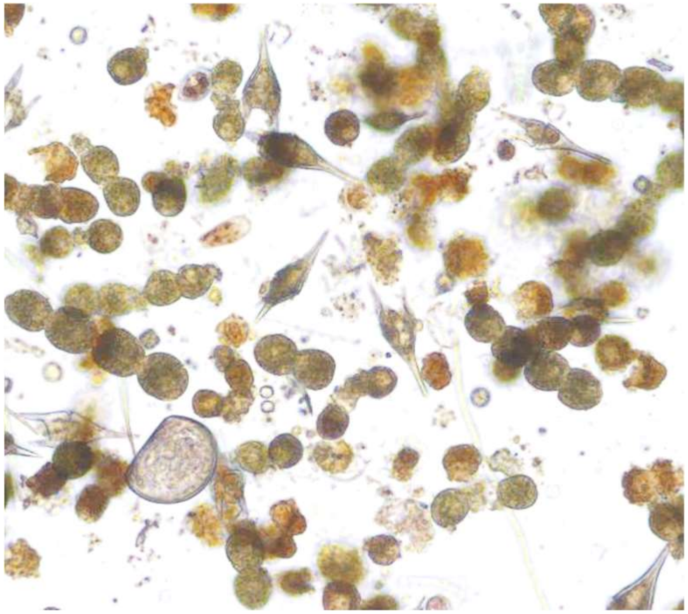


PICES

BRIN
BADAN RISET
DAN INOVASI NASIONAL

INSTITUT TEKNOLOGI INDONESIA
The Technology-based Entrepreneur University

UNIVERSITAS MATARAM

Ciguatera Indonesia II

PREPARED BY RIIM-4 CIGUATERA II INDONESIAN TEAM

RIIM-4

DEVELOPING THE DISASTER MITIGATION MODELS FOR CIGUATERA FISH POISONING (CFP) AND HARMFUL ALGAL BLOOMS (HABS) IN THE MARINE TOURISM PARK OF GILI MATRA, LOMBOK

Harmful Algal Blooms (HABs)

Harmful Algal Blooms (HABs) → one among 10 Plagues of the Seas → 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

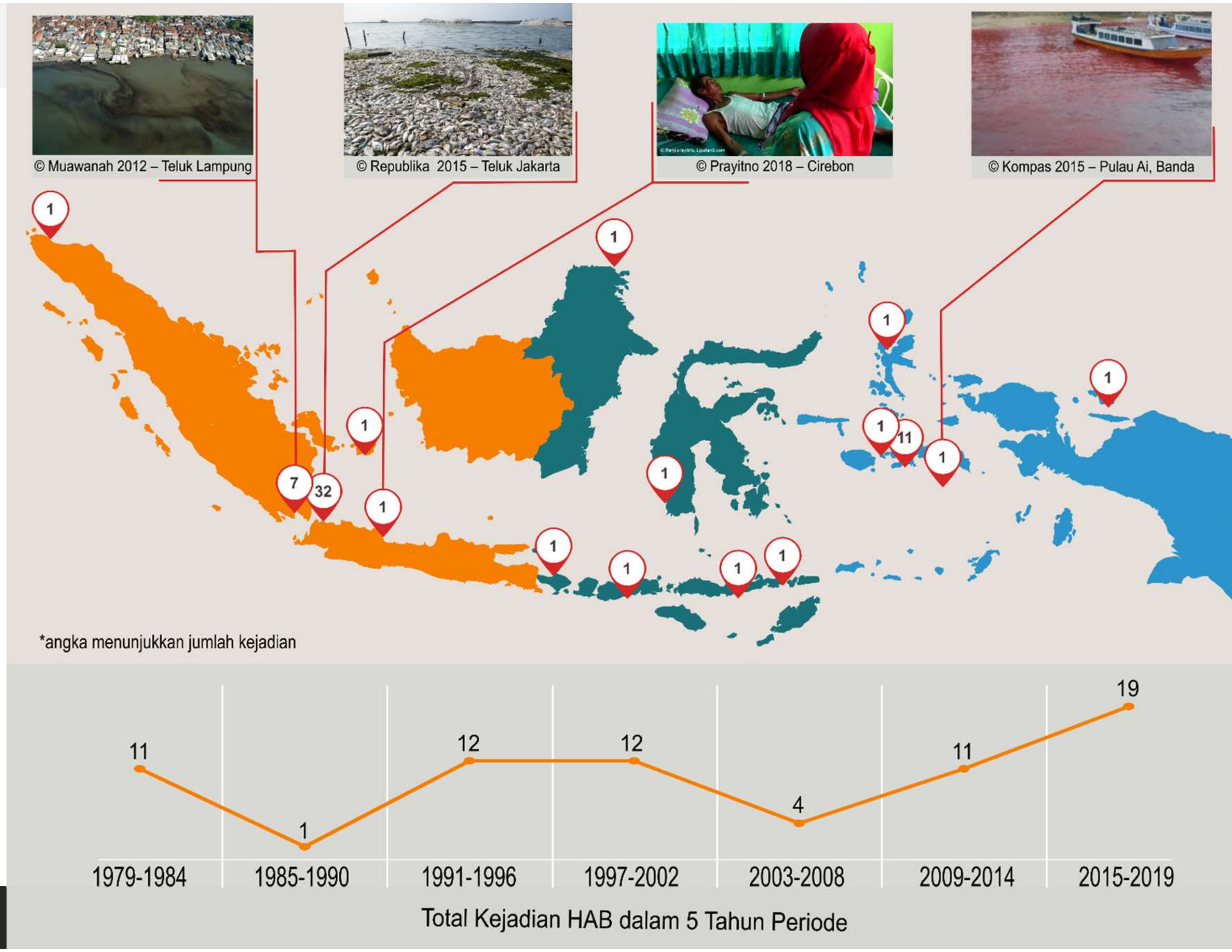
HAB Dashboard

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***



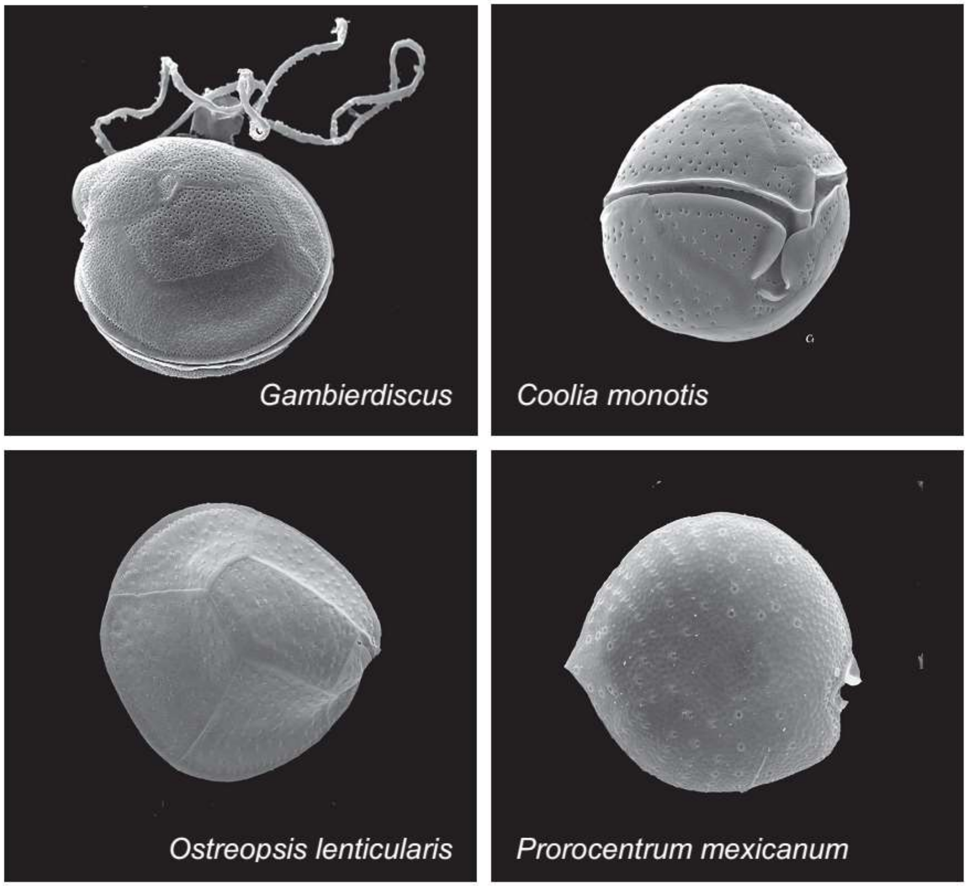


Figure 4. Morphology of *Gambierdiscus*, *Coolia*, *Ostreopsis* and *Prorocentrum* is illustrated in scanning electron micrographs. Cell dimension are estimated by the length and width of the species: *Gambierdiscus* 53-85 µm x 44-58 µm; *Coolia monotis* 23-49 µm x 23-38 µm; *Ostreopsis lenticularis 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)

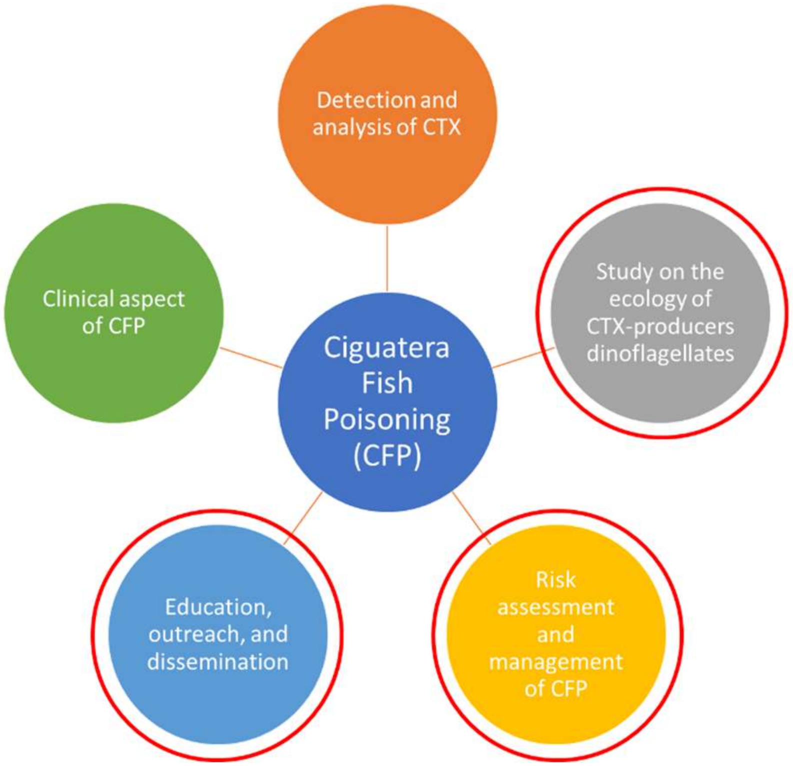
Records of benthic dinoflagellate species associated with CFP

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

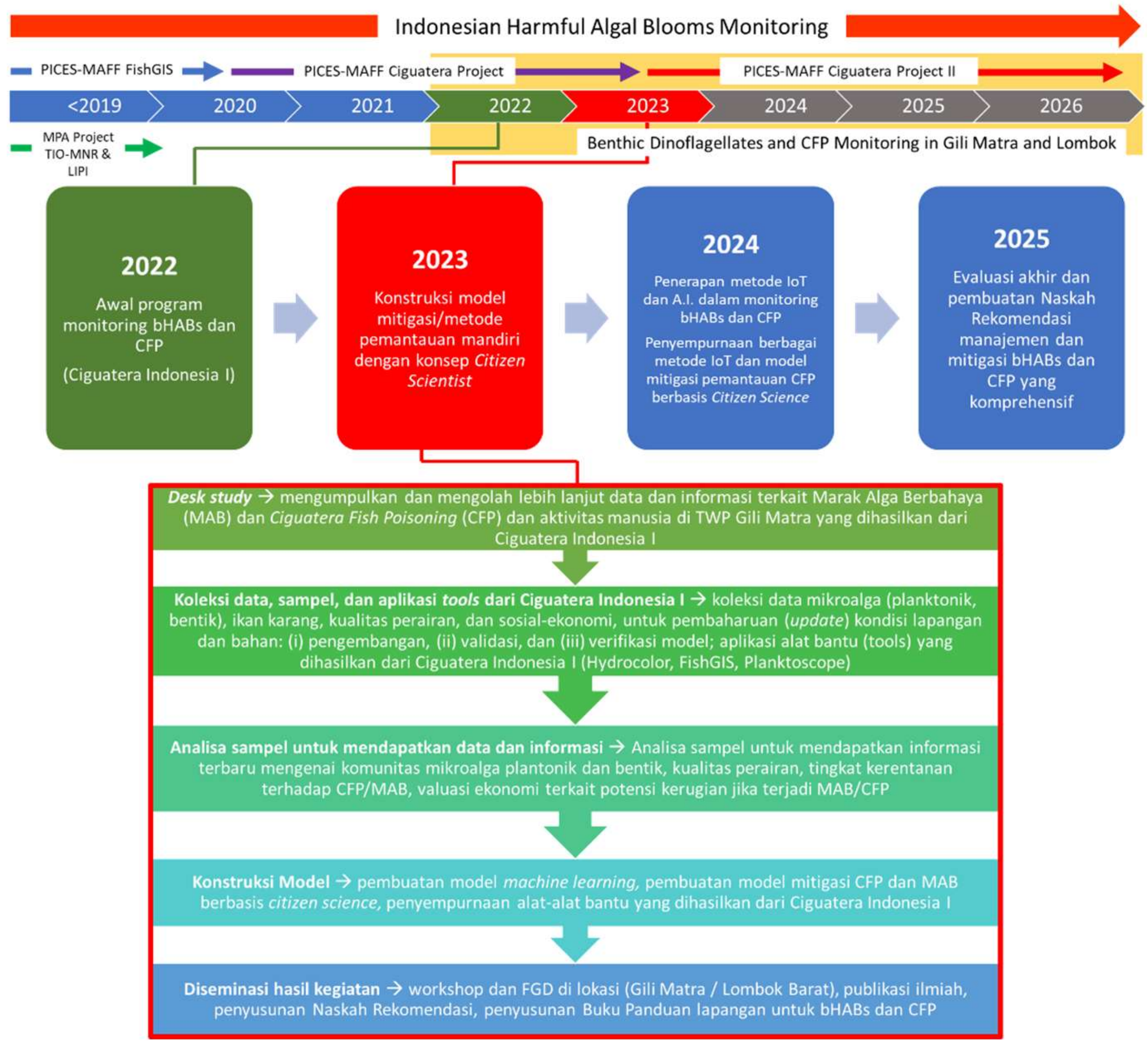


Integrated Multidisciplinary Research

- **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

Ciguatera Project II

Continuation of the Ciguatera Project I (2022-2023) → focused on the monitoring and mitigation strategies for bHABs and CFP



Research Aims

Ciguatera Indonesia II

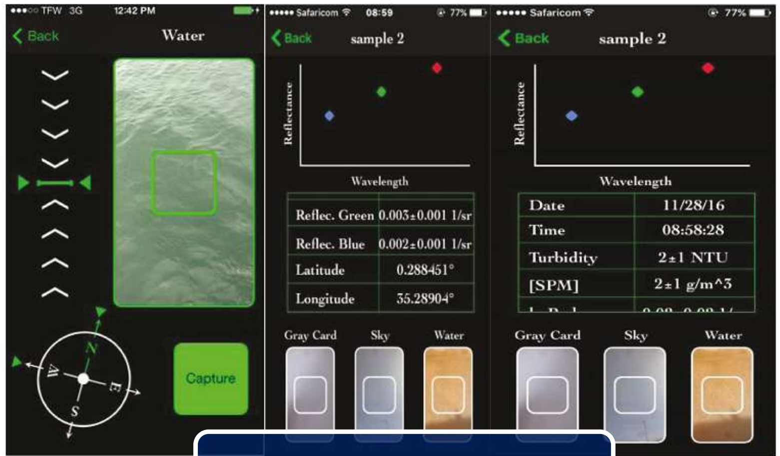
To continue the study the benthic dinoflagellate communities which could potentially cause CFP and their relationship with anthropogenic pressure and the habitat condition

To continue to disseminate information and increase the local public awareness on the potential health and economic impacts of HABs and CFP

To develop independent Citizen Science monitoring and mitigation strategies using available tools from the previous project (Ciguatera Project I)

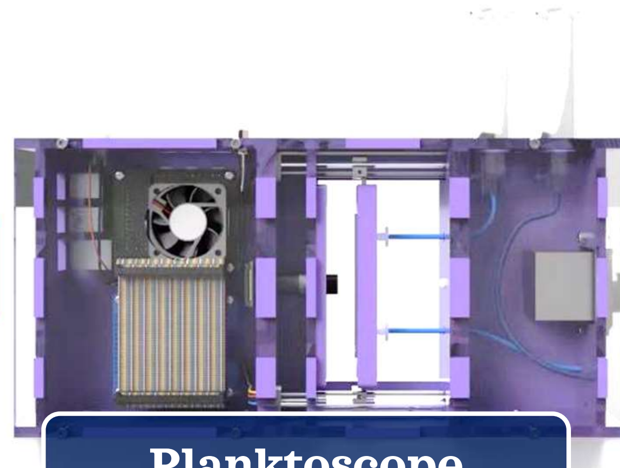
Integration of monitoring tools to monitor and mitigate the Harmful Algal Blooms (HABs)

