

Using Autonomous Reef Monitoring Structures (ARMS) for metagenomics-based studies of aquatic hard-bottom benthic communities

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BACKGROUND

Biota inhabiting **cryptic spaces** in the **hard-bottom benthic matrix** comprise a **major part of marine biodiversity**. In coral reefs, for example, up to 75% of diversity is represented by small-sized organisms inhabiting the cryptic spaces in the reef framework; they are often referred to as the **'reef cryptobiome'** (Carvalho *et al.*, 2019). The analysis of **environmental & bulk-community DNA** has revolutionized the study of such biotic communities. However, **standardized and non-destructive sampling** of complex benthic communities remain **challenging**. Therefore, the **"cryptobiome"** and **microbiome** remain largely **understudied**. **Autonomous Reef Monitoring Structures (ARMS)** are used **globally** in combination with **molecular tools** to close this knowledge gap. These structures are **deployed for months/years** and upon **retrieval & disassembly**, **DNA metabarcoding and/or shotgun-metagenomics** are performed on the **biota colonizing the units**.

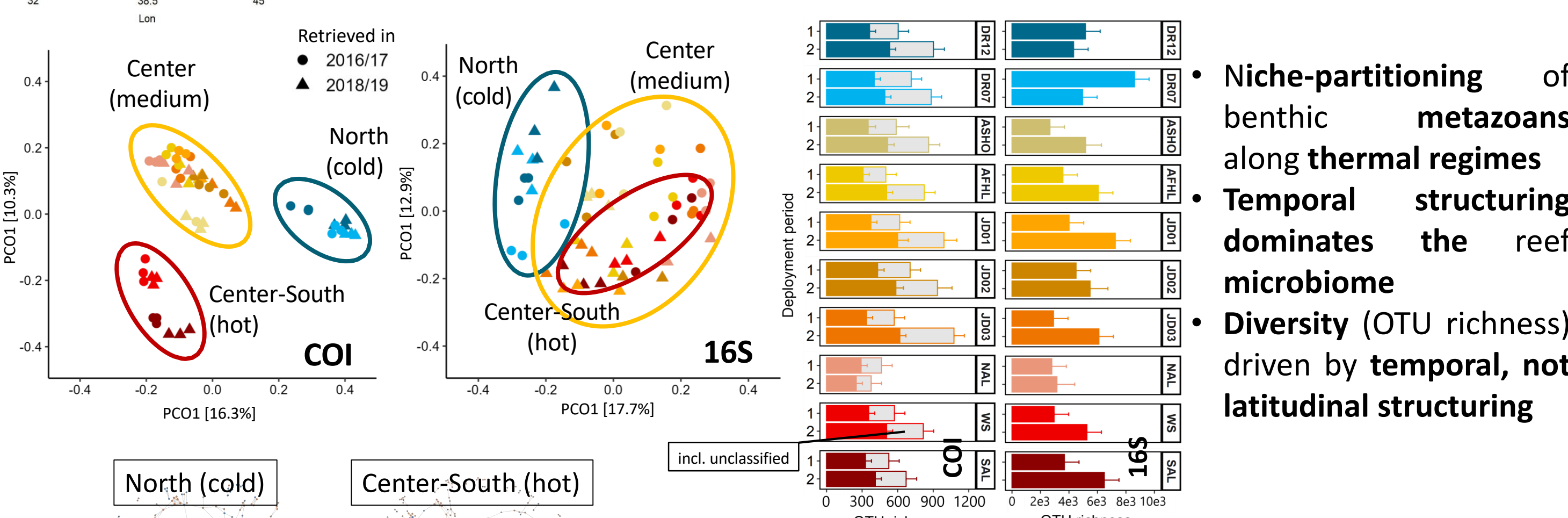


Pearman et al. 2018

ARMS plate with attached (sessile) biota

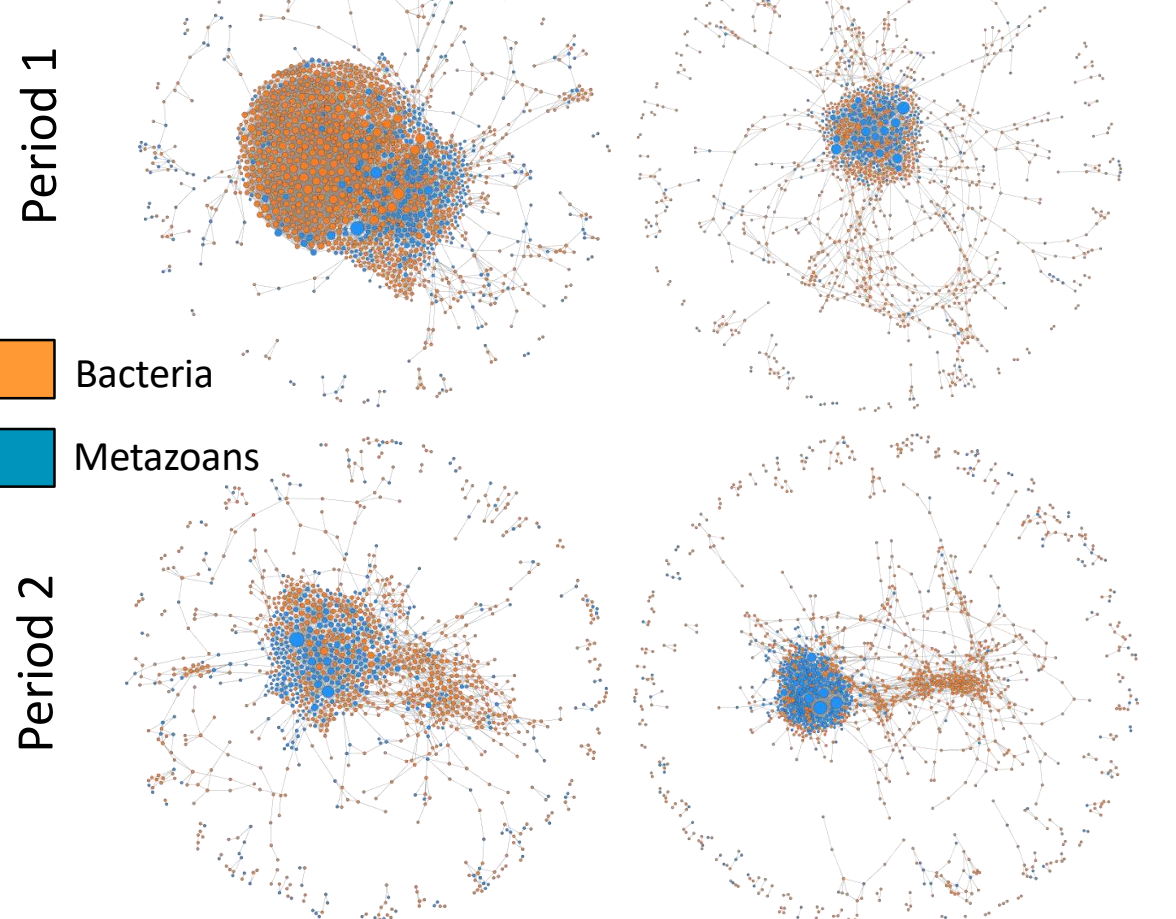
BACTERIAL-METAZOAN DIVERSITY AND CO-OCCURRENCE NETWORKS

Along a north-south **bleaching** (El Niño 2015/16) & natural **SST gradient**, **59 ARMS** were deployed as **triplicates on 10 reefs in the Red Sea** for two consecutive periods (2014/15-16/17 & 2016/17-18/19). Based on SST data and coral bleaching observations, sites were grouped into **North (cold)**, **Center (medium-hot)** & **Center-South (hot, heavily bleaching affected)**. **DNA metabarcoding** (16S rRNA – **prokaryotes**, COI – **metazoans**) was performed on the **sessile fraction** (biota attached to plates) of ARMS. **Diversity, community structure & inter-domain co-occurrence networks** were analyzed to assess how the cryptic reef benthos responds to **environmental gradients and disturbances**.

