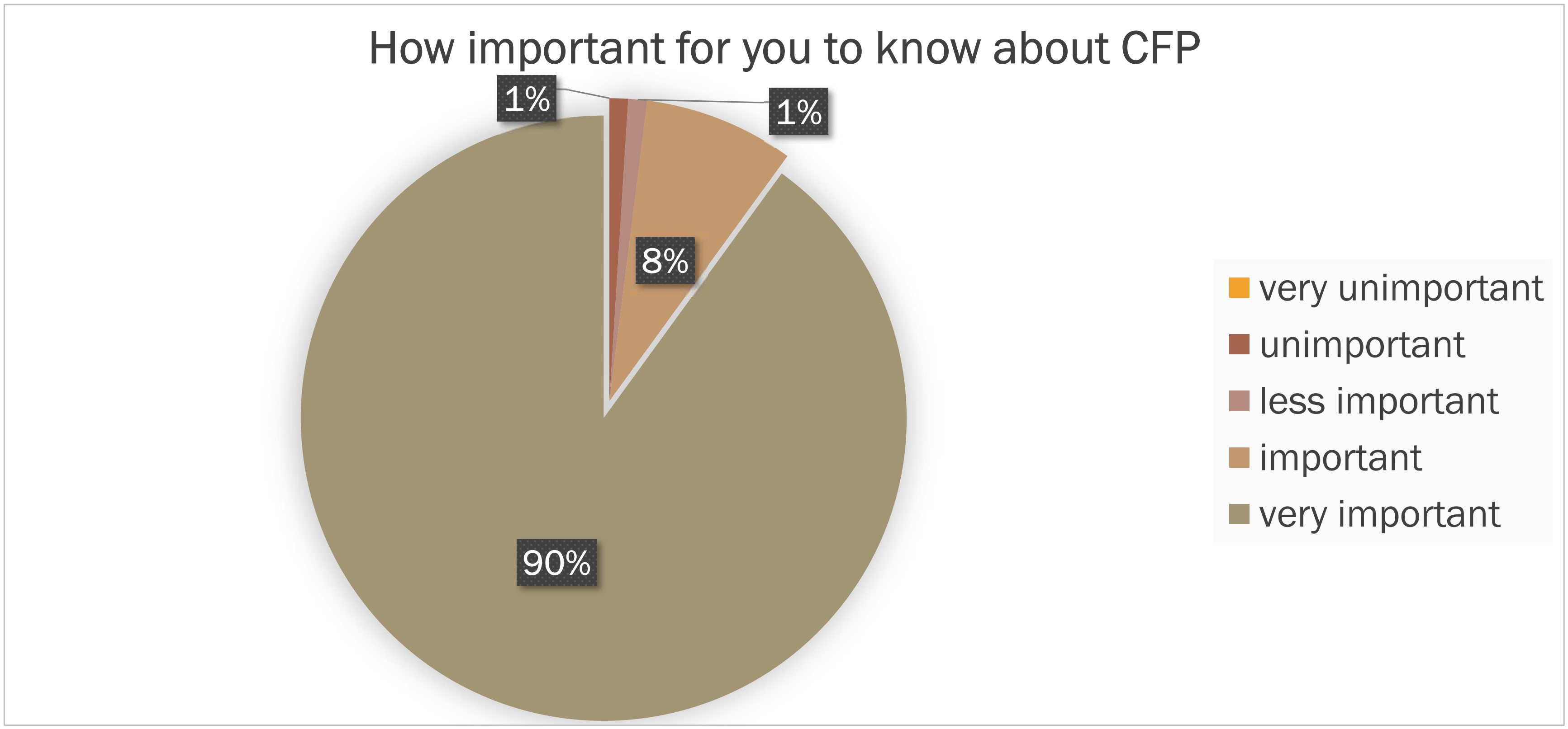
Information related to Ciguatera

There has never been any information related to Ciguatera

People also never been told, what to do when there is a change on the sea color nor when suddenly massive fish dead occurs.


Things people want to know
70
60
50
40
30
20
10
0
65
16
7
11
1
what cause CFP
syndrome or sign of CFP
Action to do when CFP occurs
How to address CFP
Impact of CFP


Targetted group for CFP introduction
100
90
80
70
60
50
40
30
20
10
0
89
4
0
3
4
0
Fisherman
Household
School
Village government
Religion Leader
Regional Government unit


How important for you to know about CFP
1%
1%
8%
90%
very unimportant
unimportant
less important
important
very important

Conclusions (temporary)

- Phytoplankton community in Gili Meno (part of Gili Matra) → dominated by diatoms and cyanobacteria → the dominance shifting along with seasonal changes → diatom dominance in wet season and transitional season I → trophic level in dry season is oligotrophic and it change into higher mesotrophic in wet season
- 17 species of phytoplankton → considered potentially harmful in Indonesia → have records to cause blooms, ocean discoloration, and/or fish mass mortality
- 5 targeted bHABs genera was found in Gili Matra → *Prorocentrum sp.*, *Ostreopsis sp.*, *Coolia sp.*, *Amphidinium sp.,* and *Gamiberdiscus sp.*→ *Prorocentrum* sp. was the most abundant target genera; *Gambierdiscus* sp. and *Amphidinium* sp. were only found in Gili Trawangan (so far)
- Ciguatoxin → not detected / no observable symptom or lethality in mouse bioassay → might exist in low concentration → below limit to cause symptom in laboratory mouse/mice
- Threat of CFP or HABs in Gili Matra so far → considered as low → due to:
  - *Low density of potentially harmful benthic dinoflagellate*
  - *Low density of potentially harmful phytoplankton species*
  - *Ciguatoxin not detected or no observable symptom or lethality in mouse bioassay*
- This is ongoing research → analysis of many samples are still ongoing

Acknowledgements

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THANK YOU

Scribo ergo sum