FISH-GIS

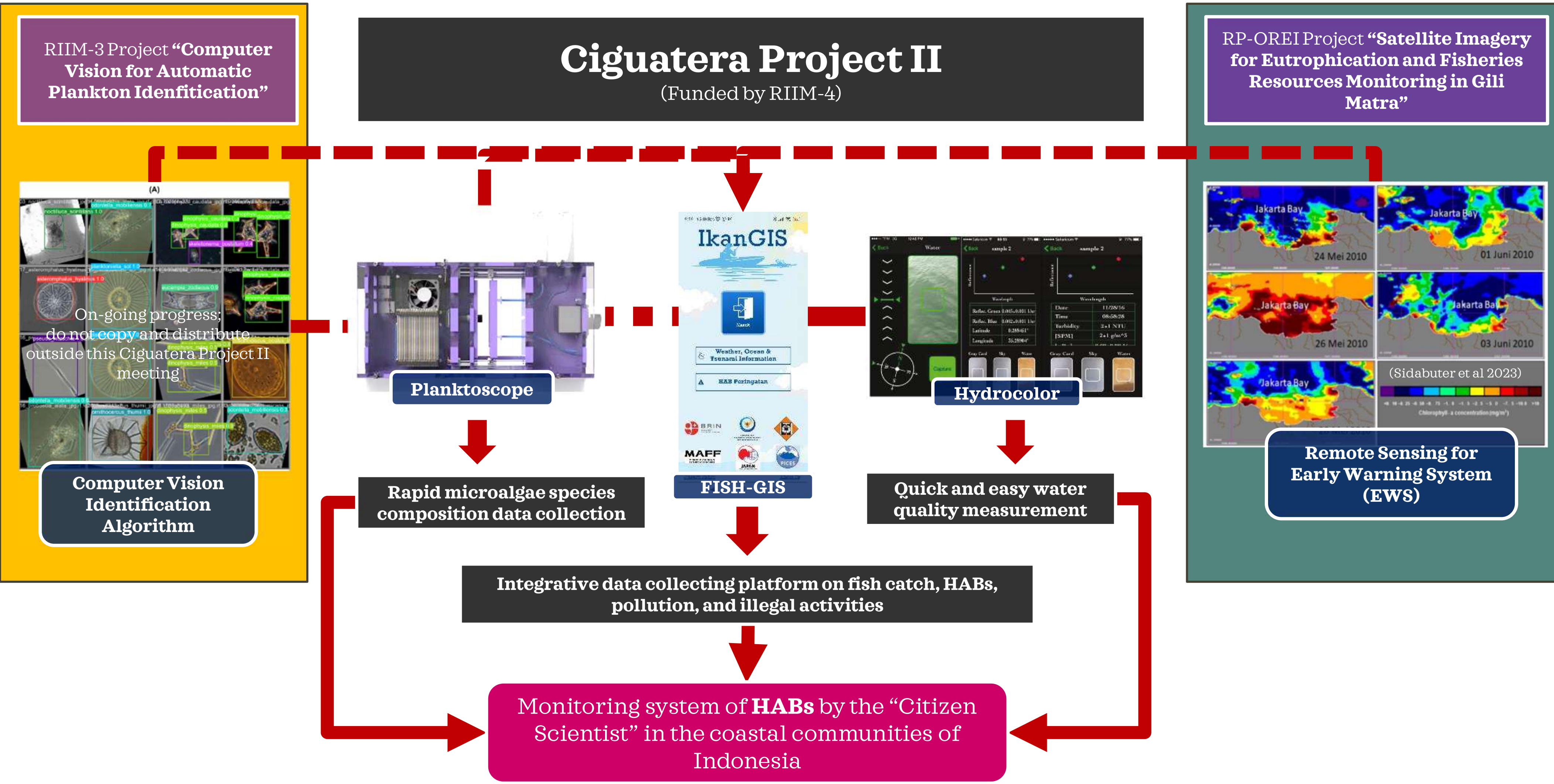


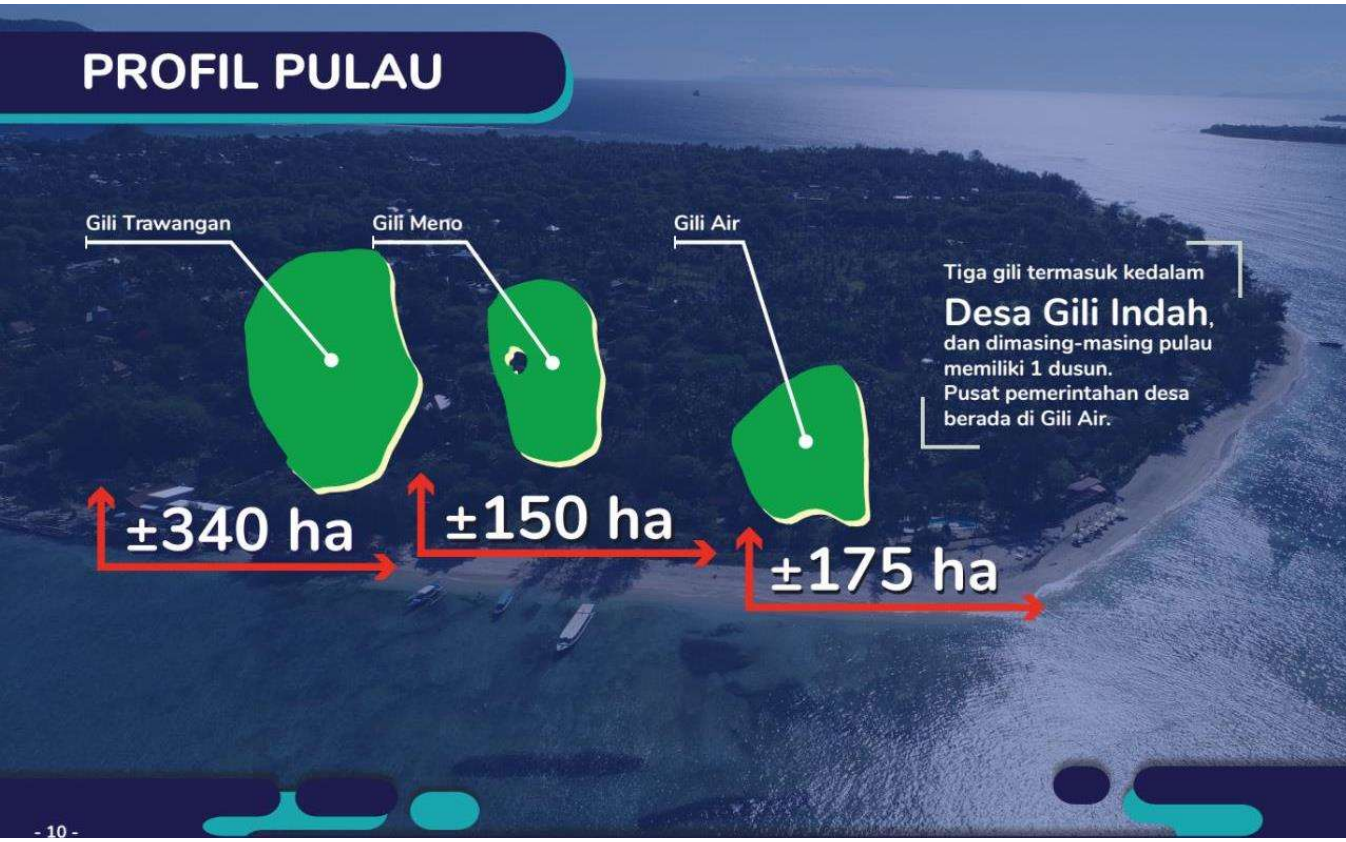
Hydrocolor

Monitoring system of **HABs** by the "Citizen Scientist" in the coastal communities in Indonesia



Planktoscope


Ciguatera Project II
(Funded by RIIM-4)
RIIM-3 Project "Computer Vision for Automatic Plankton Idenfitication"
On-going progress; do not copy and distribute outside this Ciguatera Project II meeting
Computer Vision Identification Algorithm
Planktoscope
Rapid microalgae species composition data collection
IkanGIS
Weather, Ocean & Tsunami Information
HAB Peringatan
MAFF
FISH-GIS
Hydrocolor
Quick and easy water quality measurement
RP-OREI Project "Satellite Imagery for Eutrophication and Fisheries Resources Monitoring in Gili Matra"
Jakarta Bay
24 Mei 2010
01 Juni 2010
26 Mei 2010
03 Juni 2010
(Sidabuter et al 2023)
Remote Sensing for Early Warning System (EWS)
Integrative data collecting platform on fish catch, HABs, pollution, and illegal activities
Monitoring system of HABs by the "Citizen Scientist" in the coastal communities of Indonesia



Sumber: 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**

Consist of important coastal ecosystems:

- Mangrove
- Coral Reef
- Seagrass

Have ecologically vital function 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)

Sampling Area

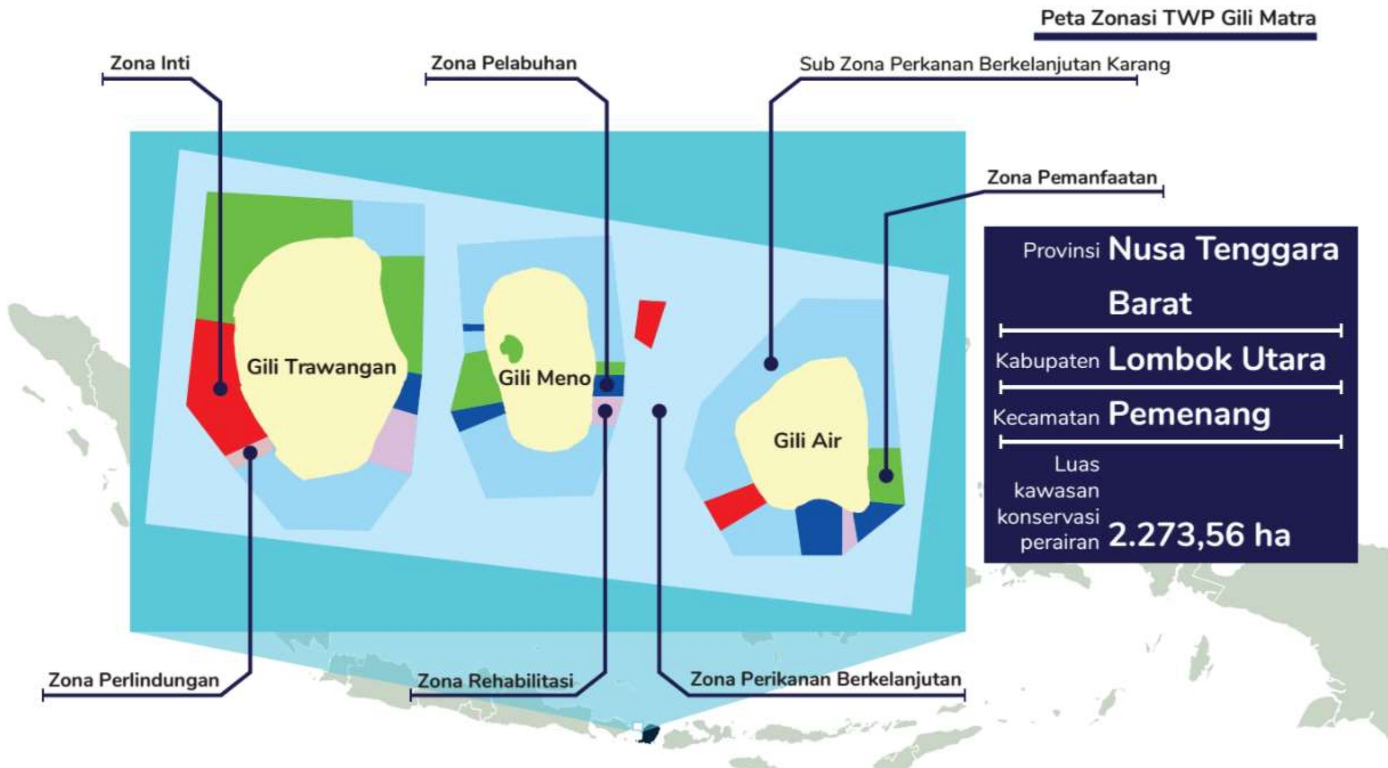
Sampling and data collection → will be conducted within selected zones around the Gili Trawangan, Gili Meno, dan Gili Air

Fieldworks within the timeframe of 2024 → March (Transition Season I) & August (Dry Season)

Fieldwork → 4 days → 2 days effective working days on field

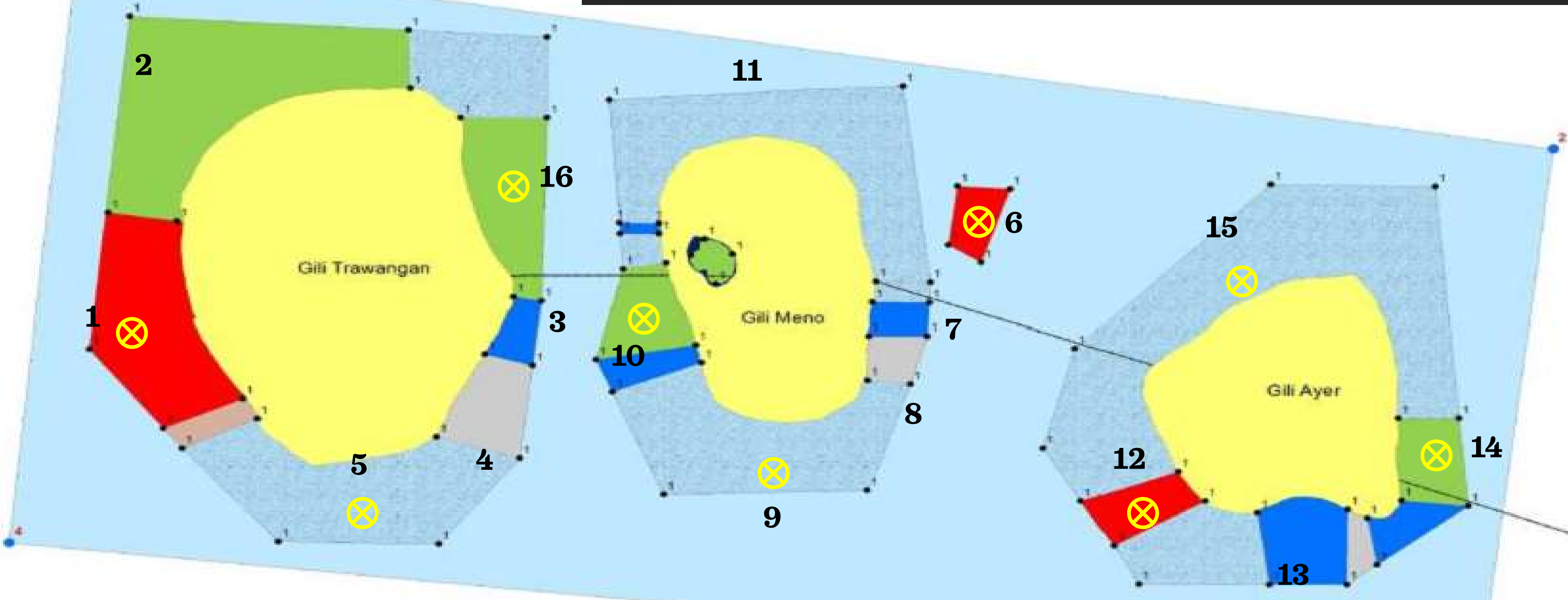
Microalgal sampling →

- Water column
- Seagrass,
- Macroalgae



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

Reduced number of sampling sites due to budget and time constraint



⊗ Water column sampling sites → **16 Sites (Ciguatera I)** → **9 sites (Cigatera II)**

- Plankton (phytoplankton + zooplankton) → **18 samples**
- Water quality (Temperature, pH, Salinity, DO, TDS) → **9 dataset**
- Nutrient concentration → **9 sampel**
- Chlorophyll-a (?
- e-DNA (?)

Focused on: the Core Zones (red), Utilization Zones (green), and Sustainable Fisheries Zones (light blue)

Station	Long (E)	Lat (S)	Colour Code	Zone	Island
1	116.0236	-8.35352	Red	Core	Gili Trawangan
5	116.0358	-8.36574	Light-blue	Sustainable Fisheries	Gili Trawangan
6	116.0681	-8.34595	Red	Core	Gili Meno
9	116.0572	-8.36373	Light-blue	Sustainable Fisheries	Gili Meno
10	116.0502	-8.35152	Green	Utilization	Gili Meno
12	116.0722	-8.36524	Red	Core	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

Water column sampling sites

Numbering of the sites will be changed later

Water column sampling

Plankton

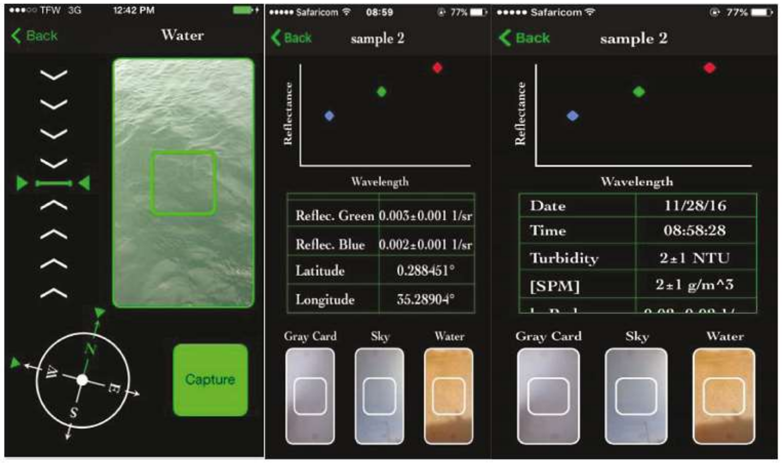
- Vertical towing with plankton net (zooplankton net, mesh 125 um; phytoplankton net, mesh 20 um)

Water (nutrient, chlorophyll-a, eDNA?)