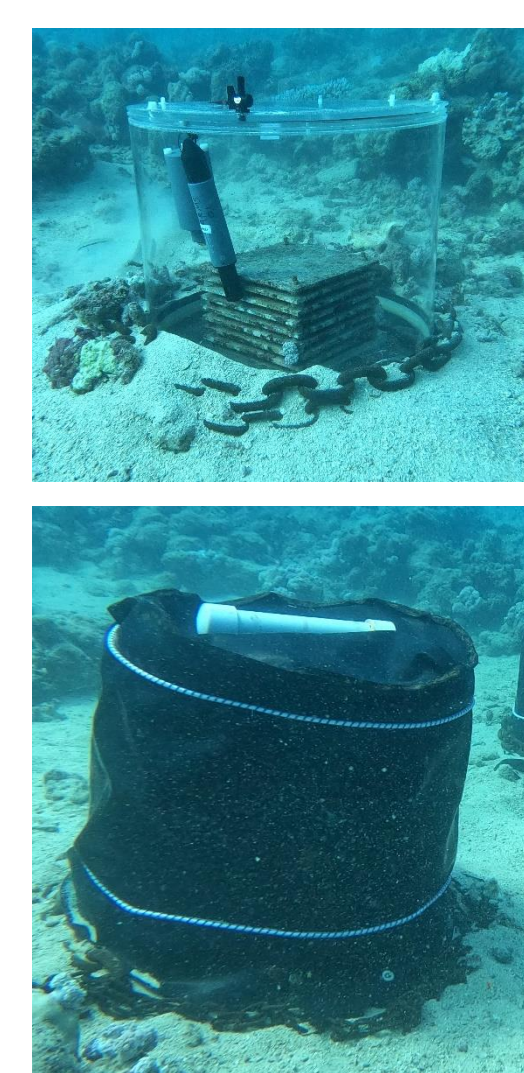


- Niche-partitioning of benthic metazoans along thermal regimes
- Temporal structuring dominates the reef microbiome
- Diversity (OTU richness) driven by temporal, not latitudinal structuring

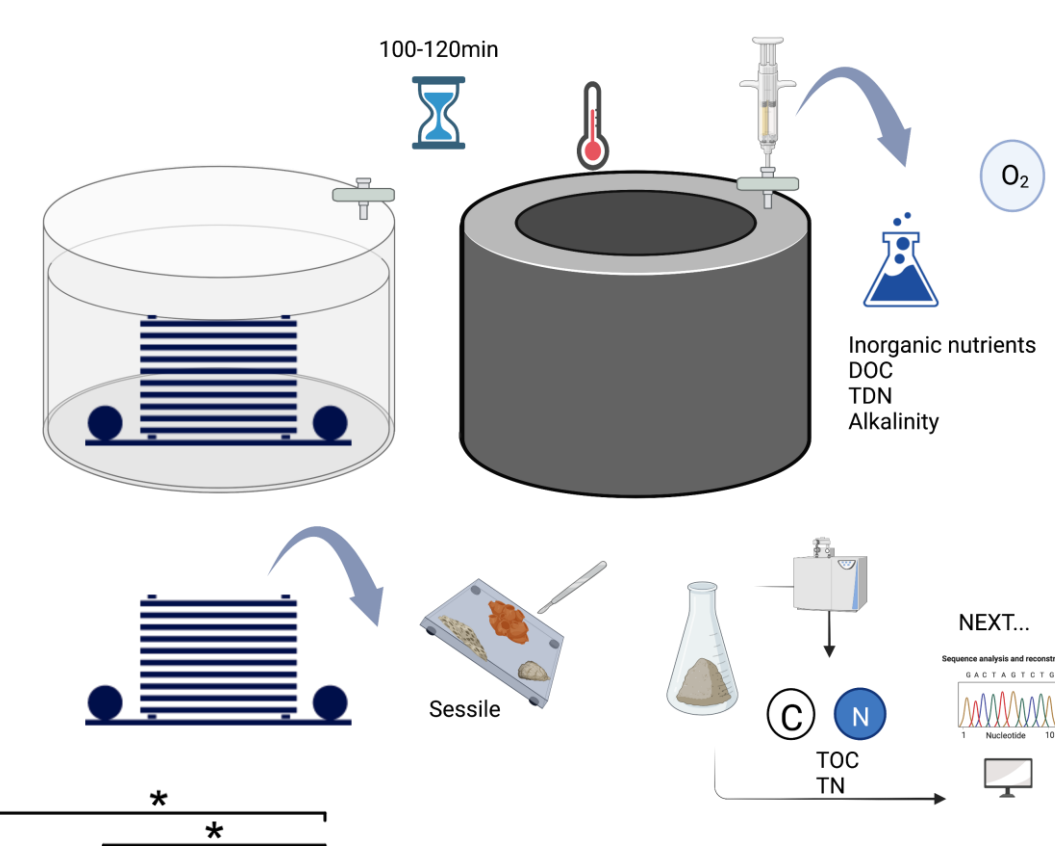
- More complex & homogenous inter-domain co-occurrence networks in the north
- Bleaching affected reefs in Center-South more heterogeneous & dominated by negative interactions -> connectivity maintained by fewer taxa & competition / niche-differentiation more intense
- Co-occurrence networks reveal community complexity aspects that go beyond regular diversity-based assessments