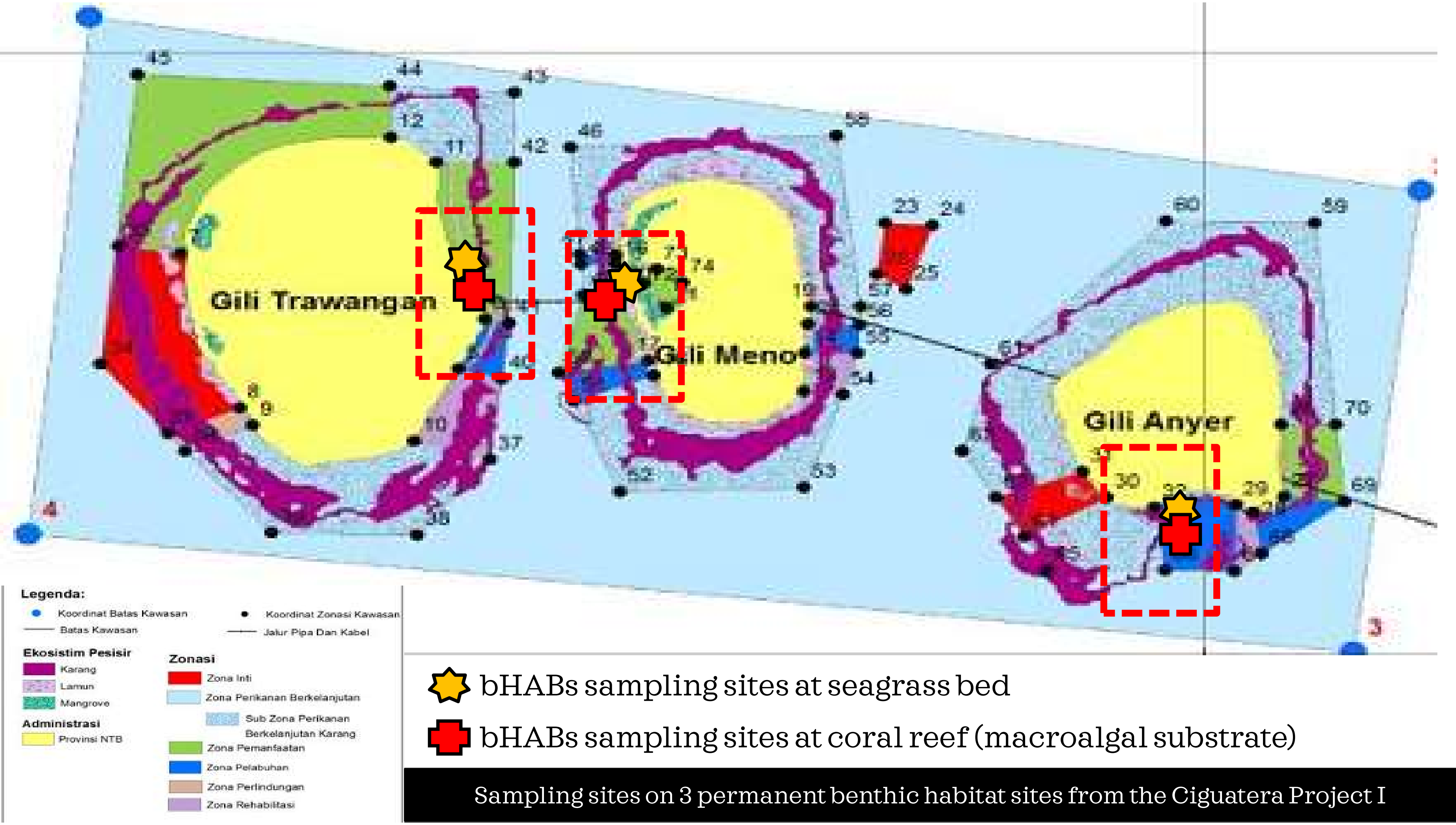
- Van Dorn / Nansen bottle → at minimum, 1 sample at surface layer (0.5 – 1 m depth); if possible, 2 sample (surface + near bottom)

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),
  - Hydrocolor



Water column sampling



Sampling sites on 3 permanent benthic habitat sites from the Ciguatera Project I

Benthic microalgal sampling

Benthic microalgae

- *Natural substrat (free dive)*

Air (analisis nutrient, klorofil dan eDNA)

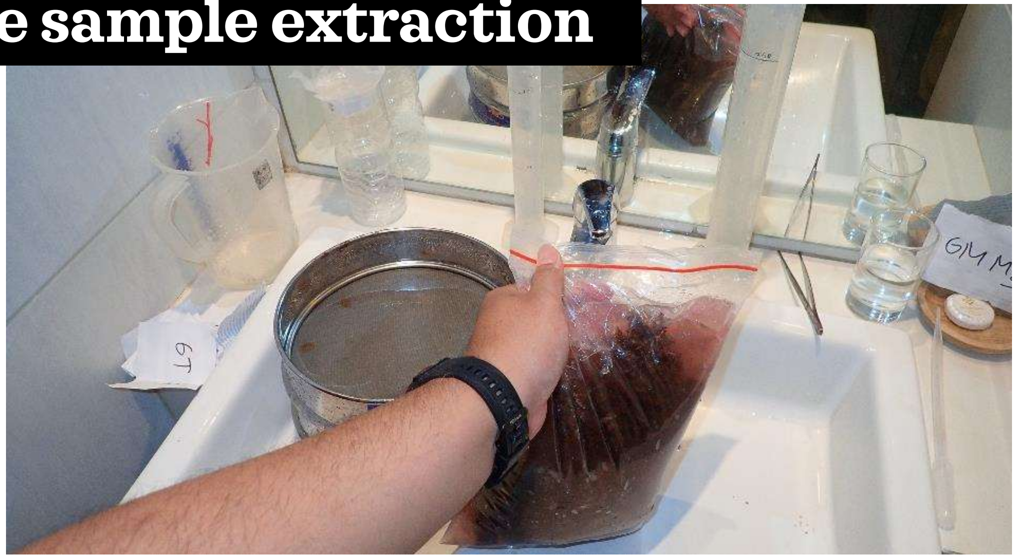
- *Van Dorn / Nansen bottle → at minimum, 1 sample at middle column (0.5 – 1m from the bottom/habitat/substrate)*

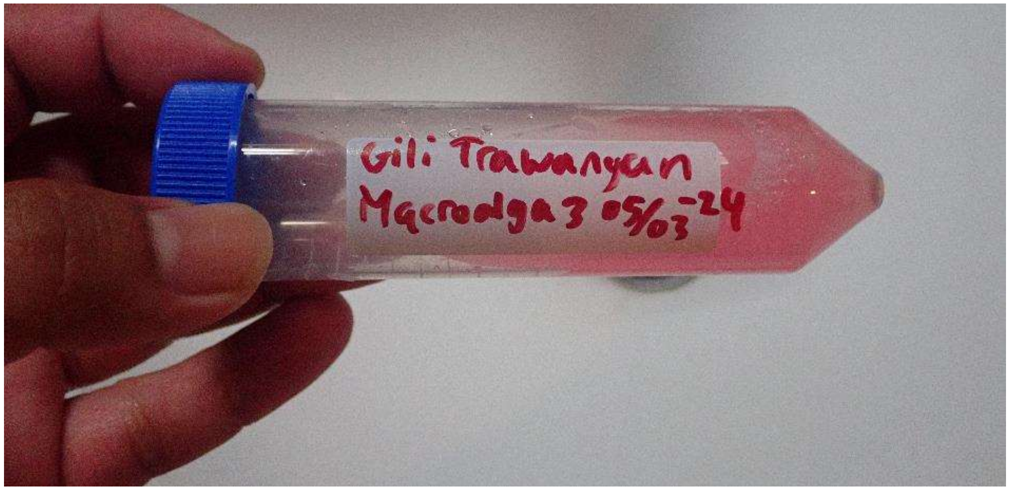
Water quality

- *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),
  - Hydrocolor

Benthic dinoflagellate sampling

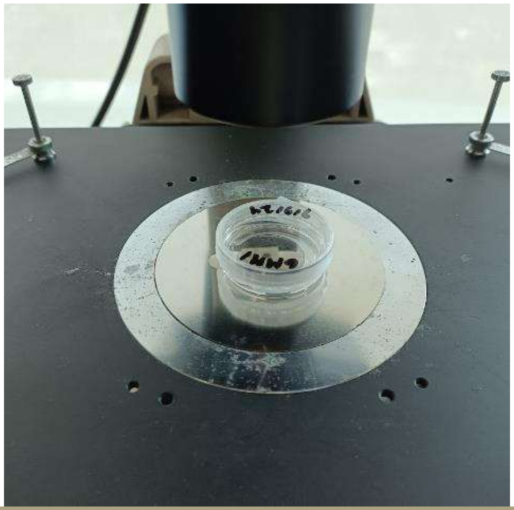
Benthic dinoflagellate sample extraction

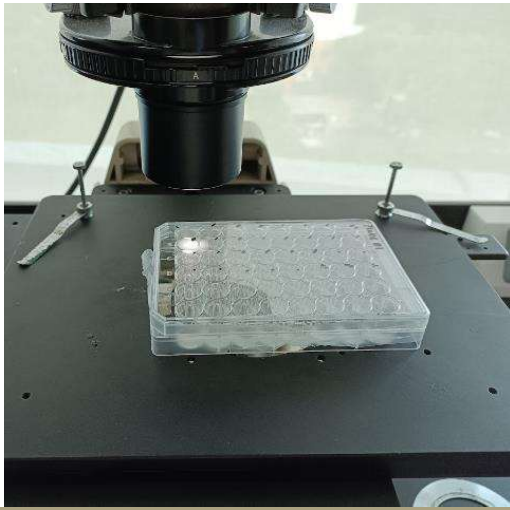




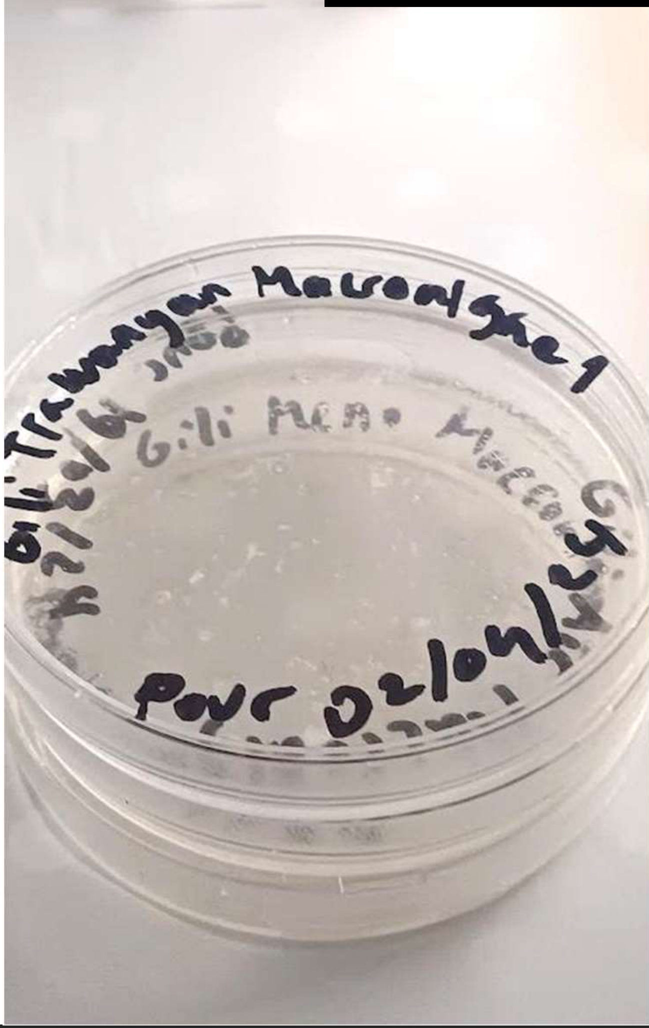
Benthic dinoflagellates culture experiments

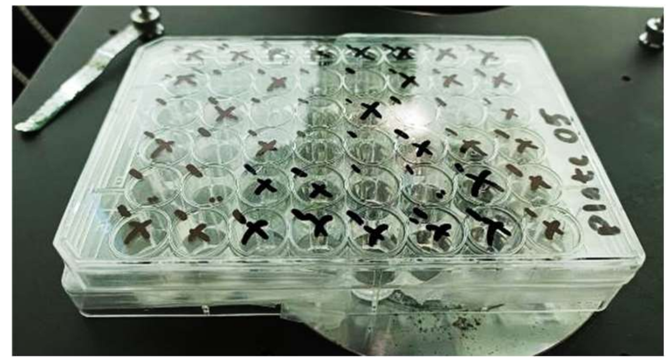
- Live benthic dinoflagellate samples from macroalgae were cultured → adding 0.5 – 1 mL live sample and filtered and syringe-sterilized ambient seawater in a 25 cm^2 culture flask
- Raw cultures were incubated in a culture chamber with side mounted dual-tone LED light at 26°C, 12:12h Light:Dark period, at around 1000 – 2000 LUX (17 – 34 umol/s/m^2) for 15- 30 days → 1mL ENSW medium (ambient sterile seawater + F/2 or F/4 medium) was added every 7 – 10 days
- Raw cultures with targeted species were transferred to TC-treated petri dish → cells were isolated with capillary Pasteur pipette → washed 2-3 times in sterile seawater droplet → inoculated in 35mm petri dish with ENSW → between 5 – 20 cells per petri dish → left to grow for 5 – 7 days
- Living and healthy cells in 35mm pre-culture petri → isolated → washed 2 times → inoculated in 48-well TC-treated culture plate → 100 uL ENSW medium added every 5-7 days

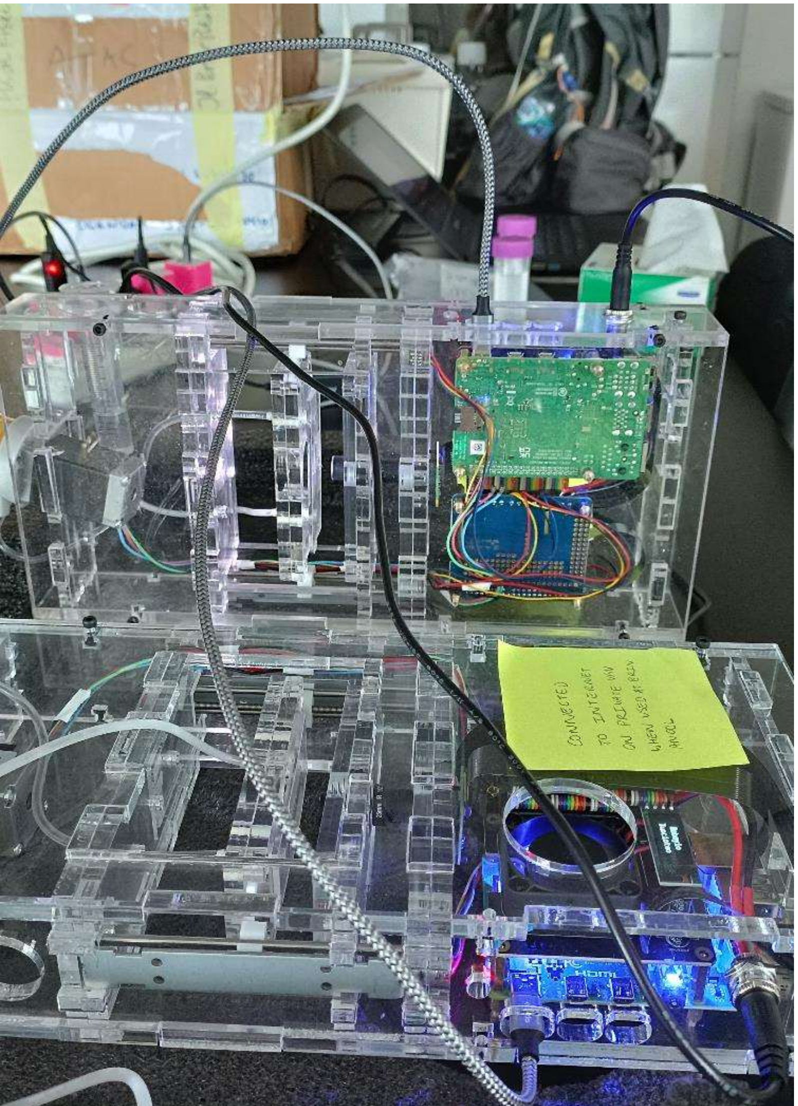




Benthic dinoflagellate culture experiment







Planktoscope analysis

Plankton samples for analysis will be collected from

- Plankton Net samples
- Water samples
- Macrophyte natural samples

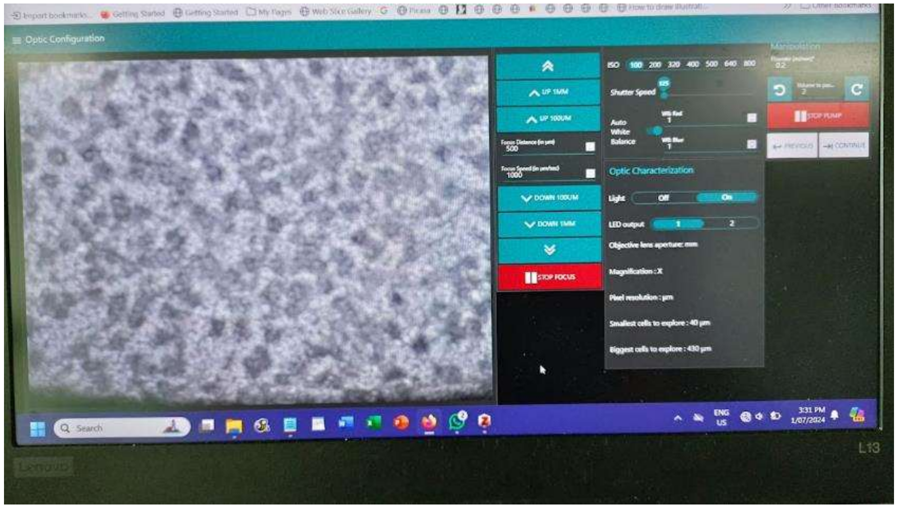
Samples will not be preserved → to avoid staining in the microfluidic column