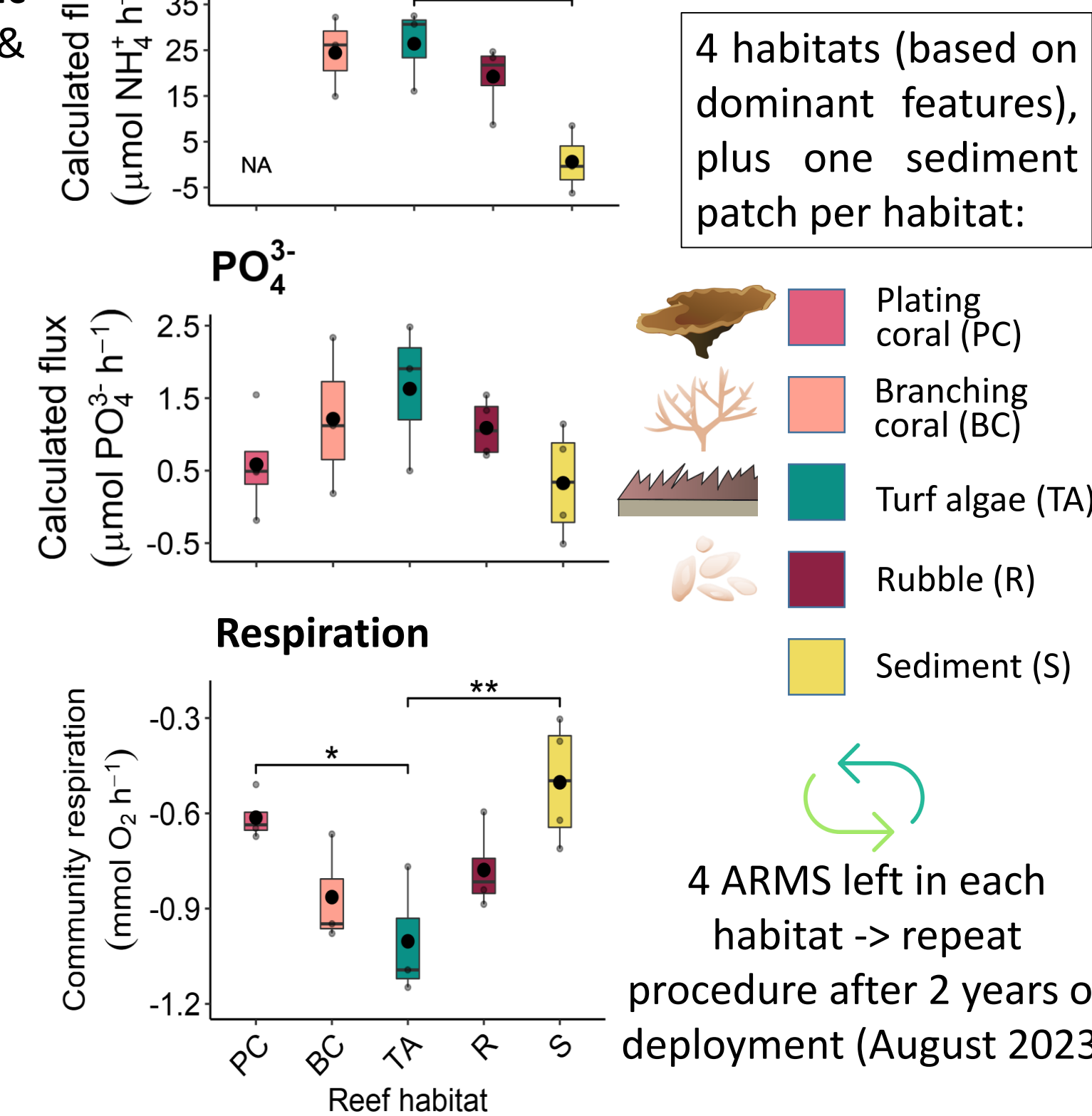
BIOGEOCHEMICAL FUNCTIONING OF THE REEF CRYPTO- & MICROBIOME



The reef crypto- & microbiome play an important role in **carbon (C) & nitrogen (N) (re-)cycling**, sustaining the high biodiversity of coral reefs in **oligotrophic environments**. Our aim is to assess how **pioneer benthic communities** associated with **contrasting benthic habitats** contribute differently to reef **biogeochemical functioning**. We performed **in situ incubations** (Roth *et al.*, 2019) of ARMS to measure **biogeochemical fluxes** and are using **16S rRNA amplicon and shotgun metagenomic sequencing** for taxonomic & functional analysis (in progress).



- Four replicate ARMS deployed at four well-defined habitats in Tahala reef (central Red Sea, 22°N)
- Habitats had the same depth profile & similar exposure conditions (exposed)
- ARMS deployed for 7 months & dark-incubated ~2h in situ before retrieval; water samples taken at beginning & end of incubation from the chambers to measure biogeochemical fluxes
- incubations also performed for one sediment patch per habitat



NEXT...
Sequence analysis and reconstruction
...16S rRNA amplicon & shotgun metagenomic sequencing in progress!

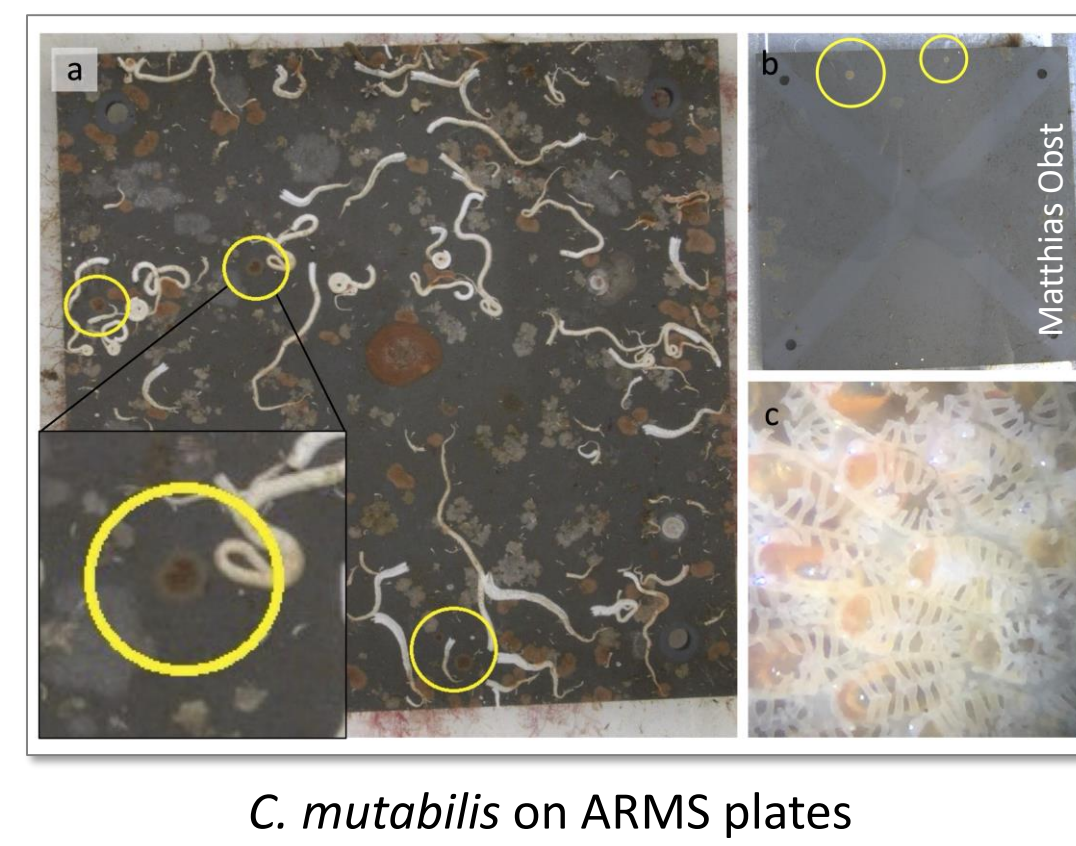
CORAL REEFS OF THE RED SEA

THE EUROPEAN ARMS PROGRAMME

FROM METABARCODING TO METAPHYLOGEOGRAPHY

The European ARMS programme (ARMS-MBON; Obst *et al.*, 2020) is a **network of ARMS** placed nearby **marine stations, ports, marinas, and Long-Term Ecological Research (LTER) sites** across **Europe and polar regions**. The aim of ARMS-MBON is to assess the status of, and changes in, **hard-bottom communities of near-coast environments**, using **DNA metabarcoding (COI & 18S rRNA)** in combination with **image analysis**. Such extensive monitoring initiatives result in a wealth of **genetic data on large temporal and spatial scales**. Here, we tested how widely used amplicon sequencing data of the protein-coding **COI marker gene** - which is **highly variable on the intraspecific level** - can be used for **population genetic & phylogeographic studies**.