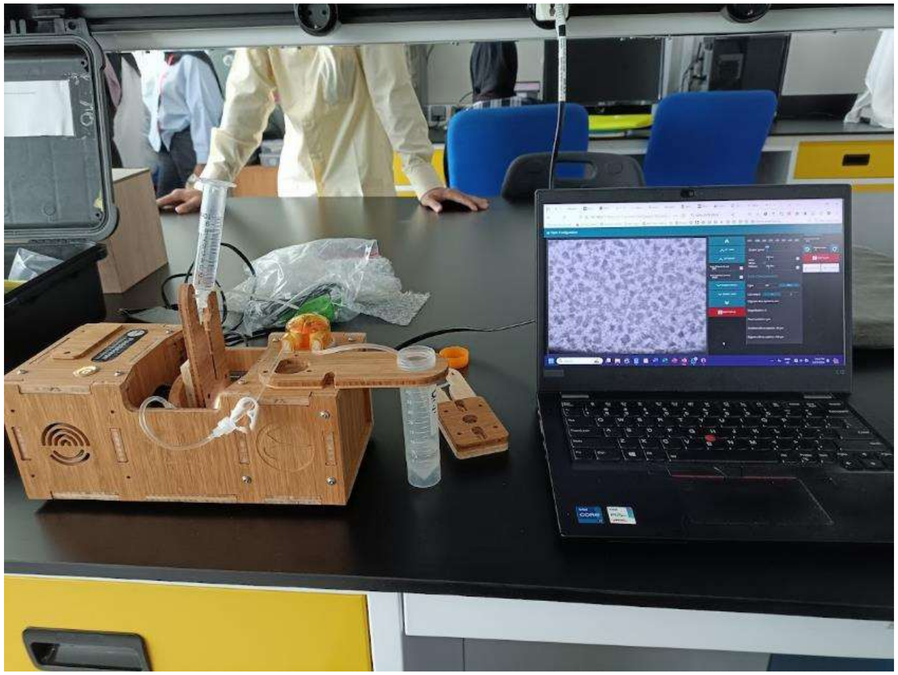
Fresh sample will be analyzed with Planktoscope at hotel

If the analysis can't be carried out directly at the same day → sample can be stored in low temperature (4°C) to reduce the rate of decomposition

Samples that have been analyzed → will be preserved with Alcohol 70%

Planktoscope analysis





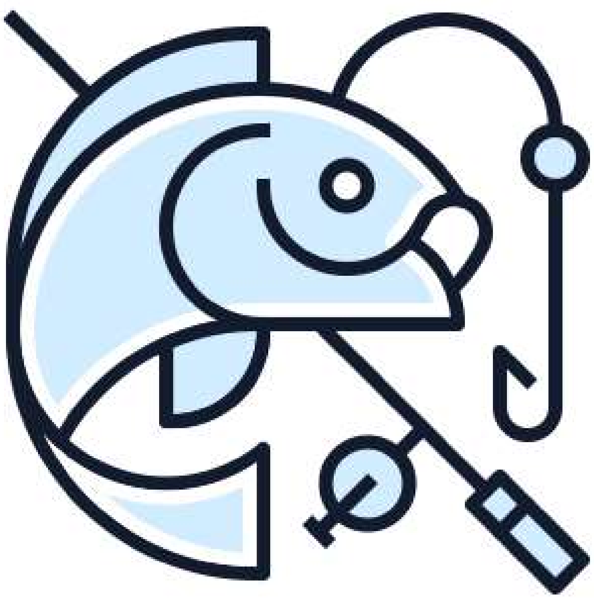
- Currently have issue with **<u>fungi on lens</u>** due to high humidity → wooden casing prone to trap humidity? → acrylic case might be the best in tropical countries
- **Planktoscopes** → currently in the care of ITI to disassemble and to learn to fix and improve the design/function

Social-economy sampling/data collection

On-site survey	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
Questionaire	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
Focus Group Discussion	Discussion with local community or other important/relevant stakeholders (local government, academics, NGO, fisheries department, conservation department, etc)
Secondary data	Collecting secondary data from related institutions or local government

Social-economy field survey by Mataram University students

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)

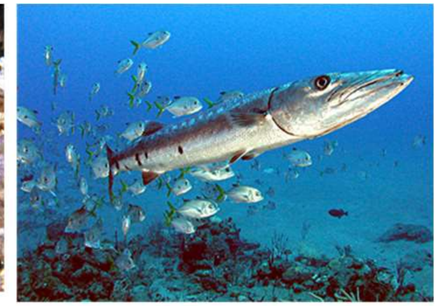
- Fish from local market in Lombok or in Gili Matra (Gili Trawangan & Air)
- Viscera, gill, body flesh (min. 500gr) → collected from each targeted species
- Ciguatoxin analysis → mouse bioassay (the laboratory of the Fish Quarantine and Inspection Agency, Ministry of Marine Affairs and Fisheries, Indonesia)

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 (Predator)
- Snapper (*Lutjanus* spp.) – Ikan Kakap
- Mackerel (*Scomberomorus* spp.) – Ikan Kembung
- Parrotfish (*Scarus* spp.) – Ikan Kakatua (Herbivore/Grazer)
- 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

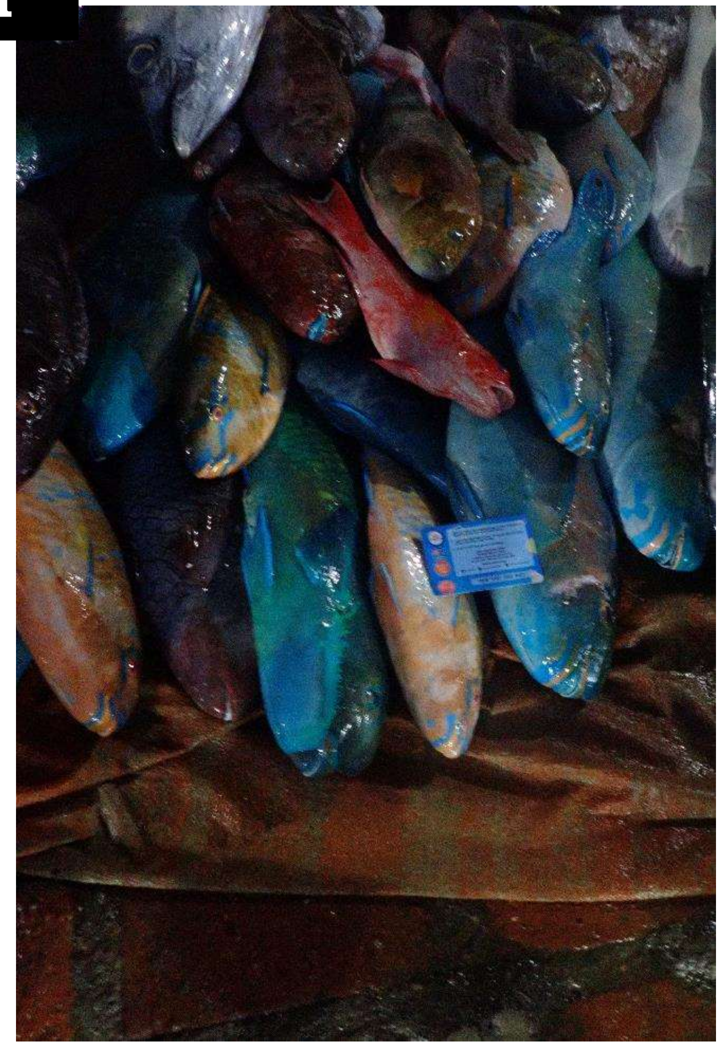





IkanGIS (FishGIS) data collection

- Fish data collection will be conducted in the local fish markets in Mataram and in Gili Trawangan
- All fish photo must be accompanied by a scale → ruler, pen, pencil, card, etc
- Any anomalies on field → floating debris/garbage, algal blooms, and unusual ship activities → will also be reported using IkanGIS apps

FISH-GIS data collection

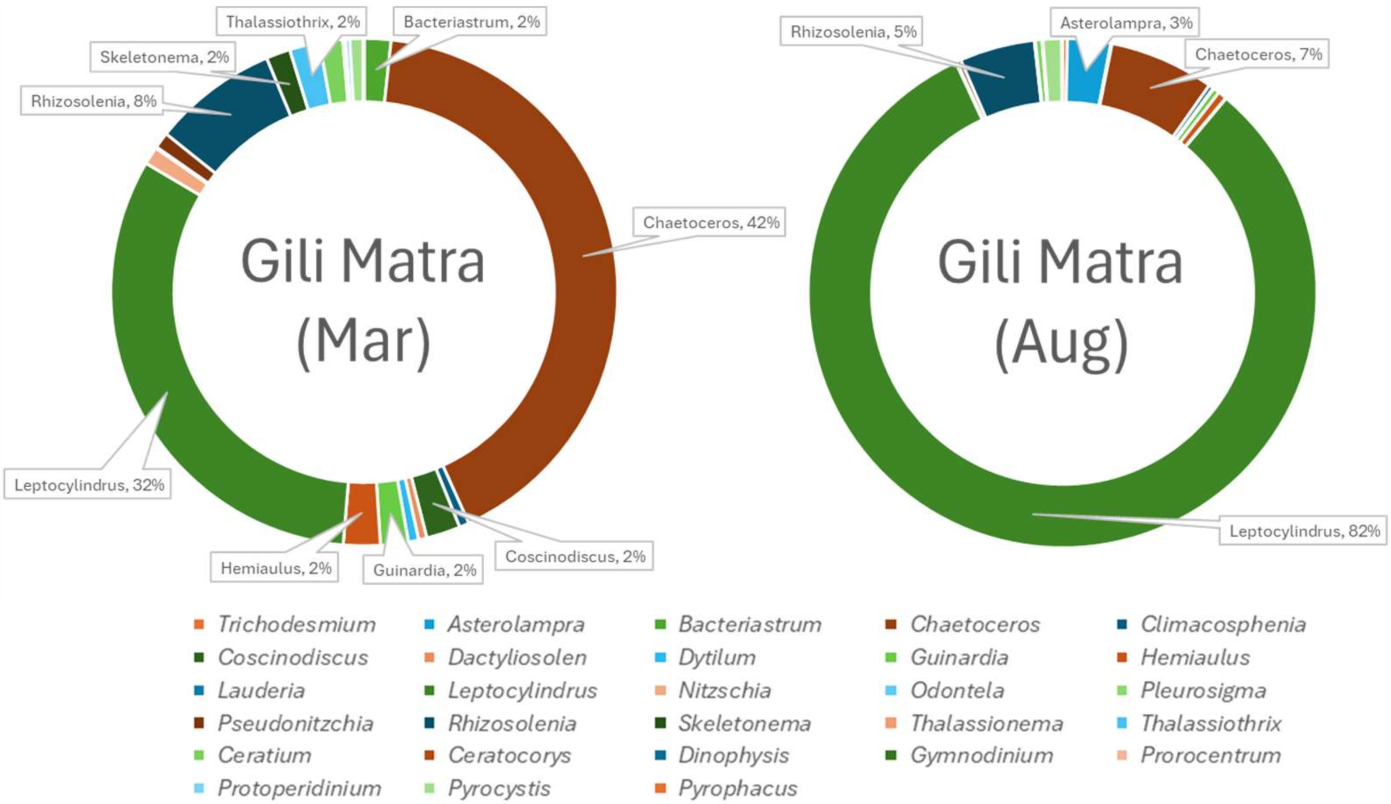


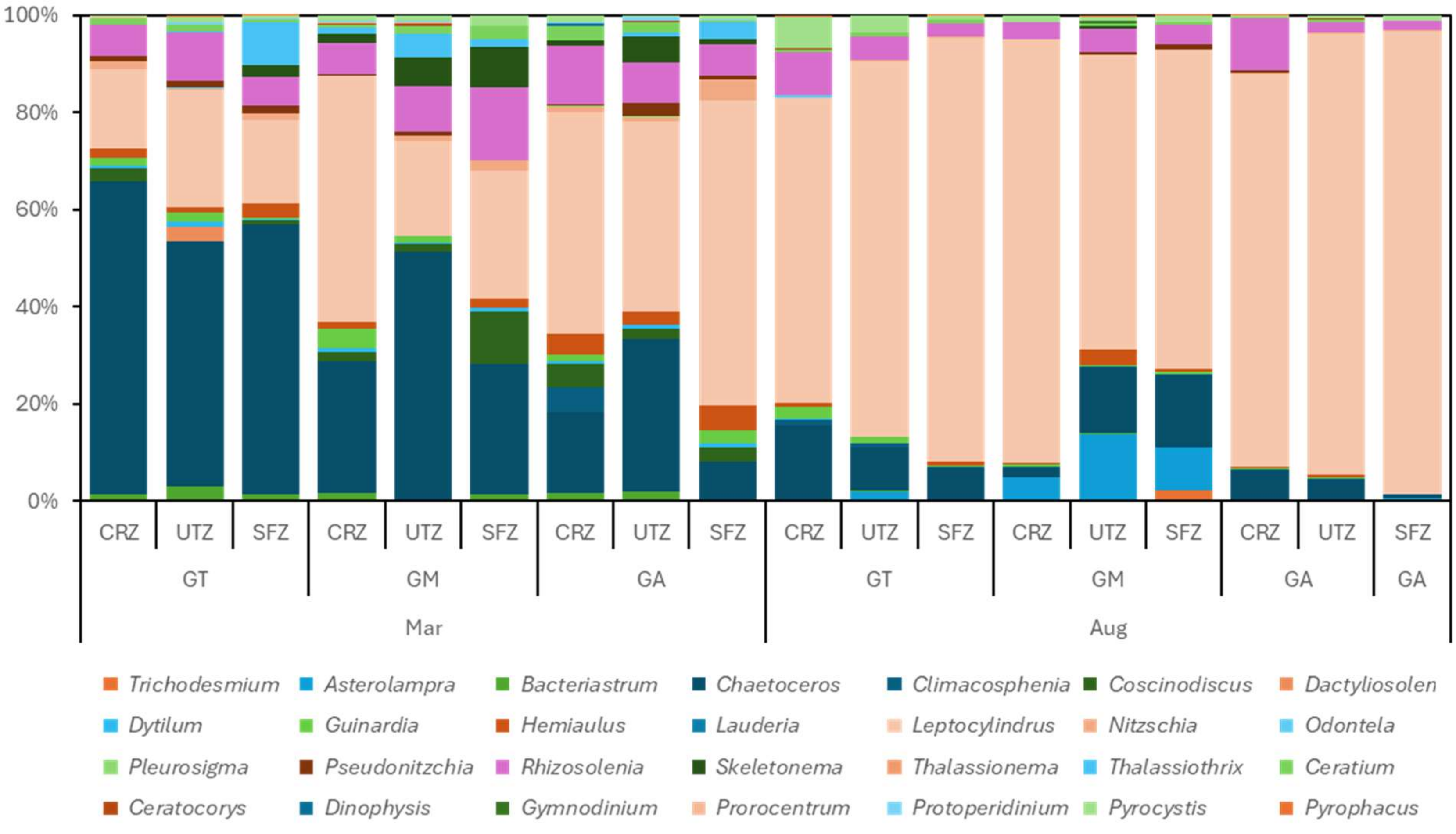
Results

CIGUATERA II
FIELDWORKS AND
LABORATORY
EXPERIMENTS

General phytoplankton assemblages

- Mainly dominated by Diatoms→ *Chaetoceros* and *Leptocylindrus*
- Other commonly abundant genus → *Rhizosolenia, Skeletonema*, *Thalassiothrix*, *Bacteriastrum*, and *Asterolampra*
- Significant differences in the phytoplankton assemblages between March and August