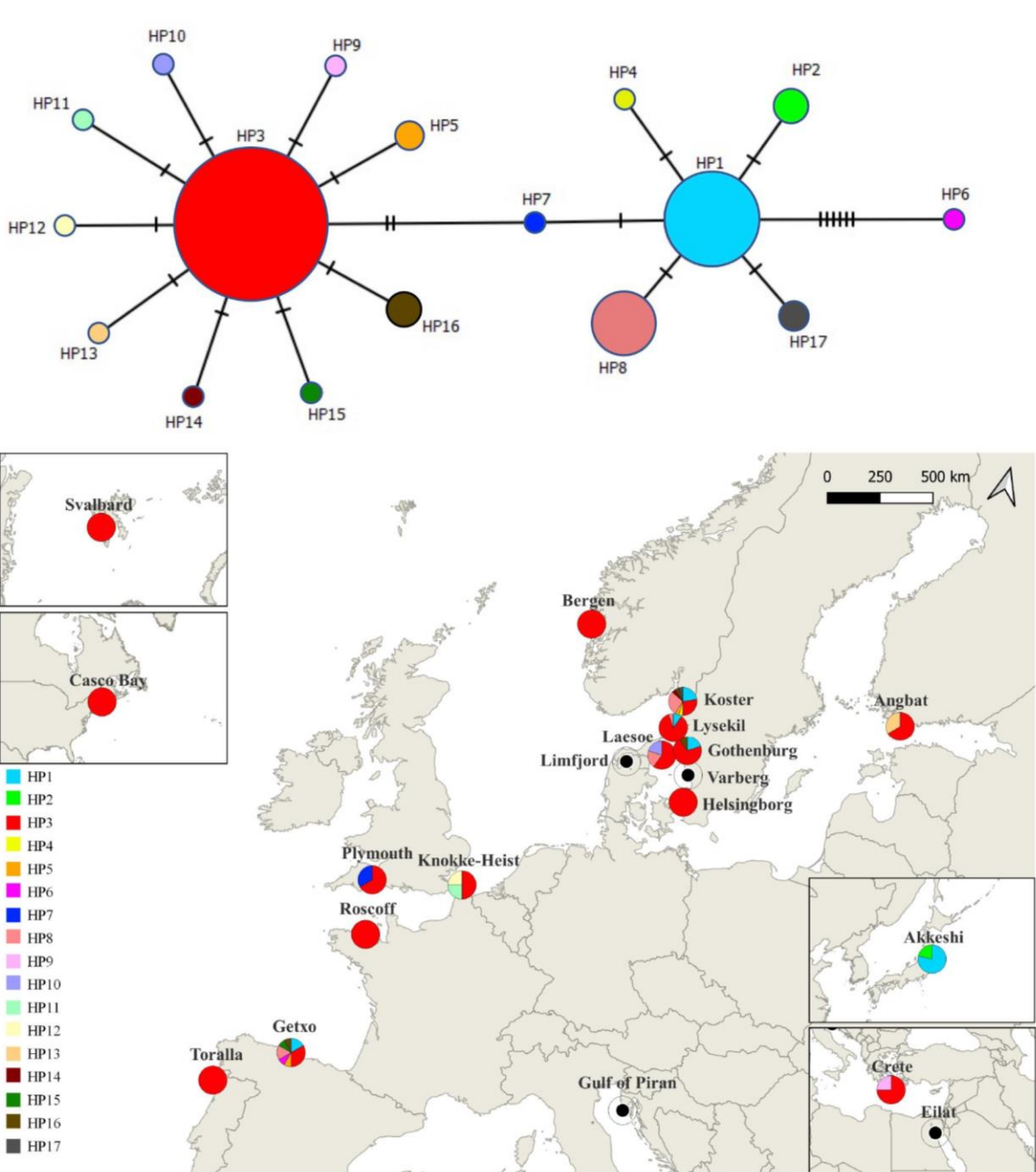
- COI amplicon sequencing data of motile & sessile fauna colonizing 66 ARMS deployed at 18 locations, from Svalbard to the Red Sea
- Bryozoan *Cribrilina (Juxtacribrilina) mutabilis* (Ito, Onishi & Dick 2015) identified in this dataset, and on ARMS plate images (see right)
- first described from eelgrass beds (*Zostera marina*) in Akkeshi, Japan, likely introduced to US east coast (Casco Bay) and northern Europe through shipping (Dick *et al.*, 2020)



C. mutabilis on ARMS plates

- 21 amplicon sequence variants (ASVs) identified as C. mutabilis
- ASVs (313 bp) aligned to publicly available sequences & haplotype network was generated (see left)
- 17 haplotypes (264 bp), 16 found on ARMS, 1 unique to Japan
- C. mutabilis widely distributed across Europe
- High haplotype but low nucleotide diversity
- Rapid but recent range expansion

Metabarcoding can be used as a tool to study population genetics & phylogeography of 100s – 1000s of species simultaneously! Advances can be made with emerging long-read metabarcoding techniques (PacBio, Nanopore seq etc.)