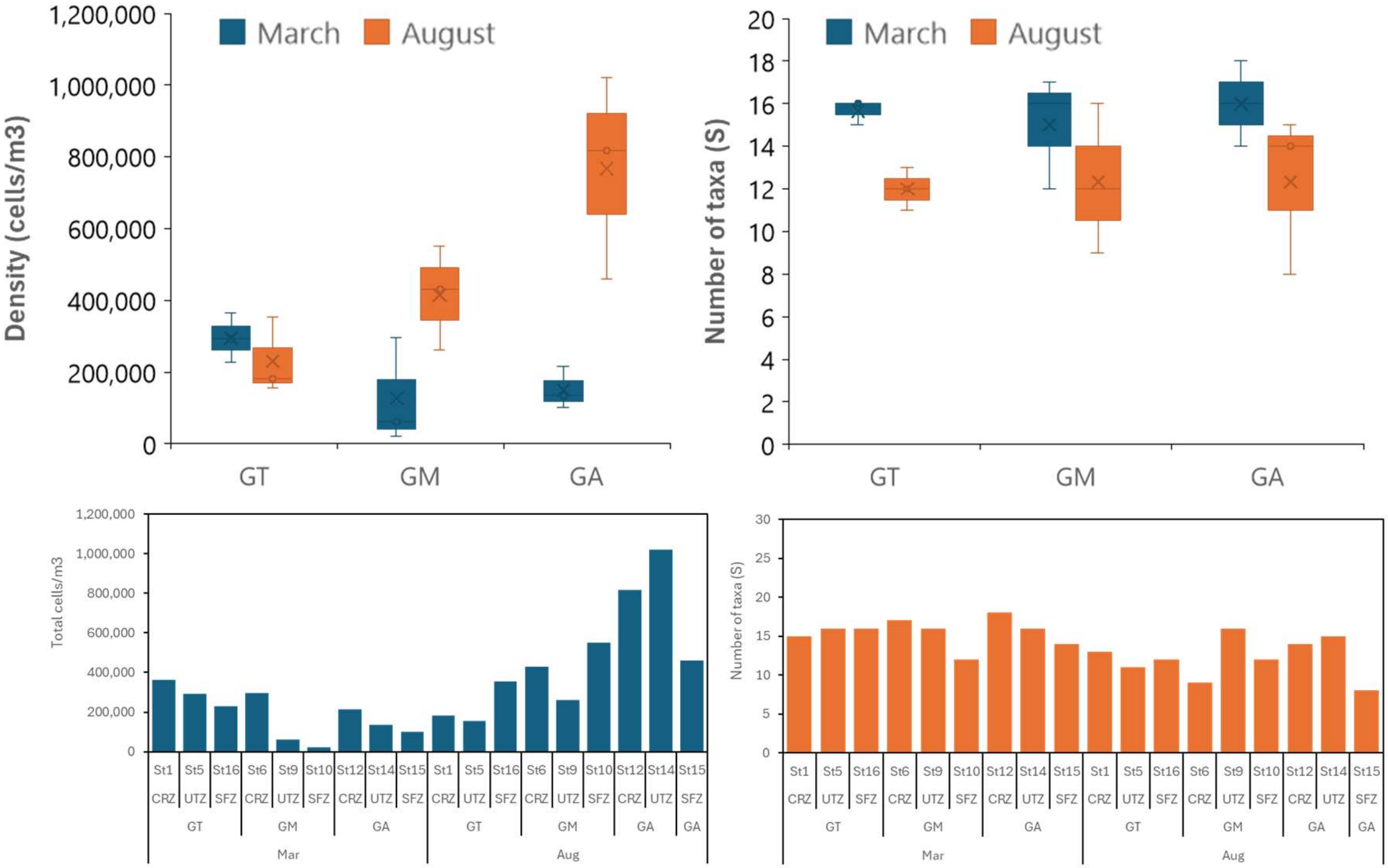




Note:

CRZ = Core Zone; UTZ = Utilization Zone; SFZ = Sustainable Fisheries Zone; GT = Gili Trawangan; GM = Gili Meno; GA = Gili AIr

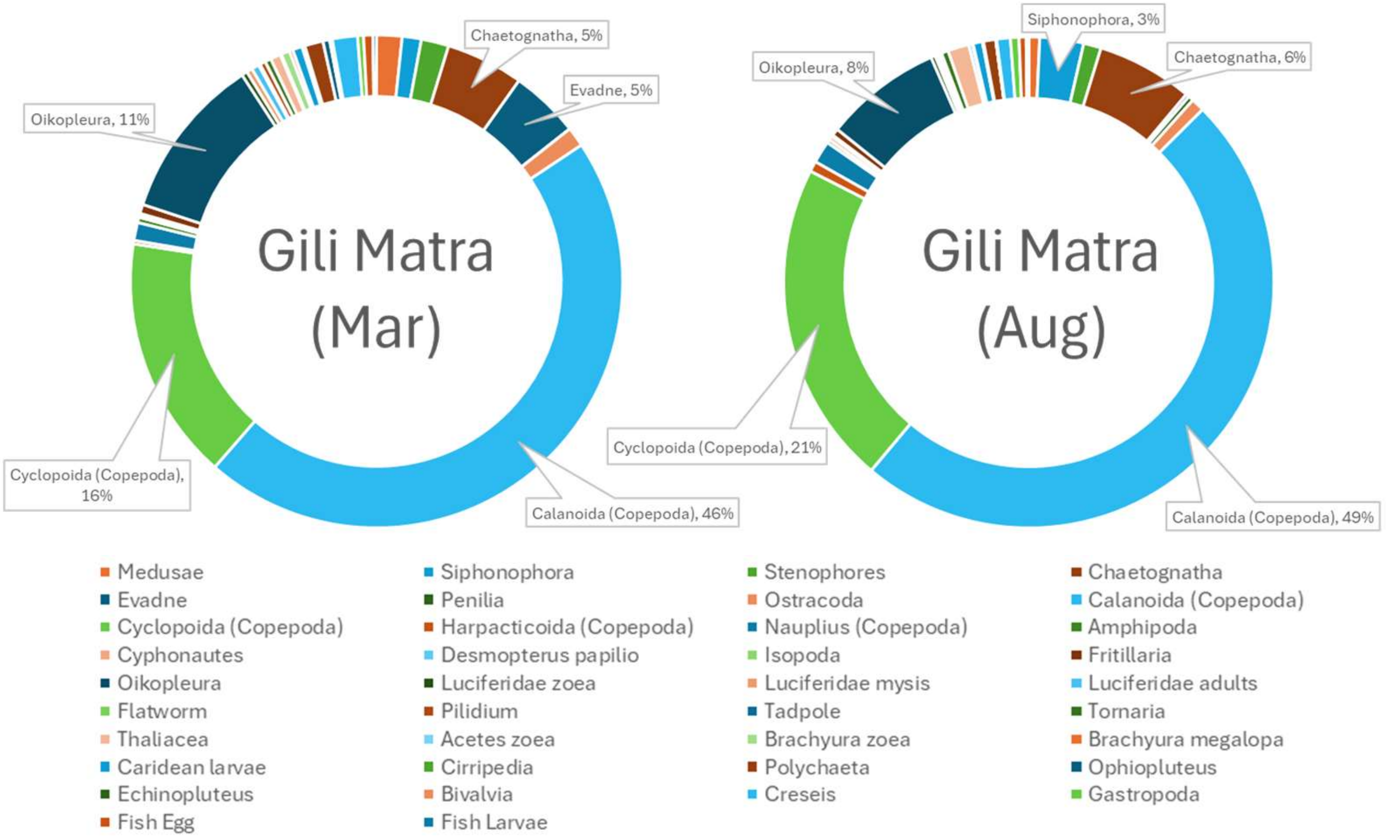
- The phytoplankton assemblages (at genus level) were significantly different in March than in August
- Co-domination of *Chaetoceros* and *Leptocylindrus* in March was completely replaced by domination of *Leptocylindrus* in August

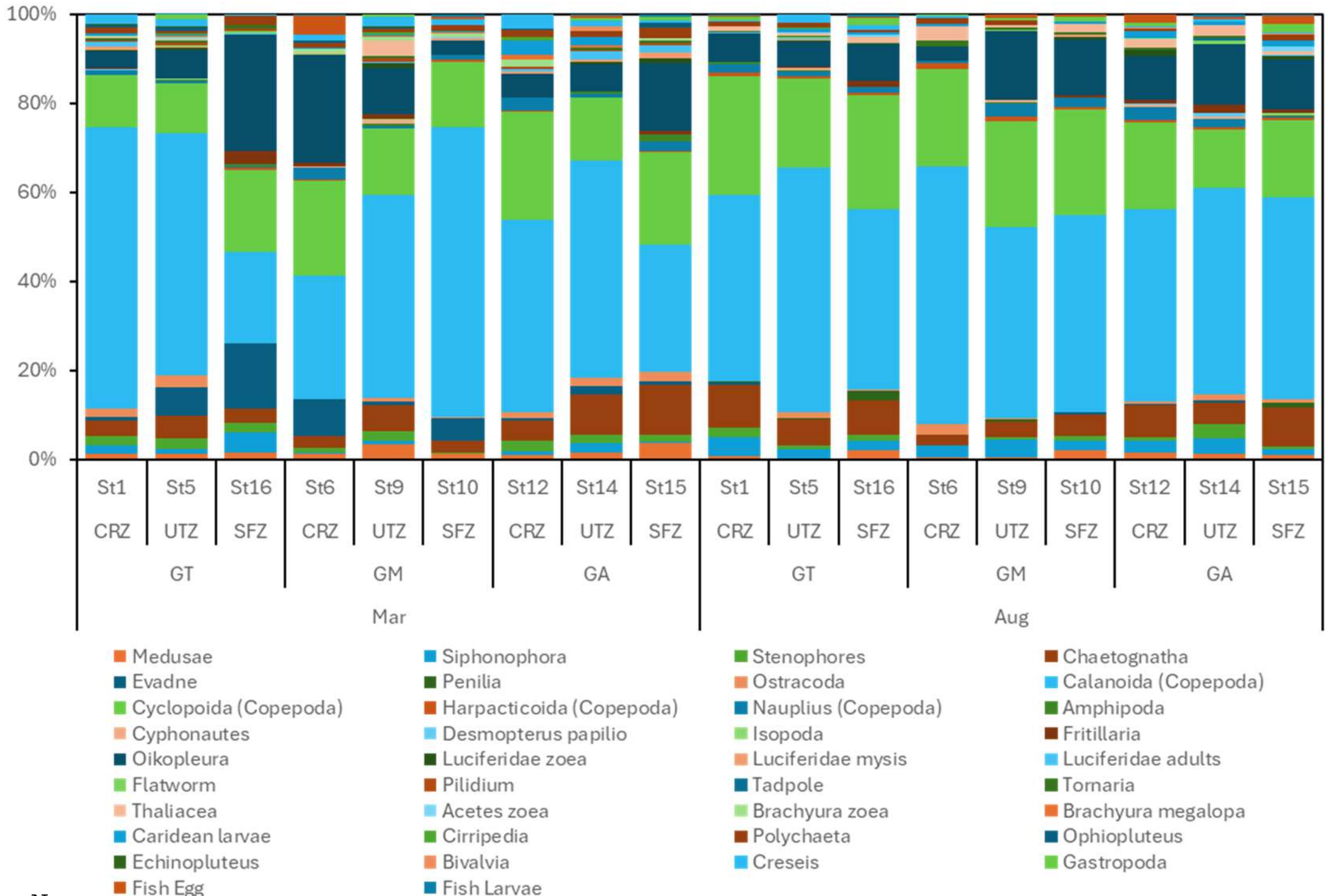


- The average density of phytoplankton was higher in August compared to in March
- Average number of taxa (genus) generally decrease due to dominance of *Leptocylindrus* in August

General zooplankton assemblages

- Mainly dominated by Copepods → Calanoida and Cyclopoida
- Other commonly abundant taxa → Oikopleura, Chaetognatha, Evadne (only in March) and Siphonophore (only in August)
- No significant differences in the zooplankton assemblages between March and August 2024

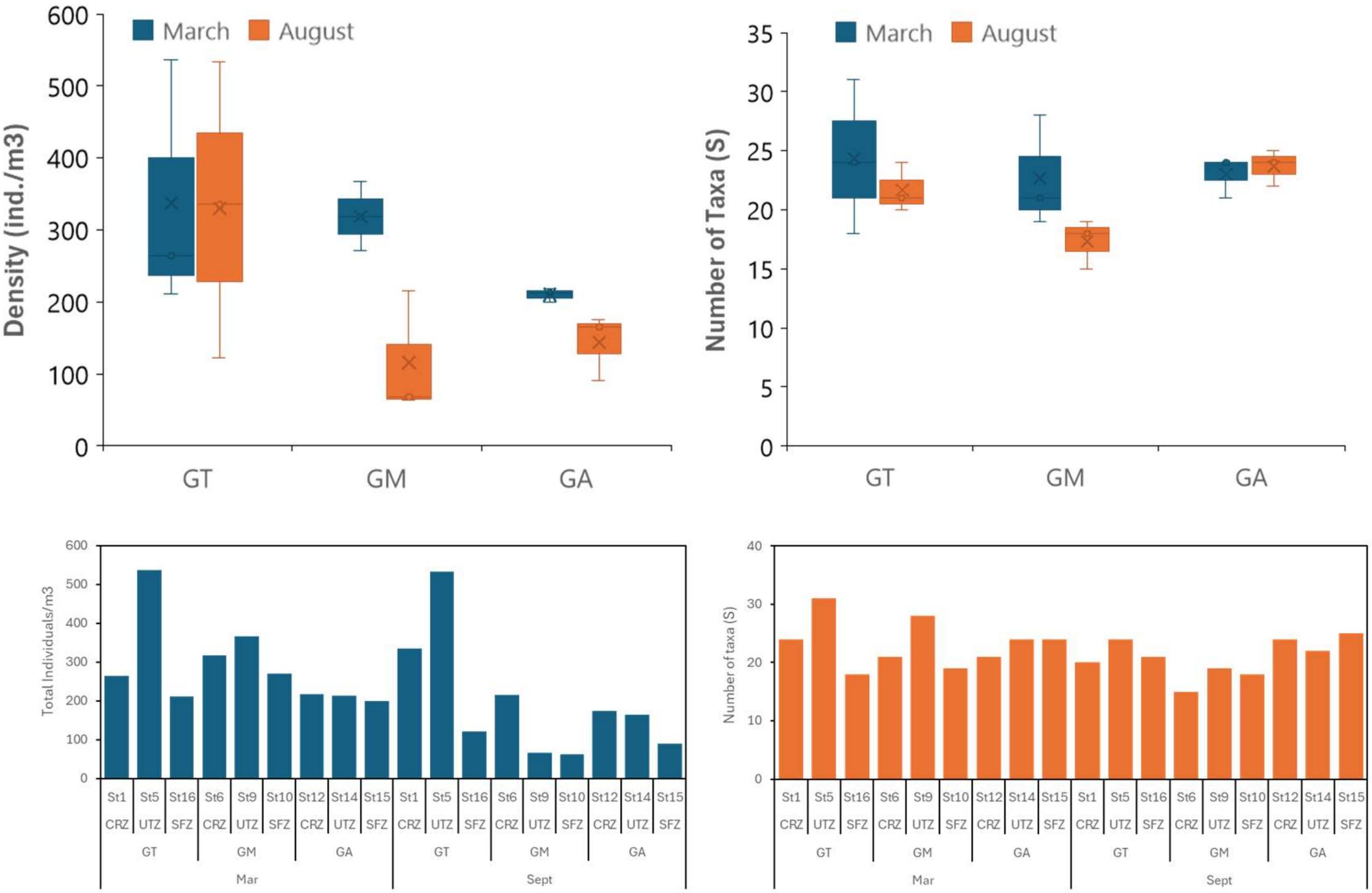




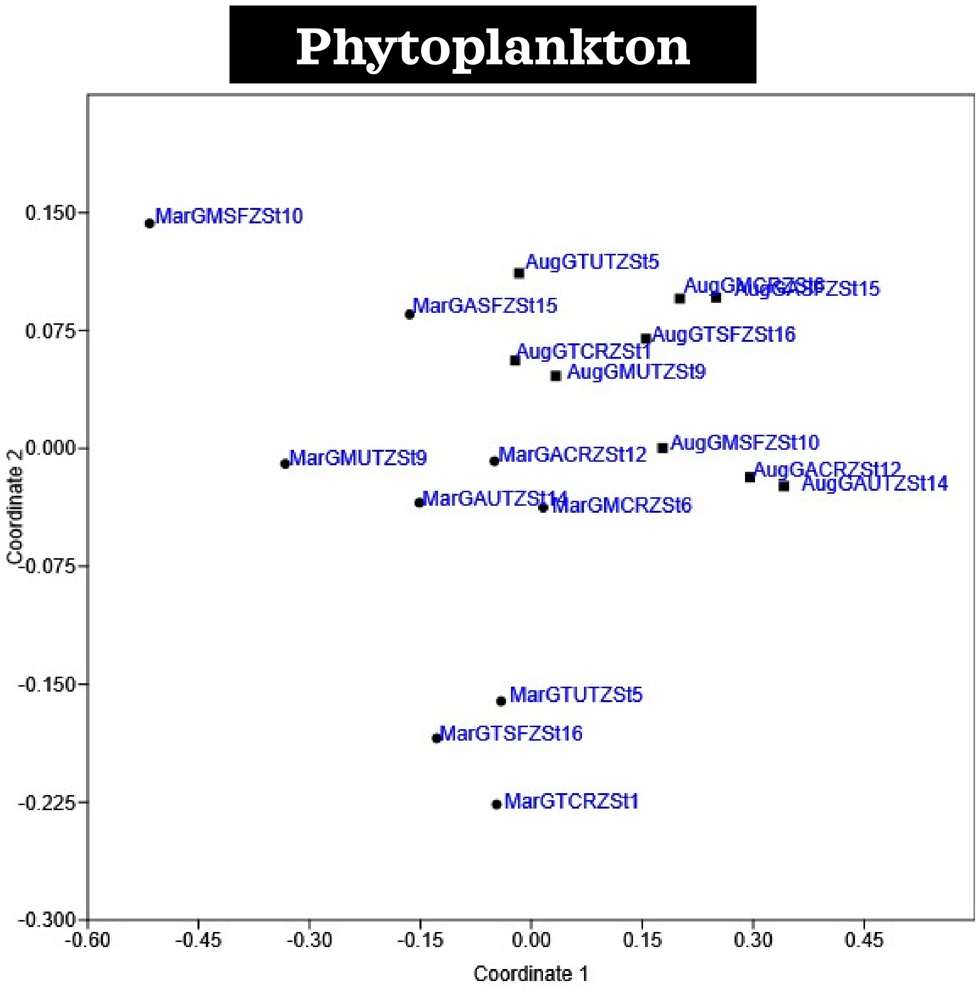
Note:

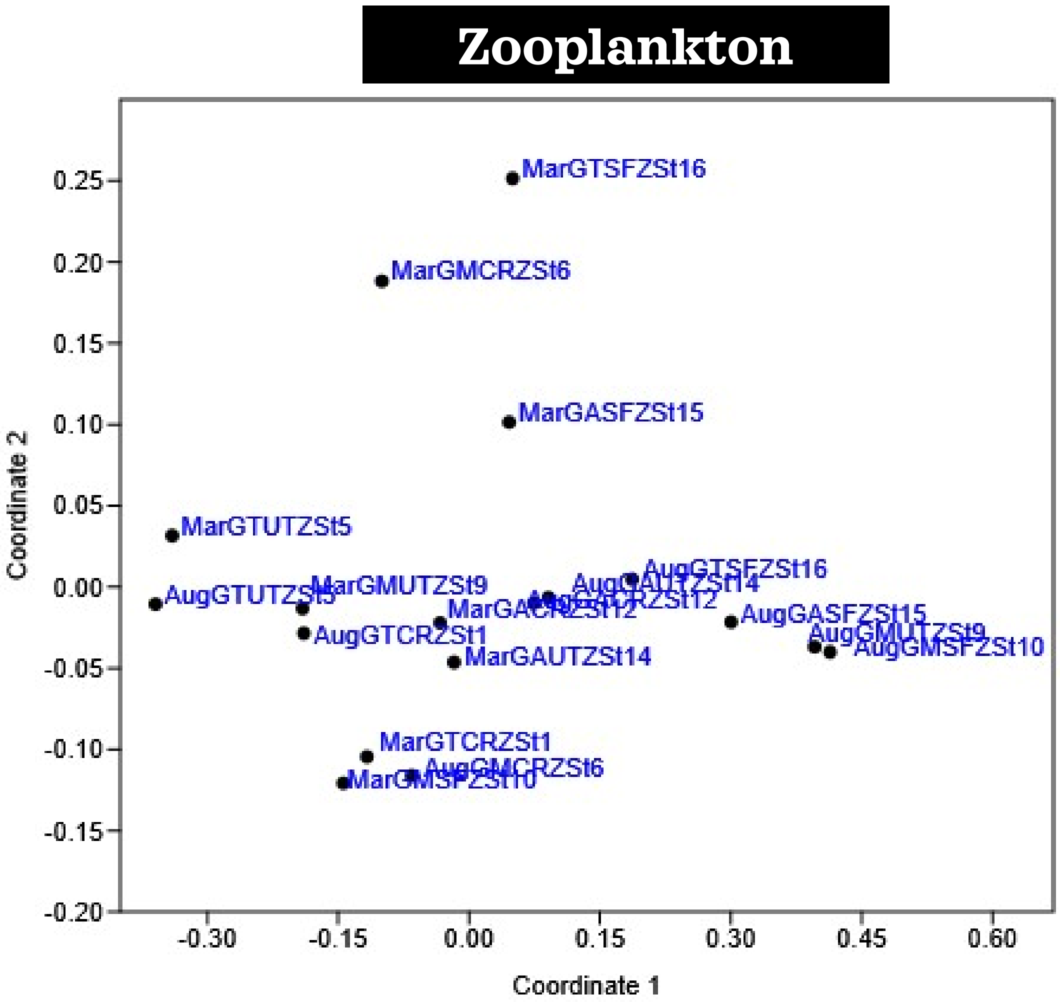
CRZ = Core Zone; UTZ = Utilization Zone; SFZ = Sustainable Fisheries Zone; GT = Gili Trawangan; GM = Gili Meno; GA = Gili AIr

- No abnormal/anomalies found between the zonation, sites, or months → GT SFZ and GM CRZ do have unique zooplankton assemblages
- Noticed that Evadne only found during March and disappear in August → mostly found in SFZ in Gili Trawangan
- The density of Siphonophora increased in August
- Oikopleura were more abundant in March → particularly in SFZ Gili Trawangan and CRZ Gili Meno

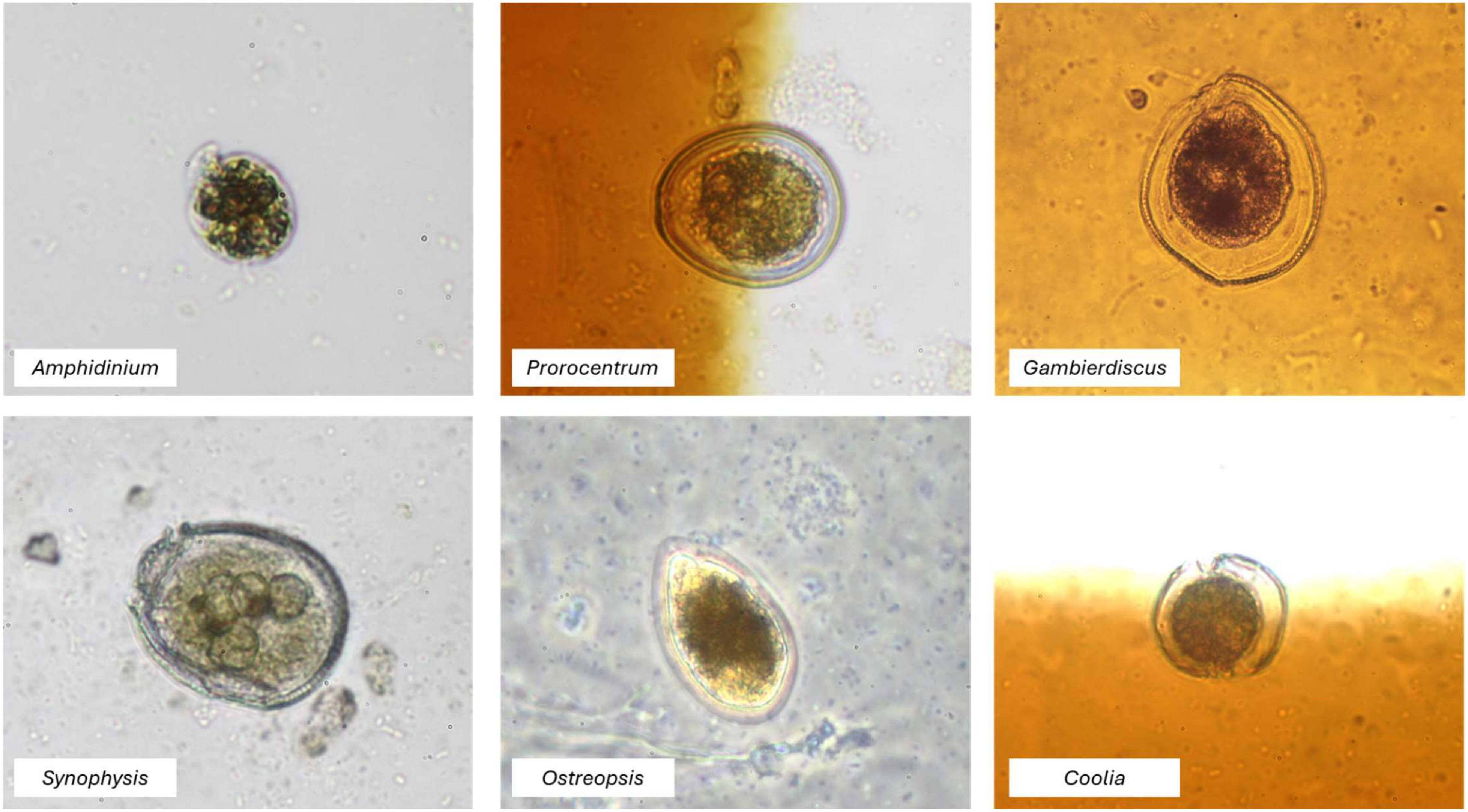


- The average density of zooplankton changes drastically especially in Gili Meno and Gili Air → Gili Trawangan showed less changed in zooplankton density
- The Core Zone in Gili Trawangan (GT CRZ) remains the hotspot for zooplankton density in both March and August sampling





Note: CRZ = Core Zone; UTZ = Utilization Zone; SFZ = Sustainable Fisheries Zone; GT = Gili Trawangan; GM = Gili Meno; GA = Gili AIr



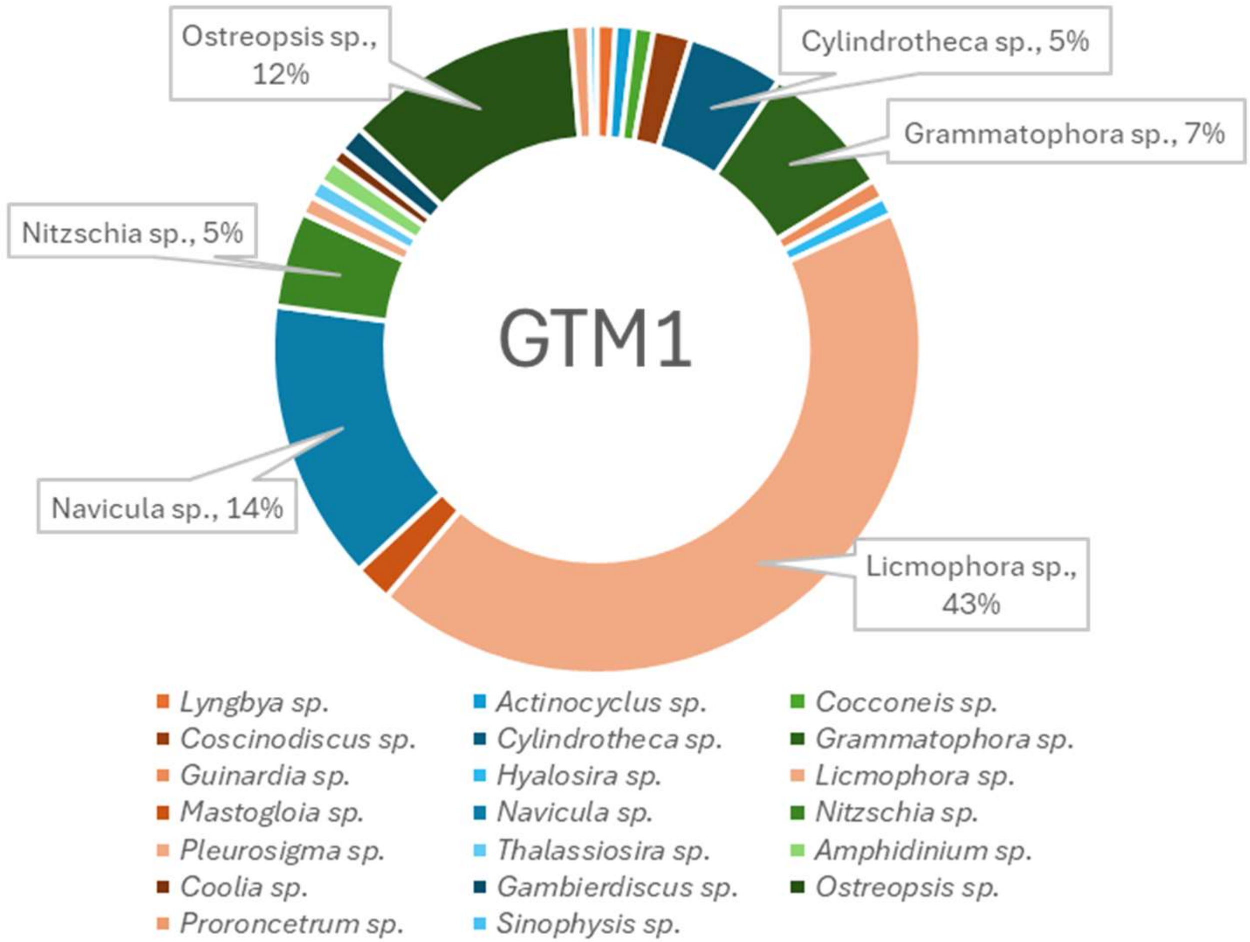
BHABs cells in preserved samples

Benthic microalgae assemblages

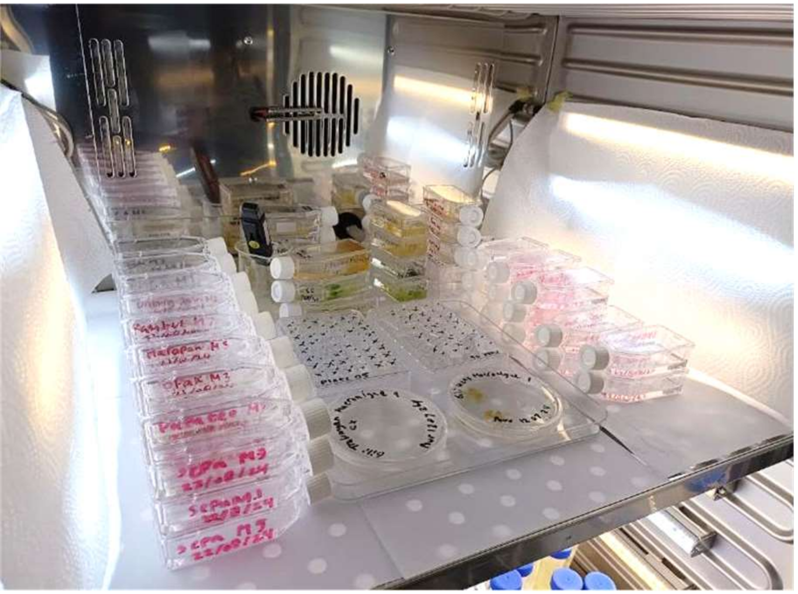
Analysis is still ongoing

Mostly dominated by benthic diatoms → *Licmophora* sp.

In Gili Trawangan Macroalgae 1 sample (GTM1; Gracilaria sp.) in March 2024 → *Ostreopsis* sp. density was high and contributes to 12% of total microalgae cell density in the sample

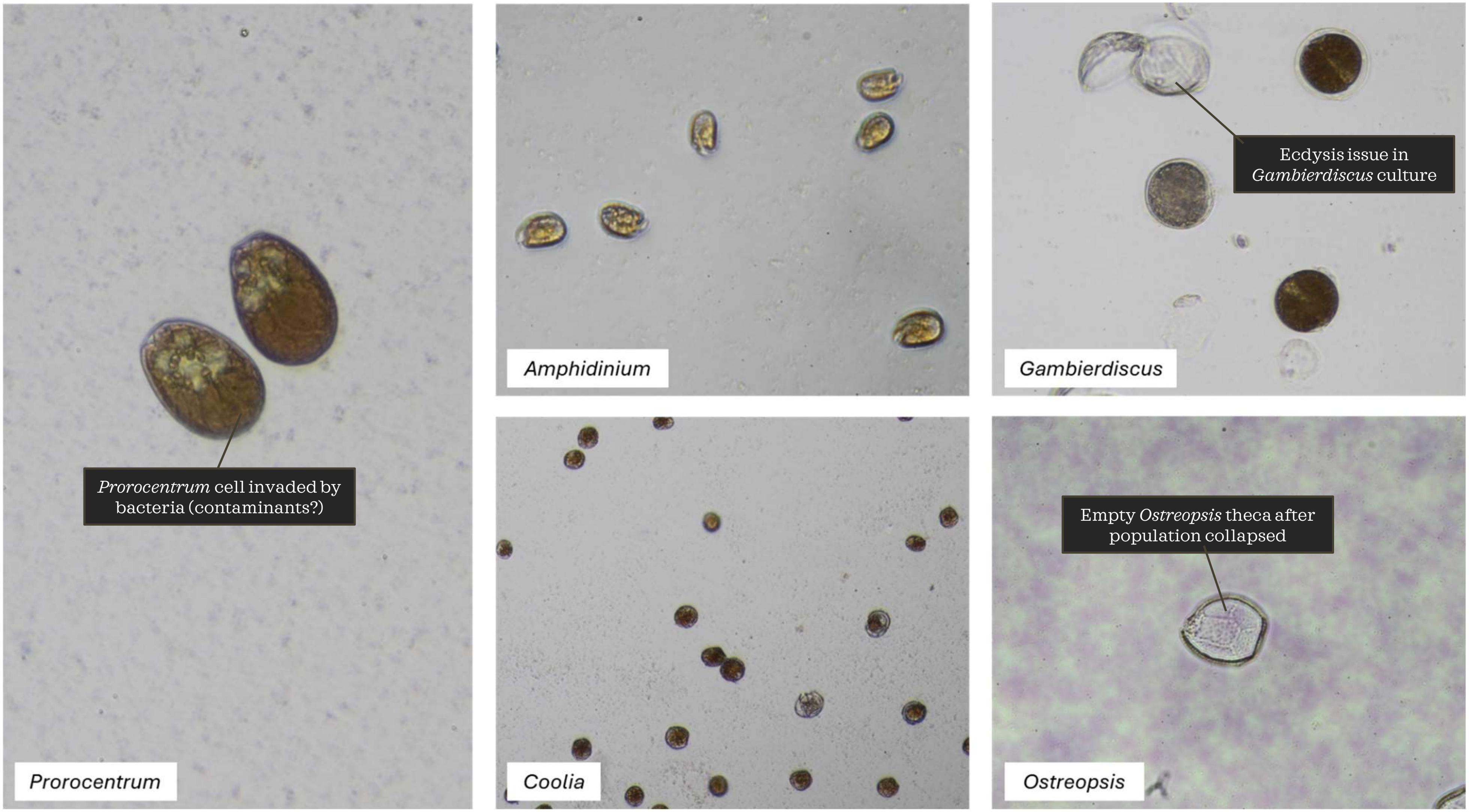


BHABs culture experiments



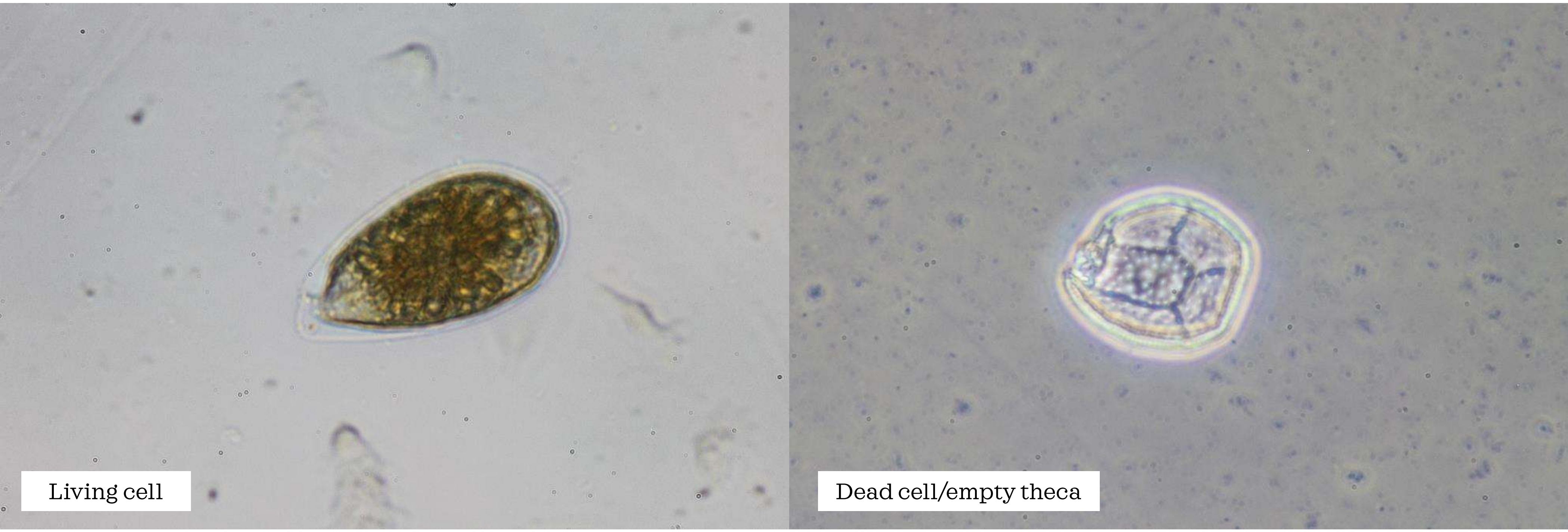
BHABs clonal culture

- From March and August samples → 96 isolates from 5 targeted genus were created → unfortunately **none survived**
  - *Gambierdiscus* → 6 isolates → at least 2 clonal culture grows up to 20 cells/well → cells undergo repeated ecdysis → died after 4-5 ecdysis
  - *Ostreopsis* → 10 isolates → one of the clonal culture grow up to >50 cells → population collapse → re-culture did not work
  - *Coolia* → 30 isolates → at least 2 clonal culture grow up to >100 cells → population collapse → re-culture did not work
  - *Amphidinium* → 25 isolates → 2 clonal cultures grow very dense and established as clonal culture → problem of rapid population collapse and abnormal cell shape → occurred after inoculation to $25cm^2$ culture flask with ENSW + F/2 medium
  - *Prorocentrum* → 25 isolates → 2 clonal cultures grow up to > 50 cells → growth stagnation and invasion by contaminant bacteria occurred and collapsing the population

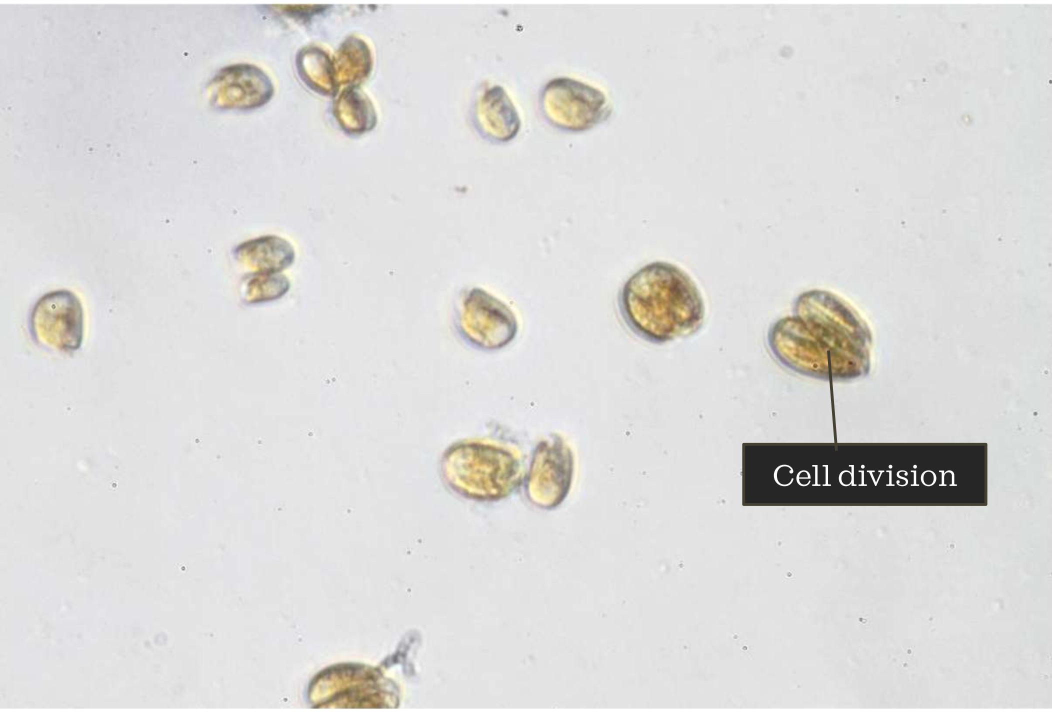


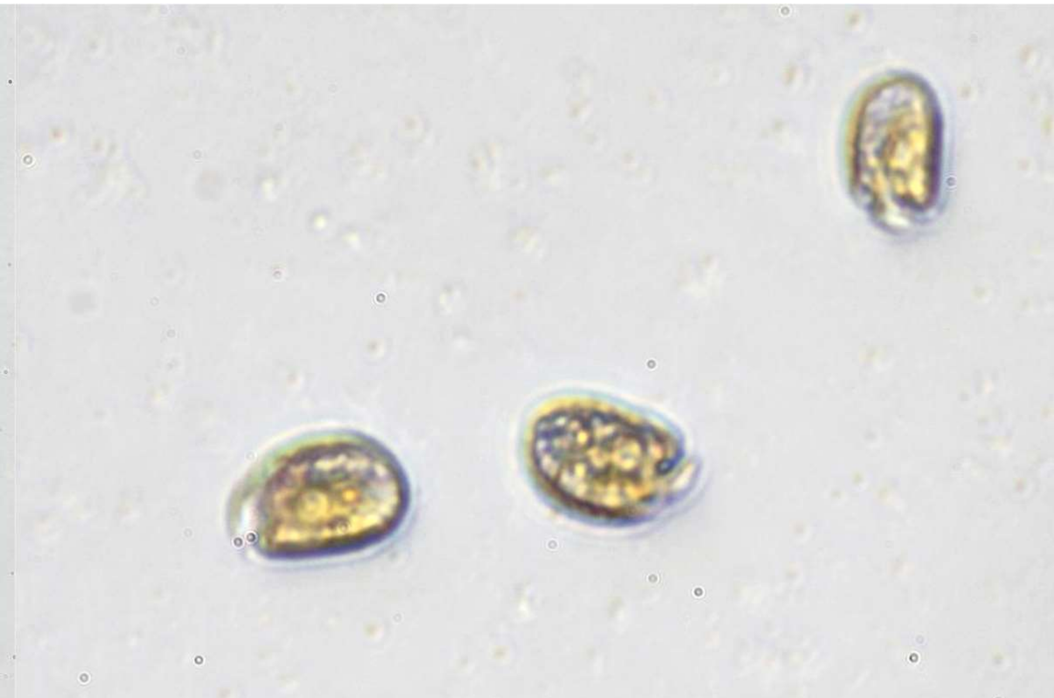
BHABs cells in cultures

Gambierdiscus

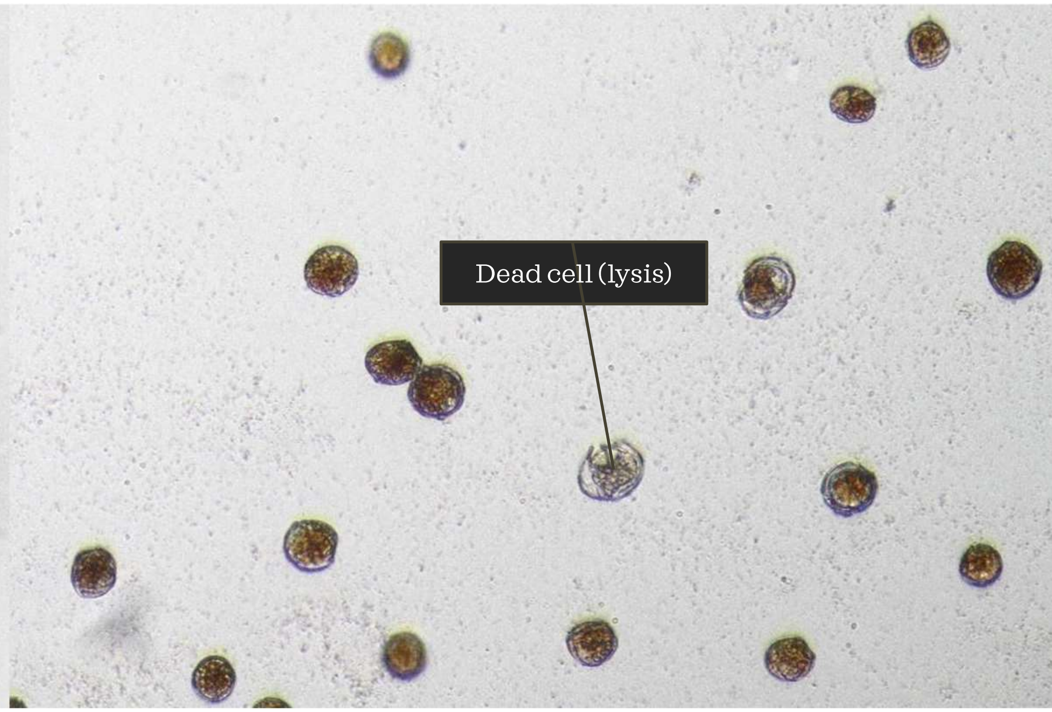


Ostreopsis

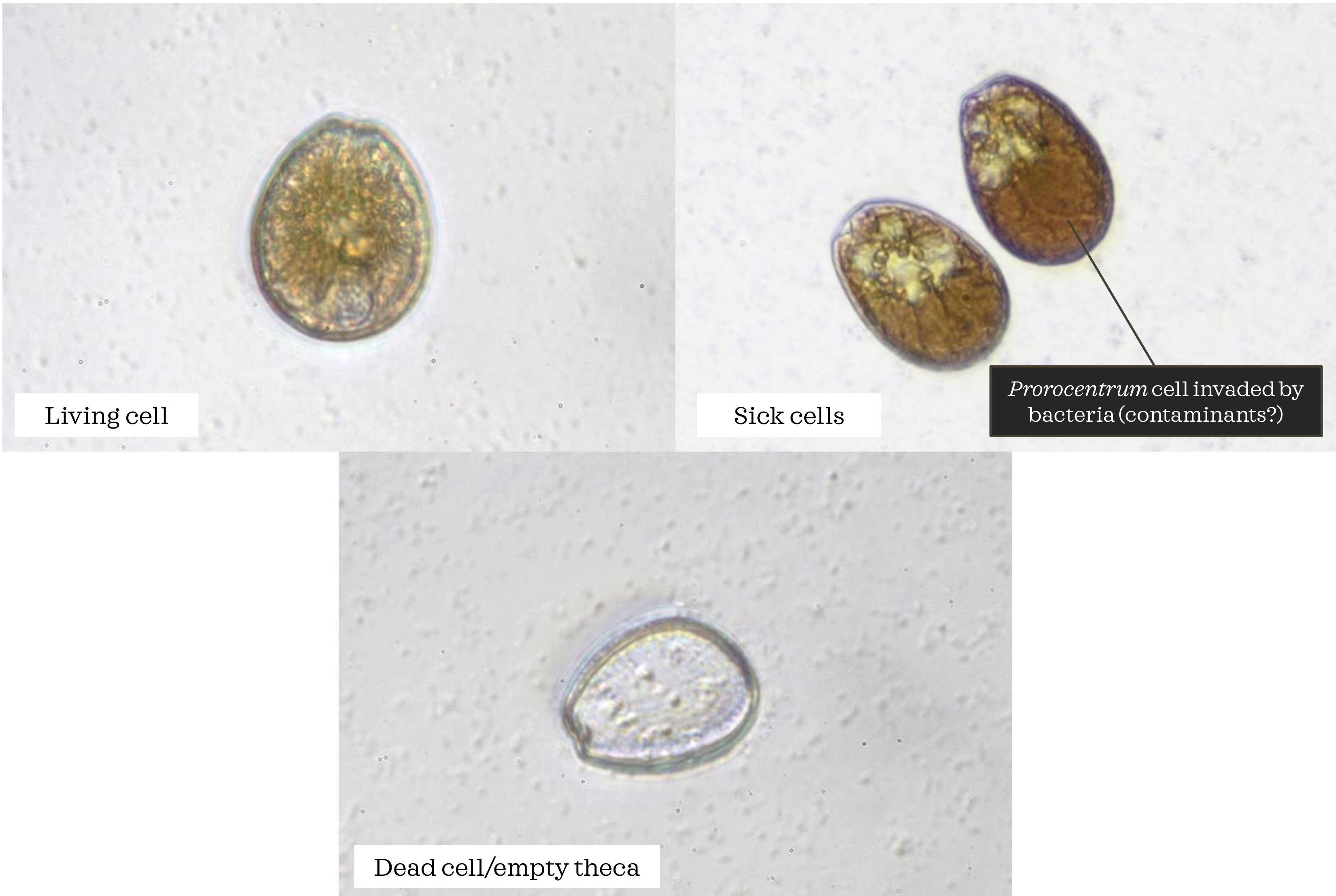




Amphidinium

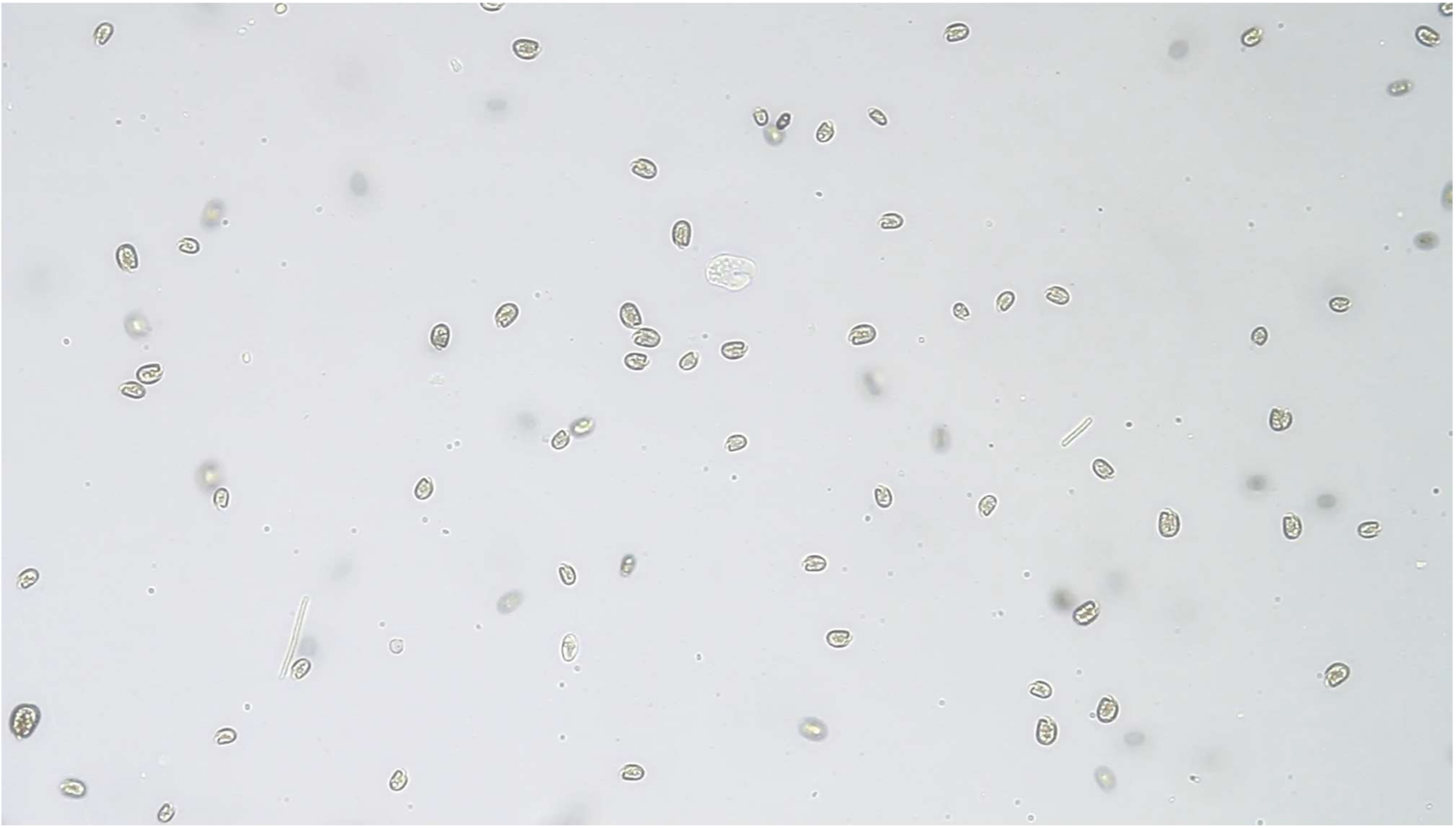


Coolia

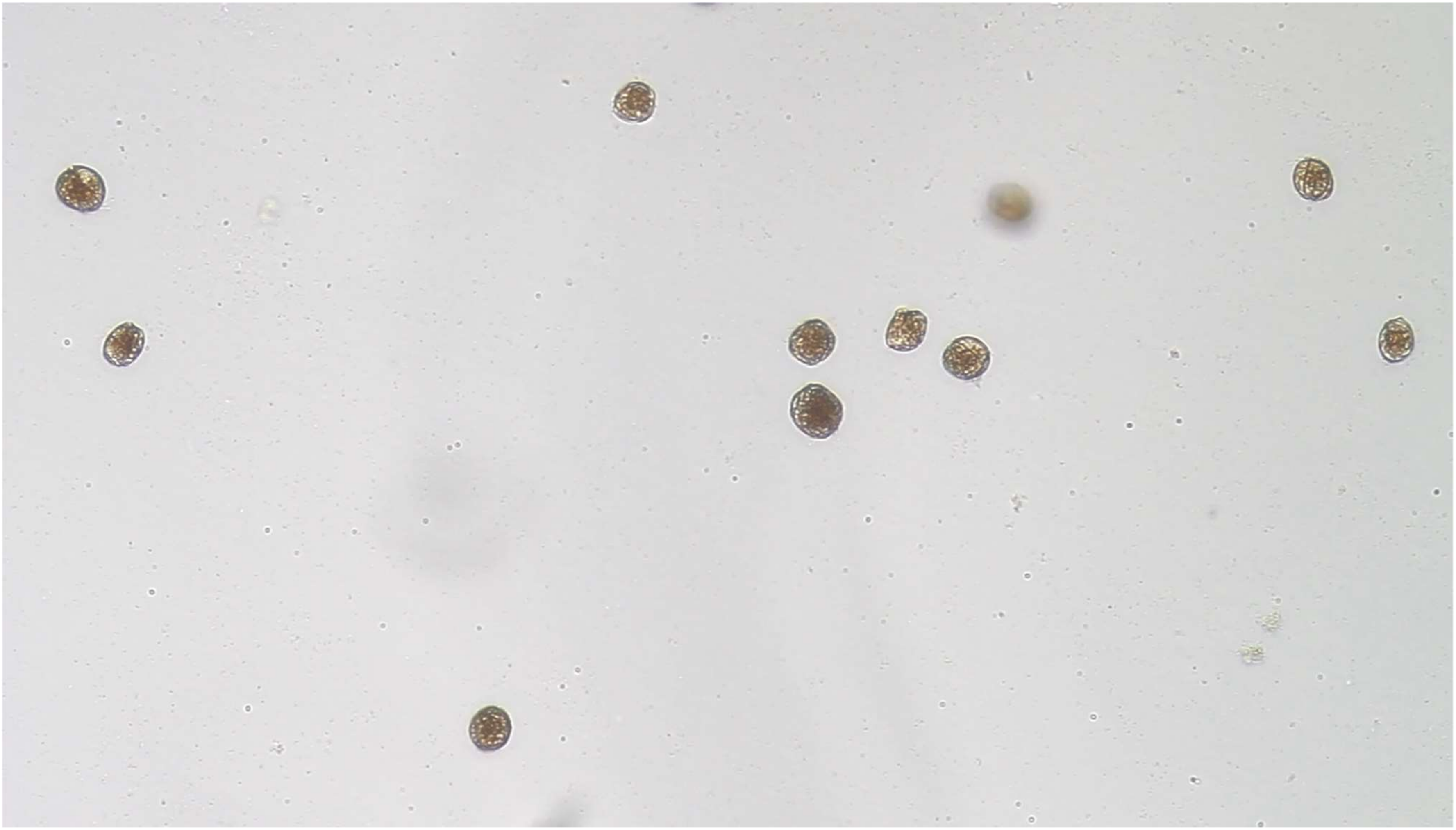


Prorocentrum

Example of dense *Amphidinium* culture



Example of *Coolia* culture



Example of *Prorocentrum* in raw/mixed culture

Problems encountered during BHABs culture experiment

- Each targeted BHABs genus have Specific growth conditions → attempt to establish clonal culture of 5 different groups of BHABs at the same time were too difficult and currently impossible due to:
  - **Limited equipment** → only 1 culture chamber in RCO-BRIN → so all isolates must be grown at the same temperature, irradiance level, and irradiance time; Laminar Air Flow cabinet often full or in use by other researchers, mainly microbiologist, which is not ideal due to high chance of cross contamination
  - **Limited personnel** → only 1 people actively doing culture experiment
  - **Limited budget for consumables** → BRIN administration system is too complicated for purchasing consumables and takes too long to buy something

Problems encountered during BHABs culture experiment (2)

- Total population collapse in all isolates might be related to:
  - **Bacterial contamination** → usually is not a problem since the culture that were tried to be established are not axenic culture and even with ‘washing’ technique, the isolated cells will always bring bacteria on the surface of cells or endogenic/endosymbiont/endoparasite
  - **Other species contamination** → in some isolates, other species, most commonly Amphidinium, often contaminate the well → might attribute to growth stagnation, ecdysis (in Gambierdiscus), or cell death due to competition/allelopathy
  - **Chemical contamination** → due to difficulties in purchasing administration → the current experiments used the culture flasks, multi-well plate, and F/2 medium that are well beyond their expiration date → the tissue-culture treated flasks and culture plates might now become toxic to some, if not, most BHABs species

Next plan for BHABs culture experiment

- Several raw/mixed culture still survive and contains cells of some targeted genus → mainly *Prorocentrum* and *Amphidinium*
- It is still possible to try to isolate and establish *Prorocentrum* and *Amphidinium* clonal culture
- Need new supply for consumables and F/2 medium
  - Currently new stock of K-medium was obtained from Arief's other research project
  - Still require culturing consumables, such as:
    - Tissue Culture multi-well plates (96, 48, 24 wells)
    - Culture flasks (25cm^2)
    - Tissue Culture sterile Petri dishes (90mm, 60mm, 35mm)
    - Pipette tips, preferably with barrier (0.1 – 10 uL, 1-100 uL, 100-1000 uL
    - Nitrile/latex non-powdered gloves

Additional Information

PhD Project

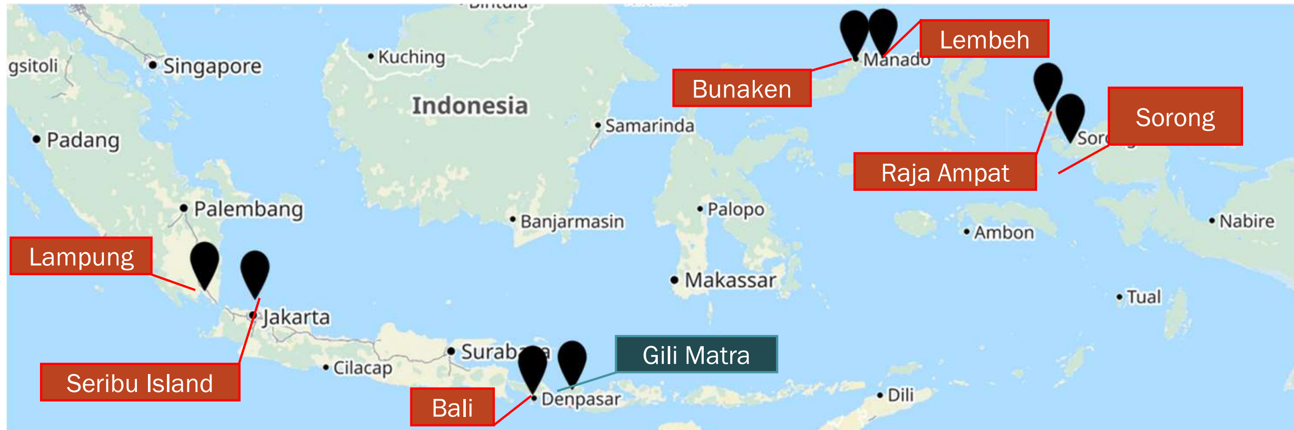
Study of the diversity, population dynamics and toxin production of Benthic Harmful Algae (BHAs) in the tropical coral reef ecosystems of Indonesia with a focus on Ciguateric species

Arief Rachman (Candidate)

Estelle Masseret (Supervisor)

Mohamed Laabir (Co-Supervisor)

Montpellier University, France



Aims

To understand the ecological characteristics of ciguateric BHA species colonizing the macrophytes within coral reef ecosystems in Indonesia and their physiological response towards variation in the water environmental parameters

Questions

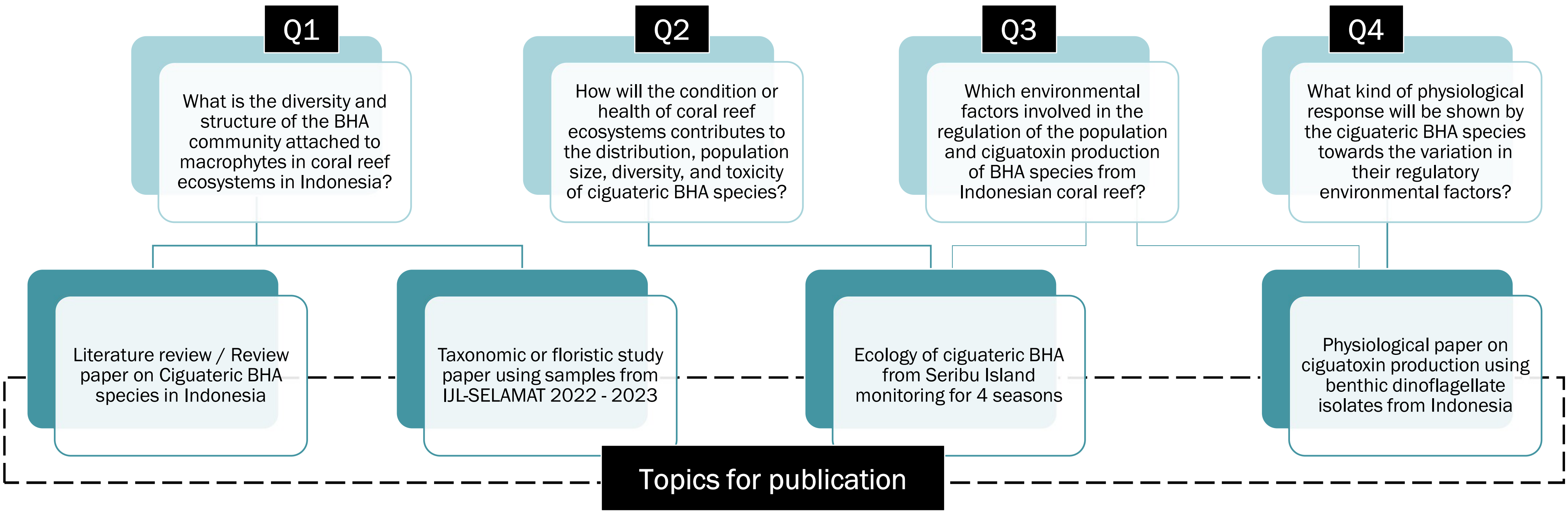
- What is the diversity and structure of the BHA community attached to macrophytes in coral reef ecosystems in Indonesia?
- How will the condition or health of coral reef ecosystems contributes to the distribution, population size, diversity, and toxicity of ciguateric BHA species?
- Which environmental factors involved in the regulation of the population and ciguatoxin production of BHA species from Indonesian coral reef?
- What kind of physiological response will be shown by the ciguateric BHA species towards the variation in their regulatory environmental factors?

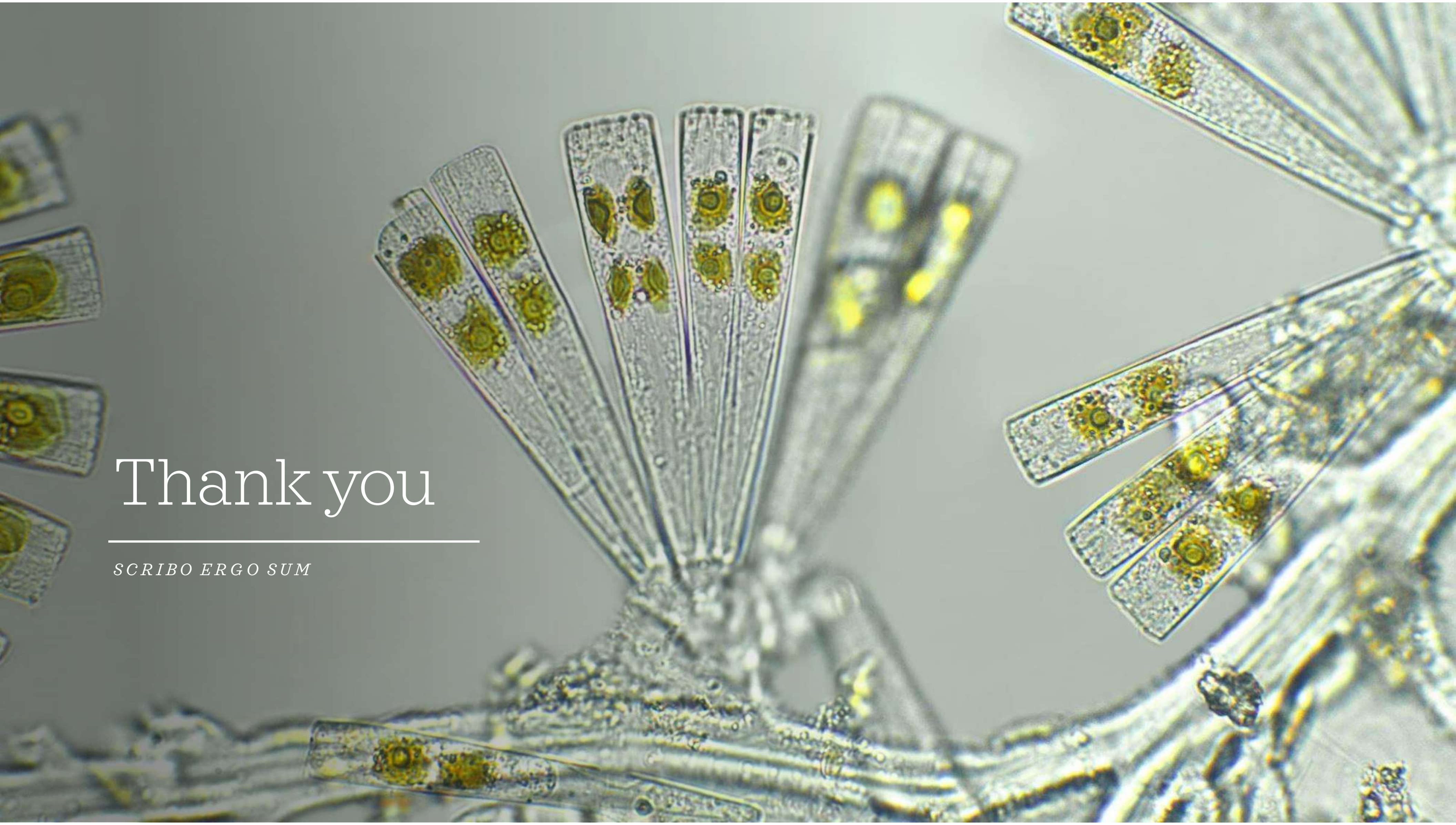
The result would fill the gap in the ecological and physiological characteristics of ciguateric BHA in Indonesia

Contributes towards the seafood security and safety management in Indonesia

Contributes towards the mitigation plan to control the BHA blooms and CFP cases in Indonesia

Structure of PhD thesis & potential publication topics




Thank you
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