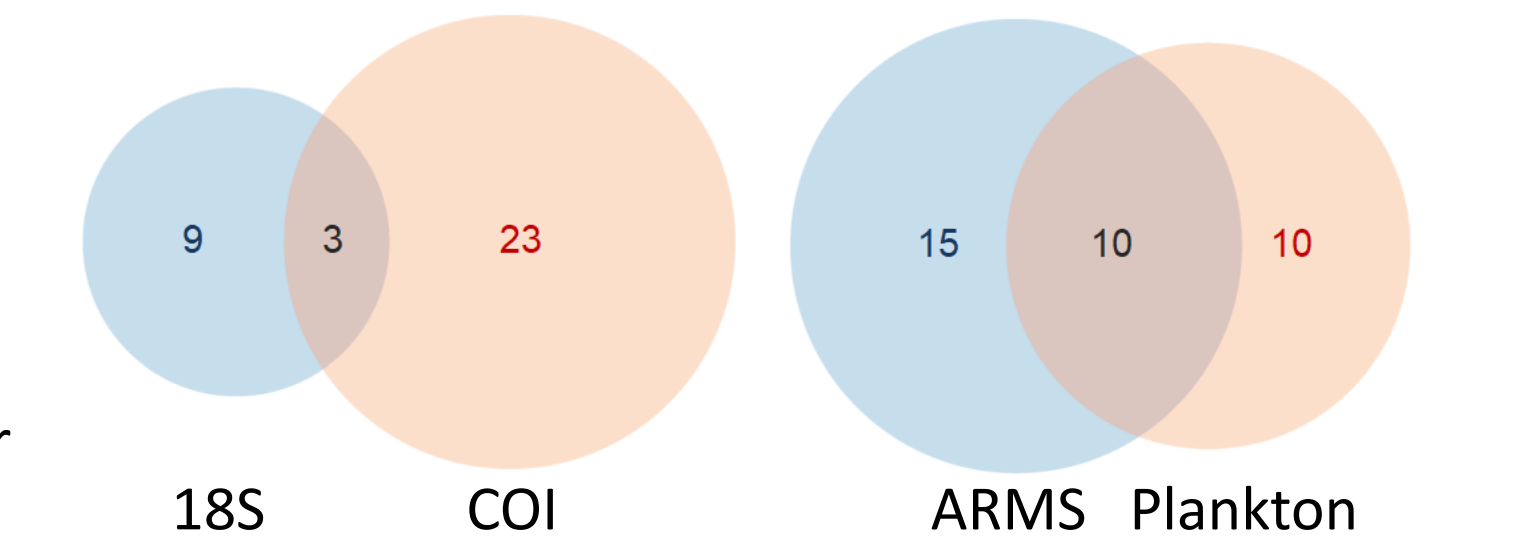
MONITORING NON-INDIGENOUS SPECIES (NIS)

Multiple vectors exit in the Anthropocene that are responsible for the **occurrence of non-indigenous species (NIS)**. The intensification of **global maritime traffic** has led to an **increase in the introduction of marine NIS** to a variety of locations. In addition, **climate change** is causing **range expansions** of marine species beyond their historical distributions. **Visual detection of NIS through routine or ad hoc surveys is challenging** and usually relies on the **presence of adult specimens at the time the survey is undertaken**. Plus, **once detected, NIS could already be established and have become invasive**. **Early, rapid and reliable detection of NIS** can therefore benefit from **genetic tools**, such as **standardized sampling using ARMS in combination with DNA metabarcoding**. In this work, **efficiency of ARMS & plankton sampling for NIS detection was tested** (Sundberg *et al.*, 2022).



- 19 ARMS & 18 plankton samples at 6 locations along Sweden's west coast (see left): blue - ARMS only, grey - ARMS and plankton
- Mainly ports / marinas as NIS hotspots, plus one MPA
- COI & 18S rRNA metabarcoding on ARMS-colonizing & plankton biota
- Species detected were screened against official catalogs to identify NIS & invasive alien species (IAS, i.e. NIS that become detrimental to environment / health / economy)

- 35 NIS from 9 phyla identified, incl. 13 IAS
- More NIS detected by ARMS than by plankton sampling
- More NIS detected by COI than by 18S rRNA marker



Number of NIS detected by each marker gene (left) & sampling method (right), plus overlap of identified species between the respective methods

Several new species in Sweden that should be risk assessed with regard to the effect on biodiversity, economy and health!

Japanese skeleton shrimp (*Caprella mutica*), detected by ARMS only. Native to NW Pacific, but introduced to Atlantic. Considered invasive, can reach high densities, outcompete native fauna & affect aquaculture.

Carvalho *et al.* (2019), *Proc. R. Soc. B* 286: 20182697
 Pearman *et al.* (2018), *Sci. Rep.* 8:8090
 Roth *et al.* (2019), *Methods Ecol. Evol.* 2019;10:712-725
 Obst *et al.* (2020), *Front. Mar. Sci.* 7:572680
 Dick *et al.* (2020), *Zool. Sci.* 37(3) : 217-231
 Sundberg *et al.* (2022), *Swedish Agency for Marine and Water Management report*; 2022: