The use of molecular approaches in marine ecosystem health assessment and research in Malaysia

Po Teen Lim, Chui Pin Leaw, Kieng Soon Hii

Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya, Bachok, Kelantan, Malayisa

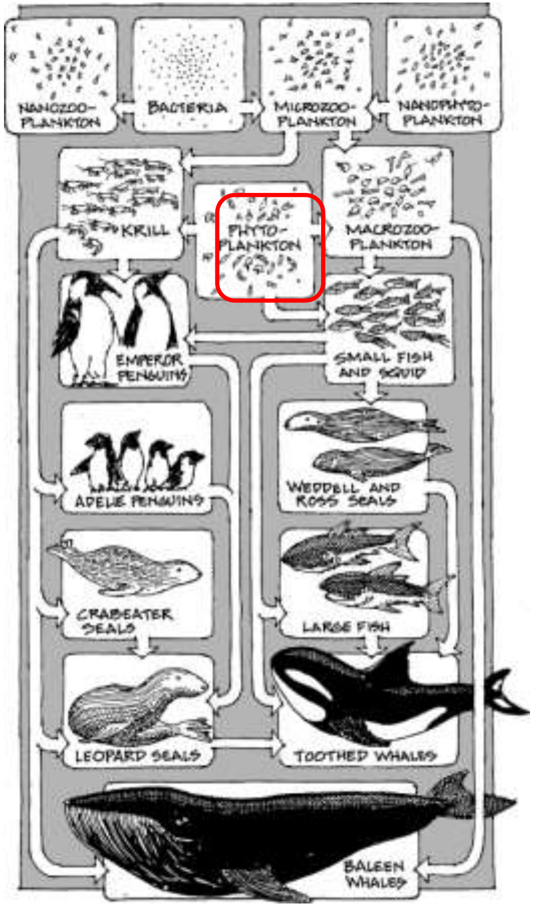
Part I

Assessing the diversity of marine phytoplankton across the South China Sea and Malacca Strait using metabarcoding approach

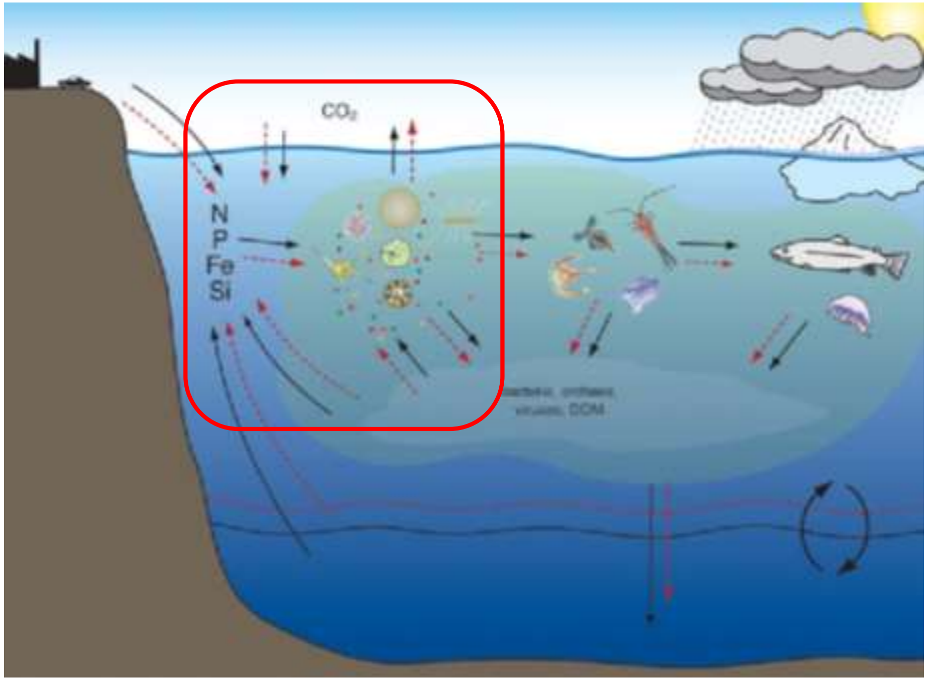
Kieng Soon Hii, Z. F. Lim, S.N. Tan, Z. Luo, H. Gu, C.P. Leaw, P.T. Lim

Importance of Phytoplankton

- They serve as the primary producers in coastal ecosystem.
- They also act as biogeochemical cycle mediator for the earth.



Sources: Gulf of Maine research Institute 2010



Sources: Finkel et. al., 2010

- Proliferation of selected phytoplankton taxa in the waters may cause tremendous impacts on human health and social-economy.

- Toxin producer taxa release biotoxin and contaminated seafood or fish kill.
- High-biomass producer taxa cause anoxia/hypoxia in waters and lead to mortality of marine life.

'Ombak Merah' boleh sebabkan kematian

Orang ramai dinasihat jangan makan kerang Sungai Geting

Tangani bahan beracu di Sungai Geting

Marine Science into the New Millennium: New Perspectives & Challenges, Phang et al. (eds.), 2004

First incidence of paralytic shellfish poisoning on the east coast of Peninsular Malaysia

Lim, P.T.[1], C.P. Leaw[2] and G. Usup[2]

Shellfish contamination in Geting River, Kelantan.

TODAY

160 tonnes of dead fish found in farms along Johor Straits

Harmful Algae

A bloom of *Karlodinium australe* (Gymnodiniales, Dinophyceae) associated with mass mortality of cage-cultured fishes in West Johor Strait, Malaysia

Mass mortality of cultured fish in Johor strait due to algal karlotoxins.

Over 50, 000 fish found dead in Teluk Bahang, fish breeder suffers RM800, 000 in losses

NATION

Sunday, 11 Aug 2019

5:07 PM MYT

By CAVINA LIM

Massive fish mortality in Teluk Bahang caused by a hypoxia-inducing algal bloom

RV Discovery, UMT

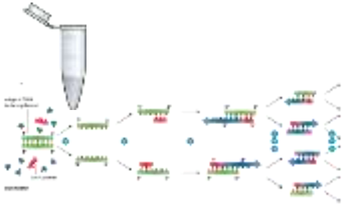
Objective

Investigate phytoplankton community assemblages across the South China Sea and Malacca Strait by genetic metabarcoding.

Methodology

72 plankton samples were collected during research cruise in July-August 2019

DNA isolation and 18S rDNA V9 region amplified



Illumina Miseq platform and data analysis

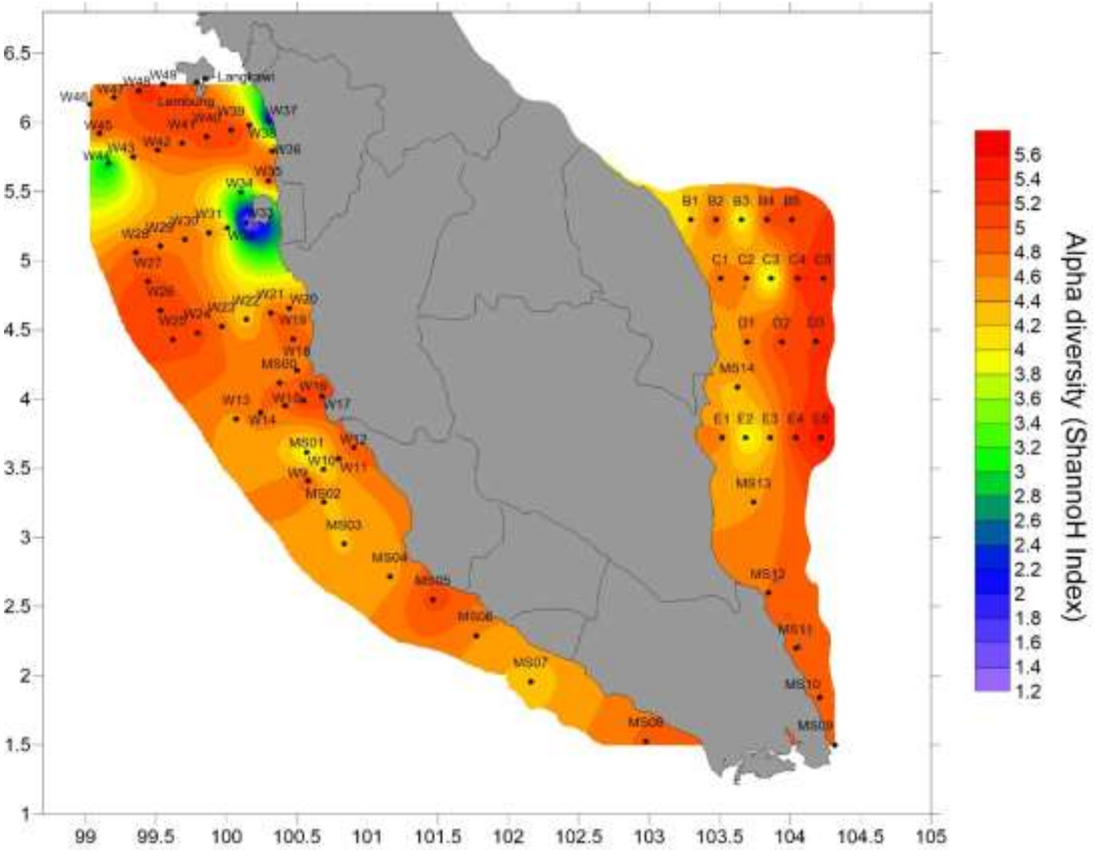
Phytoplankton community studies

- Metabarcoding is:-
  - A fast-genetic manner to elucidate the comprehensive taxonomic profiling in the environment (Kunin et al., 2008).
  - Reliable due to the taxonomic assignment are based on the sequences similarity of the references genomes in huge database such as Ref seq (Tatusova et al., 2014), PR2 (Cole et al., 2014) and SILVA (Quast et al., 2013).
- Highly accurate genetic distinction approach, metabarcoding are useful to monitor the phytoplankton/HABs species.

silva
high quality ribosomal RNA databases

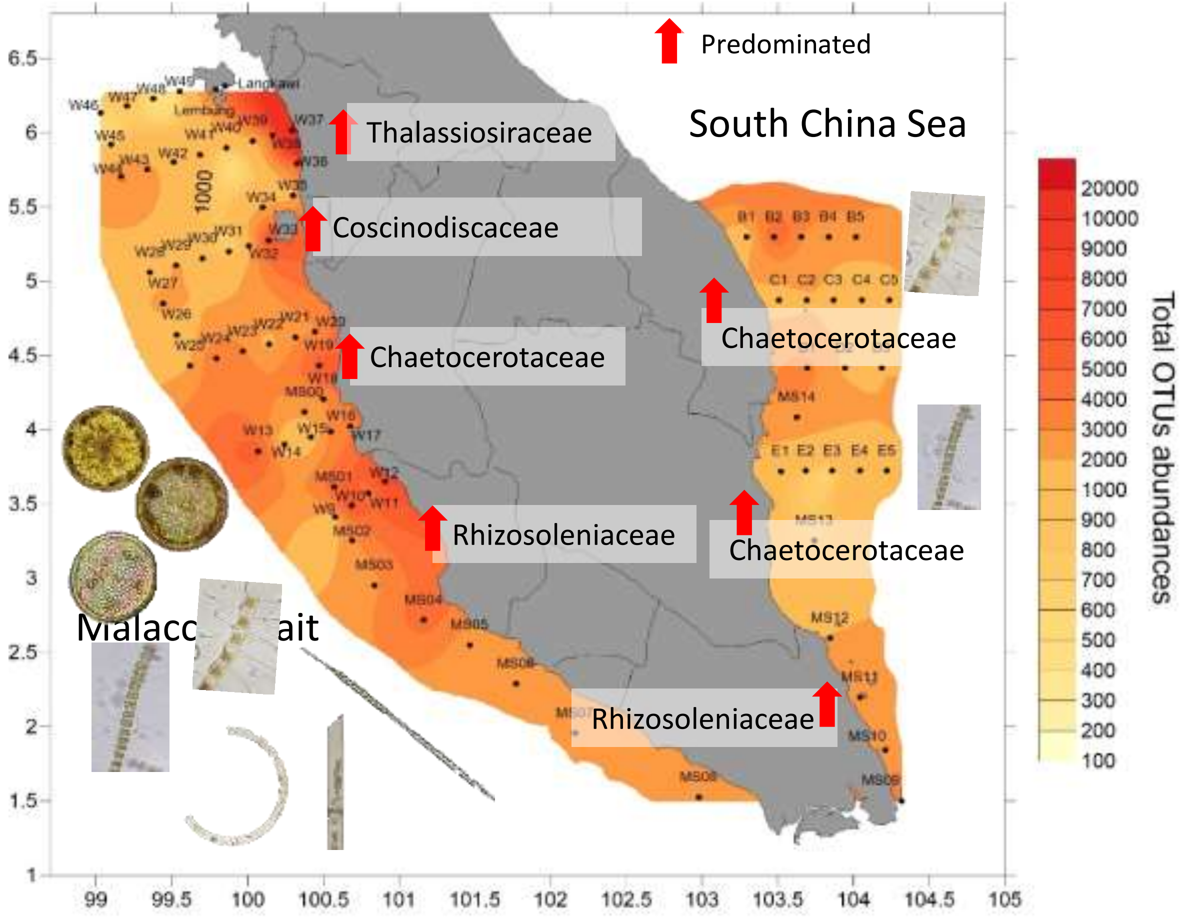
NCBI

Phytoplankton diversity



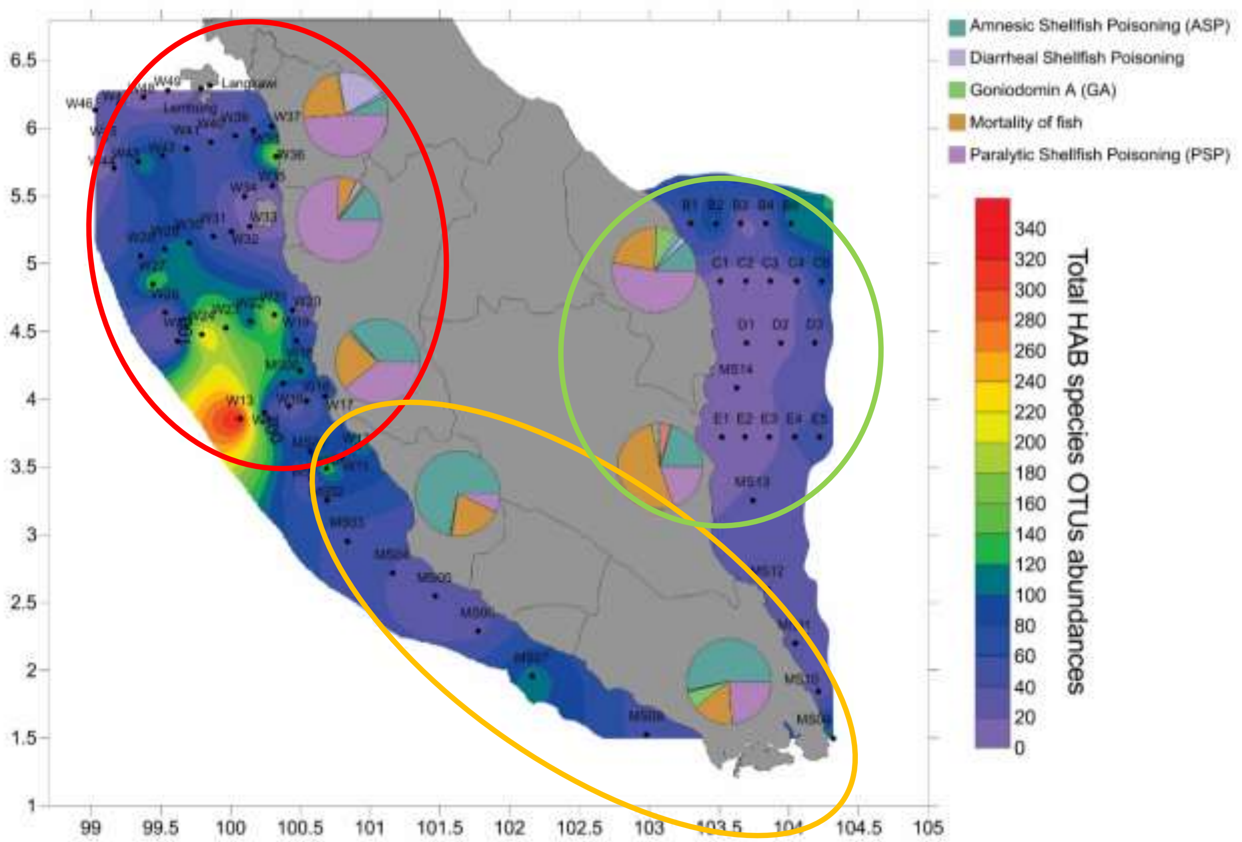
- A total of 386 phytoplankton species taxa was revealed.
- High species diversity was observed at offshore sampling station.
  - This could be due to lower nutrient at offshore.
  - High nutrient tends to shift the high-species phytoplankton diversity to monospecific.

OTUs Abundance of phytoplankton



- High OTU abundances of phytoplankton were found at shore sampling stations of Malacca Strait.
  - This was likely influenced by high nutrient loading in the region.

Harmful algal bloom species and its threats



- A total of 28 harmful algal bloom (HAB) species was annotated from OTU, with 7 new records in Malaysian waters.
- High OTU abundance of HAB species were mainly found in Malacca strait.

Conclusion

- This study provides valuable baseline inventory of marine phytoplankton diversity to evaluate long term of phytoplankton communities along the coast of Malaysia
- Metabarcoding approach is useful for HAB species detection and monitoring.

Acknowledgements

- Thanks to RV Discovery scientific cruise from UMT.
- HICoE-2014c to PT Lim.
- Thanks to Prof Gu, H. and Dr Luo, Z., TIO for the Illumina Miseq analysis.

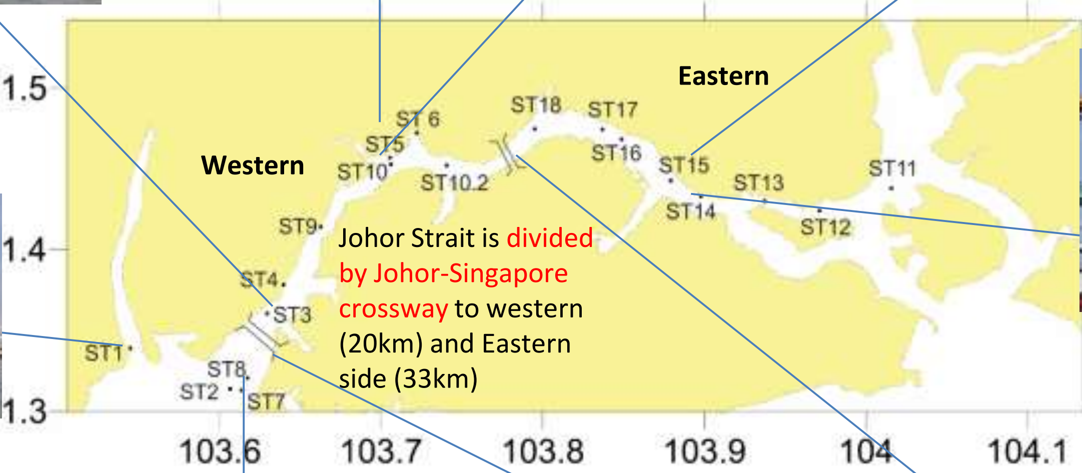
Revealing the Harmful Algal Community Shift Along the Johor Strait Using Metabarcoding Approach

Hii, K.S., Mohd-Din, M., Tan, S.N., Lim, Z.F., Lee, L.K., Luo, Z., Gu,
H., Leaw, C.P., Lim, P.T.

Study Sites: Johor Strait (JS)

ST3: Cage Fish Farm; ST5 and ST10 : Cage Fish and Green Mussel Farm

ST13-16 : Green mussel farm



Johor Strait is divided by Johor-Singapore crossway to western (20km) and Eastern side (33km)

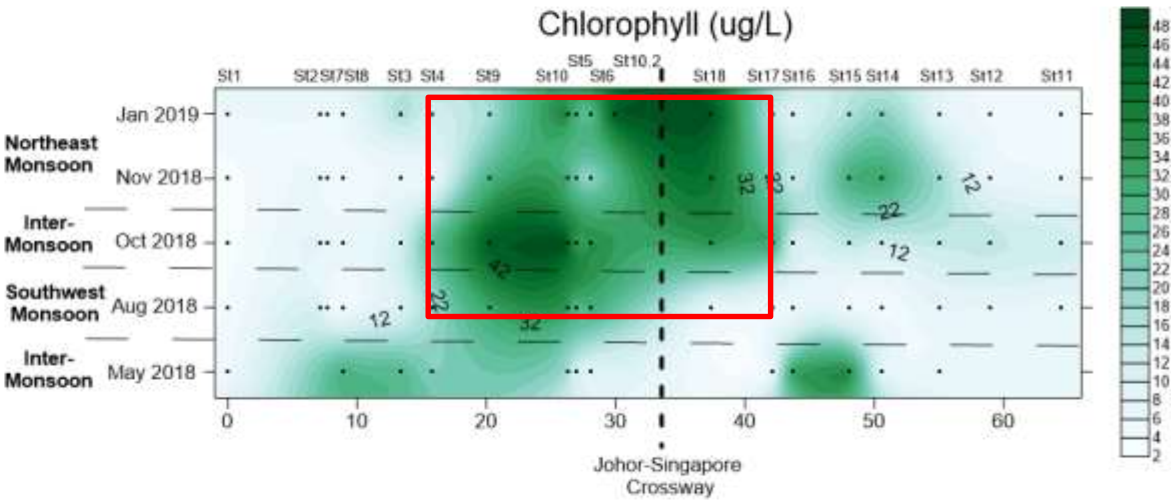
ST14-15 : Cargo port

ST1: Port of Tanjung Pelepas

ST2, ST7-ST8: Near the Merabong Island

Johor-Singapore Bridge

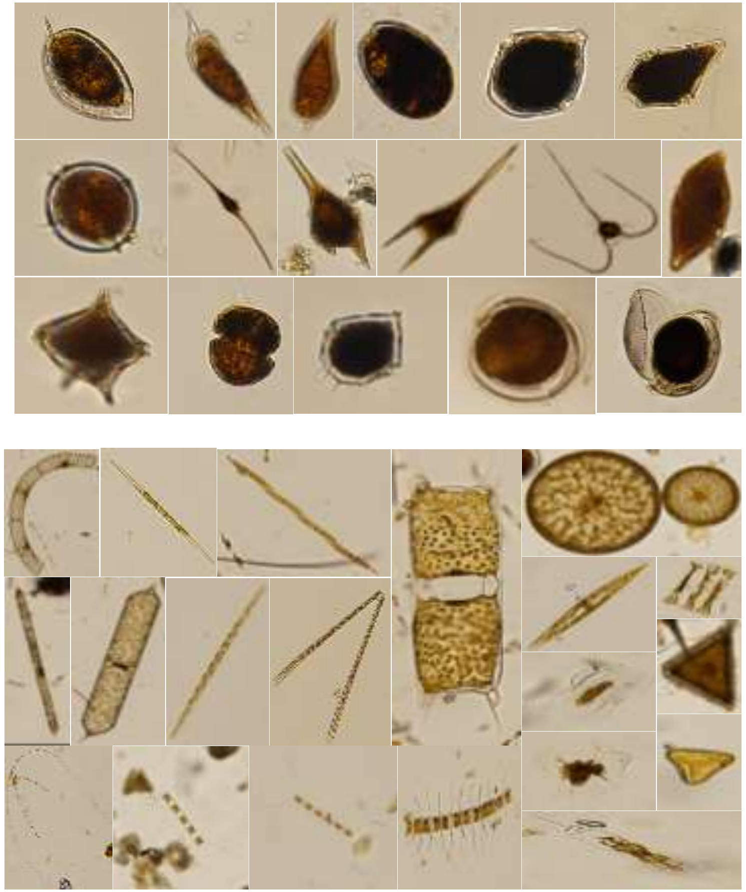
Johor-Singapore Crossway



May 2018 Inter-monsoon	Aug 2018 Southwest monsoon	Oct 2018 Inter-monsoon	Nov 2018 and Jan 2019 Northeast monsoon
Phytoplankton Blooms			
Thalassiosira antarctica *Skeletonema menzellii* *Skeletonema grevillea*	*Skeletonema grevillea* *Chaetoceros tenuissimu* *Rhizosolenia similoides*	*Chaetoceros tenuissimu* *Thalassiosira allenii* *Skeletonema grevillea* *Chaetoceros Pumilum*	*Skeletonema tropicum* *Chaetoceros tenuissimu* *Eucampia antarctica* *Thalassiosira weissflogii,* *Thalassiosira ludiana* *Thalassiosira antarcitica* *Rhizosolenia similoides*

- Chl-*a* in the Johor Strait were in the range of 48.82- 3.15 µg/L.
- The highest Chl-*a* (34-48.32 µg/L) content were observed in inner JS, where high biomass/ bloom of several diatom species in the water were observed.

Species composition based on metabarcoding



- A total of 130 phytoplankton taxa were revealed from OTUs taxonomic assignment.
    - 64 species of Dinoflagellates
    - 66 species of diatoms
- 16 know and potential HAB species

i) *Alexandrium affine*, **ii)** *A. minutum/peruvianum/ostenfeldii/insuetum*, **iii)** *A. pseudogoniaulax/hiranoi*, **iv)** *A. tamiyavanichii*, **v)** *A. leei*, **vi)** *Chattonella marina*, **vii)** *C. subsalsa*, **viii)** *Dinophysis miles/ caudata/norvegica/acuminate*, **ix)** *Gymnodinium catenatum*, **x)** *Heterosigma akashiwo*, **xi)** *Karenia selliformis*, **xii)** *Pseudo-nitzschia americana*, **xiii)** *P. brasiliana*, **xiv)***P. cuspidata/lineola/turgidula/australis*, **xv)** *P. multiseries/Pungens*, **xvi)** *P. australis*

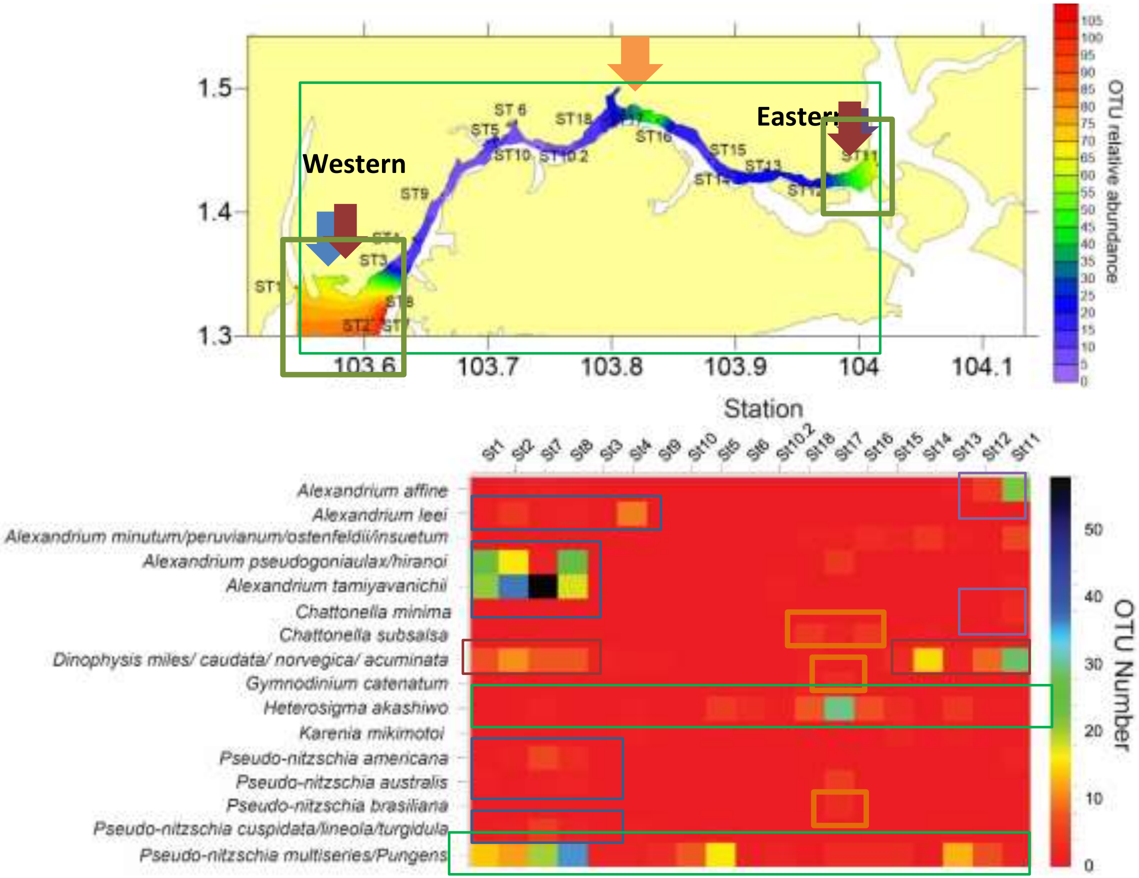
Taxonomic assignment

	Highest Blast hit	Identity (%)	Species assignment
OTU21914 Otu4410	*Dinophysis miles*	99.23	*Dinophysis miles/caudata/norvegica/acuminata*
	D. caudata		
	D. norvegica		
	D.acuminata		
Otu15209 Otu3139	*Pseudo-nitzschia pungens*	98.47	*Pseudo-nitzschia multiseries/pungens*
	P. multiseries		
Otu22780 Otu4439 Otu4441	*Alexandrium minutum*	98.46	*Alexandrium minutum/peruvianum/ostenfeldii/insuetum*
	A. peruvianum		
	A. ostenfeldii		
	A. insuetum		
Otu3088	*Pseudo-nitzschia cuspidata*	97.67	*Pseudo-nitzschia cuspidata/lineola/turgidula*
	P. lineola		
	P.turgidula		
Otu7064 Otu135	*Alexandrium pseudogoniaulax*	98	*Alexandrium pseudogoniaulax/hiranoi*
	A. hiranoi		
Otu16641	*Pseudo-nitzschia australis*	99.24	*Pseudo-nitzschia australis*
Otu21833	*Karenia mikimotoi*	97.69	*Karenia mikimotoi*
Otu23722	*Heterosigma akashiwo*	99.2	*Heterosigma akashiwo*
Otu24287	*Gymnodinium catenatum*	99.23	*Gymnodinium catenatum*
Otu4409	*Alexandrium affine*	100	*Alexandrium affine*
Otu4480	*Alexandrium leei*	97.3	*Alexandrium leei*
Otu4492	*Alexandrium tamiyavanichii*	100	*Alexandrium tamiyavanichii*
Otu4421	*Chattonella subsalsa*	100	*Chattonella subsalsa*
Otu6704	*Chattonella minima*	100	*Chattonella minima*
Otu299	*Pseudo-nitzschia brasiliana*	98.48	*Pseudo-nitzschia brasiliana*
Otu3526	*Pseudo-nitzschia americana*	100	*Pseudo-nitzschia americana*

- Only OTUs that had > 97% similarity to NCBI Nt database were included in the analysis.
- However, some of the HABs species still cannot be distinguished to species level by using V9 of 18S rDNA region.

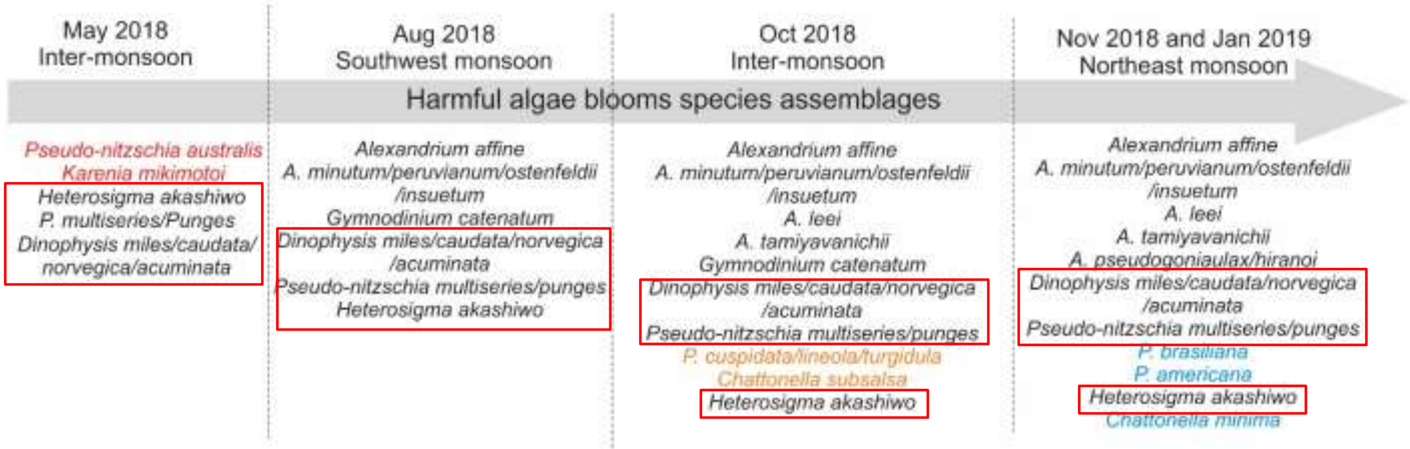
HAB species distribution in Johor Strait

Overall HABs species relative abundance throughout May2018-Jan2019



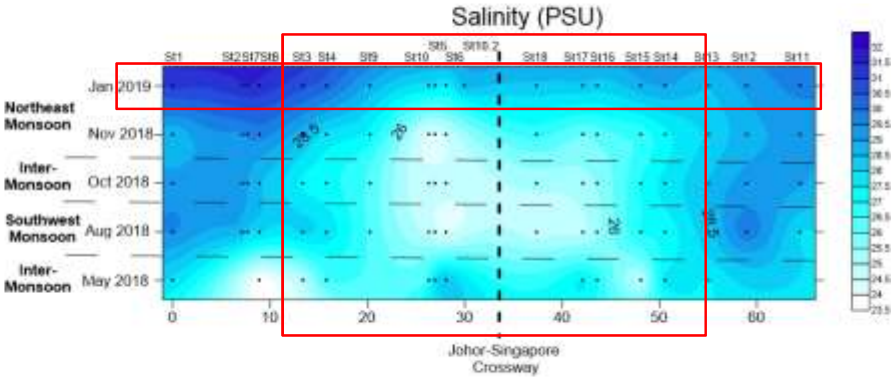
- The highest HAB species diversity and relative abundance were discovered in outer western and Eastern JS.
- *A. leei, A. pseudogoniaulax/hiranoi, A. tamiyavanichii, P. americana, P. australis* and *P. cuspidata/lineola/turgidula* were found in outer Western JS.
- *A. affine* and *C. minima* were detected in outer of Eastern JS.
- *Dinophysis* sp. was found in outer parts of Western and Eastern JS.
- *C. subsalsa, G. catenatum* and *P. brasiliana* were discovered in inner Eastern JS.
- *H. akashiwo* and *P. multiseries/punges* were detected throughout the stations in JS.

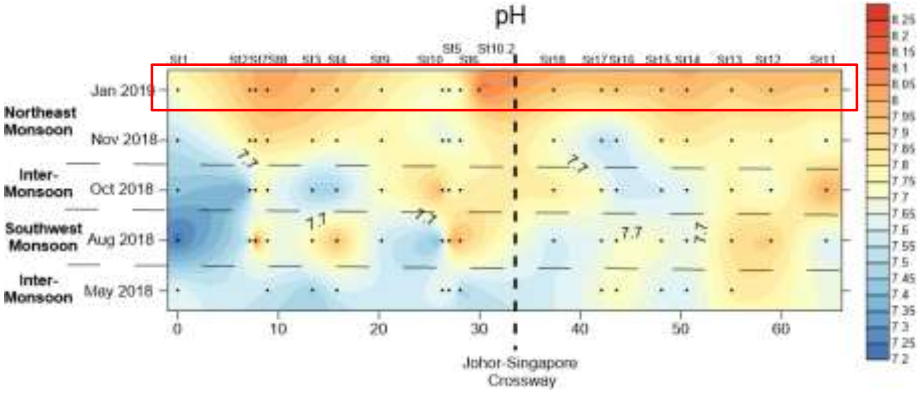
HAB community assemblages and composition shift



- HABs species diversity increased in wet season during the Northeast monsoon.

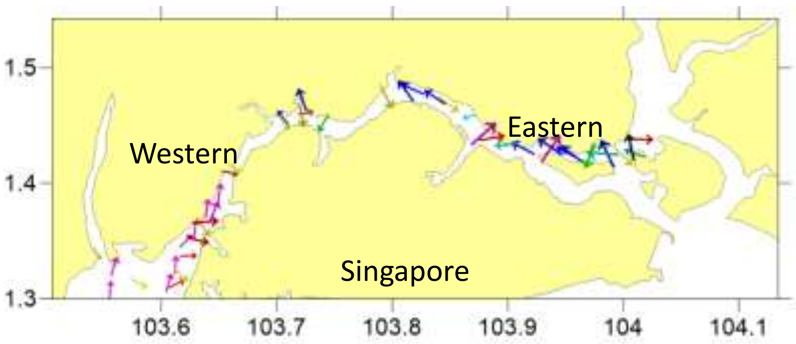
Physico-chemical variability

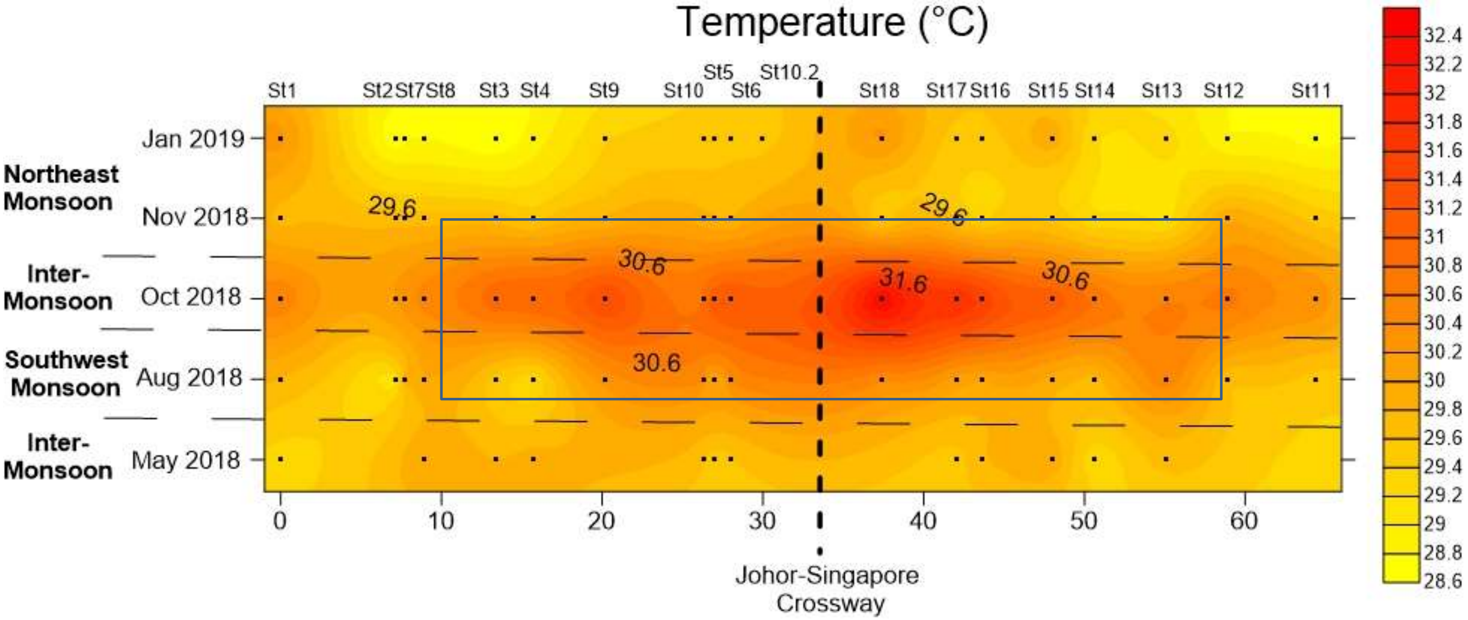




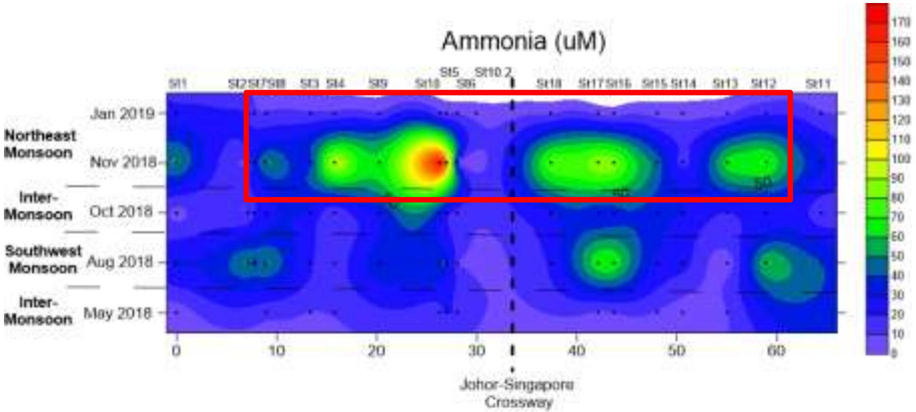
- Salinity and pH in JS were in the range of 23-32 and 7.2-8.2, respectively.
- The lowest salinity were observed in the inner JS, likely due to freshwater plumes and discharge of freshwater from reservoirs.
- The highest salinity and pH were detected in Jan 2019 during flood tide, intrusion of the seawater from South China Sea and Strait Of Malacca.

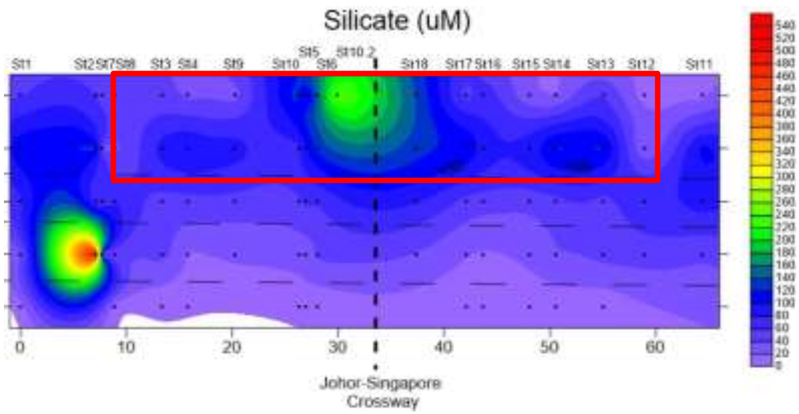
Water current movement during Jan 2019

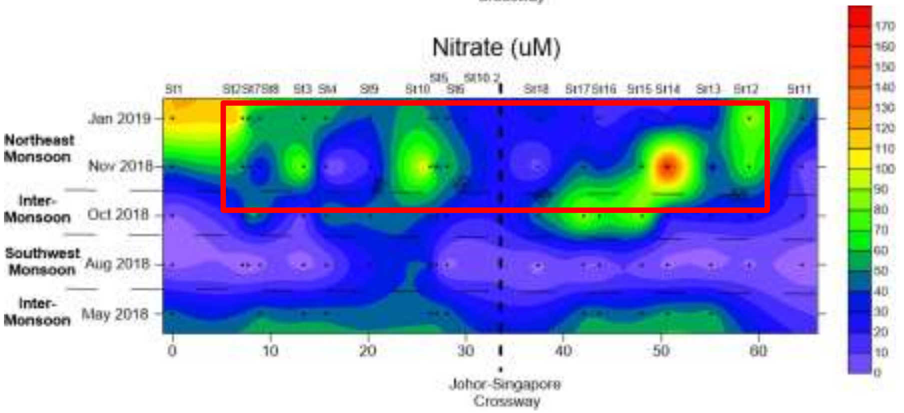


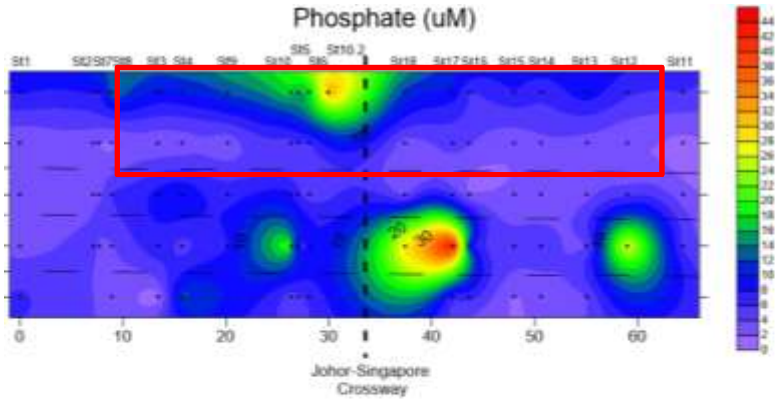


- Temperature in JS were observed in the range of 28-33°C.
- The highest temperatures (31-33 °C) were observed in the Inter-Monsoon (Oct 2018) and Southwest Monsoon.



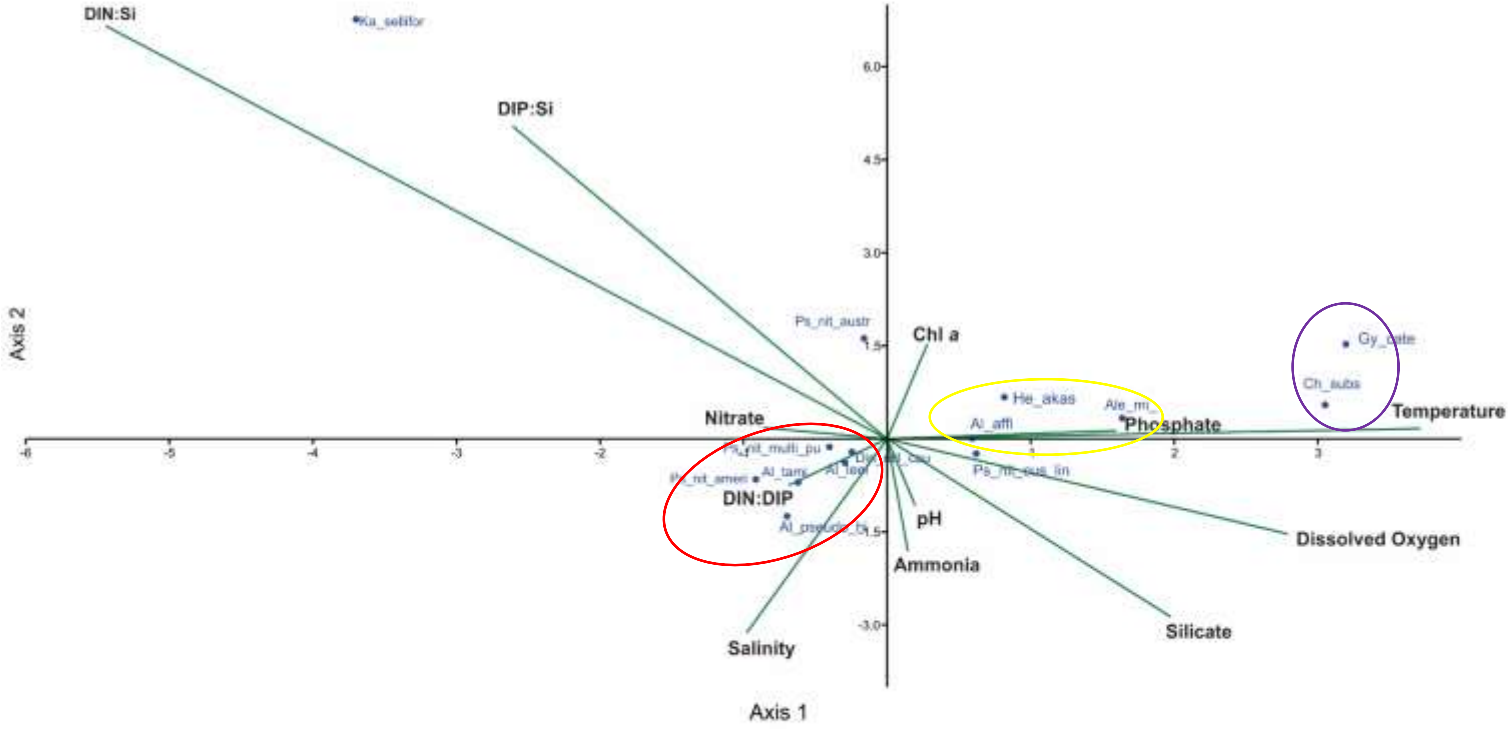






- The highest concentration of micronutrients, nitrate, ammonia, phosphate and silicate were observed during the Northeast Monsoon (Nov-Jan), postulate the nutrient sources was discharged from rivers and runoff from land in urban areas during rain.

Canonical Correlation Analysis (CCA)



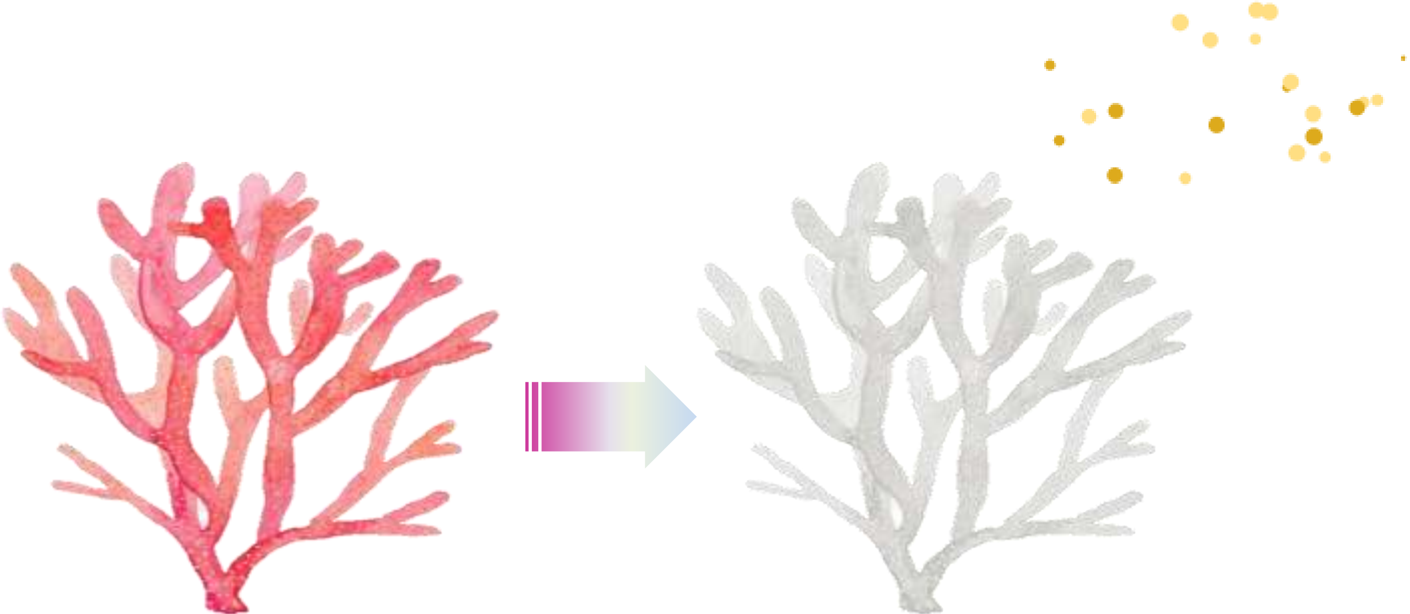
- Relative abundance of *A. pseudogoniaulax/hiranoi*, *A. tamiyavanichii*, *A. leei, P. americana, P. multiseries/pungens* and *Dinophysis* sp. were associated with increasing DIN: DIP and salinity.
- *H. akashiwo, A. affine and A. minutum/peruvianum/ostenfeldii/insuetum* were related to increasing phosphate and temperature.
- *G. catenatum* and C. *subsalsa* were highly correlated with temperature.

Part III
Exploring the diversity of zooxanthellae associated with corals of Perhentian Islands Marine Park by deep sequencing approach

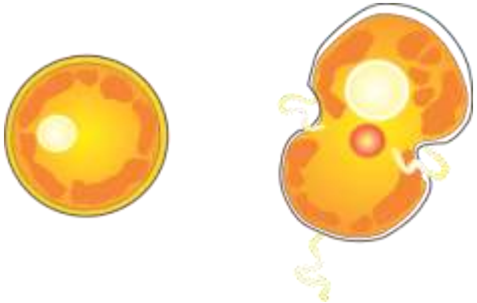
Po Teen LIM, Lee Kiat LEE, Zhen Fei LIM, Kieng Soon HII, Haifeng GU, Zhaohe Luo, Albert A. CHAN, Chui Pin LEAW

The coasts of Malaysia harbored coral reef ecosystem spanning for up to 4000km^2 in term of area (Wikinson, 2008), with estimated 500 hermatypic coral species, constituting 60% of the world described hermatypic coral species (Veron et al., 2009).

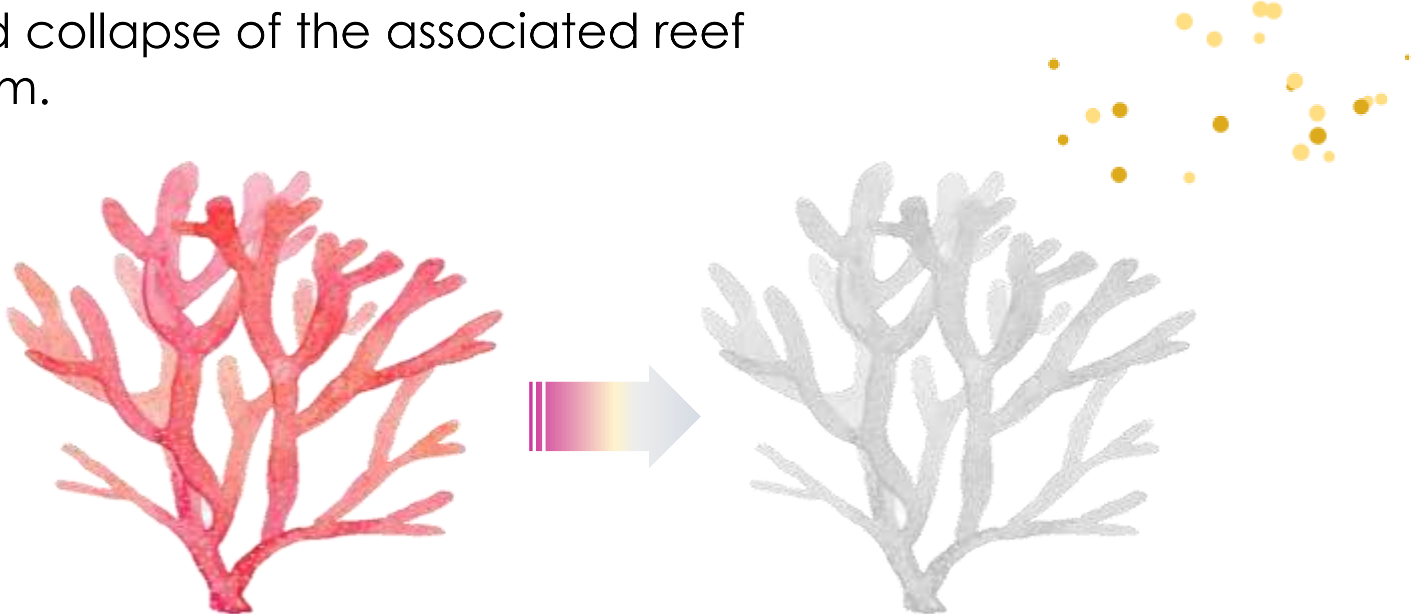
Climate change and other local stressors (eutrophication, siltation, habitat destruction and overfishing) has caused mass bleaching of coral species. eg., in year 2010.



Reef corals are hosts to a group of exceptionally diverse dinoflagellate symbionts in the family **Symbiodiniaceae.**

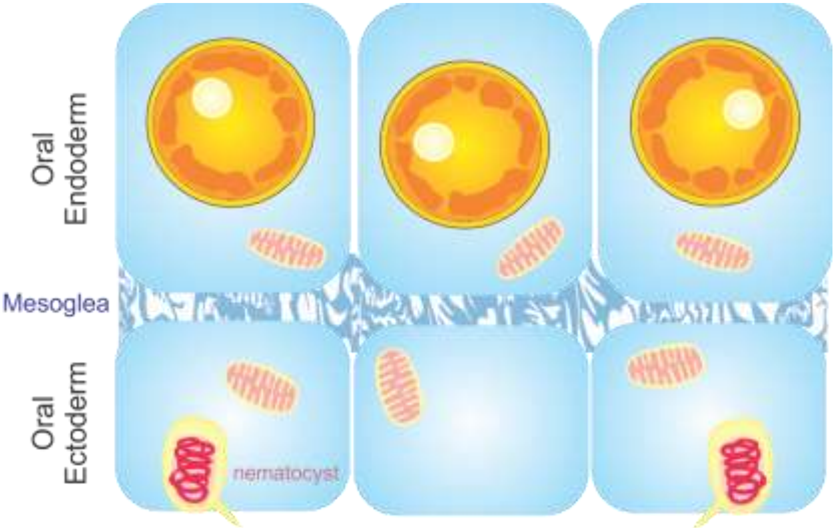


These symbionts are critical components of coral reef ecosystem. Loss of these symbionts in their coral hosts during stress-related “bleaching” events can lead to mass mortality of the coral hosts and collapse of the associated reef ecosystem.



Zooxanthellae factSheet

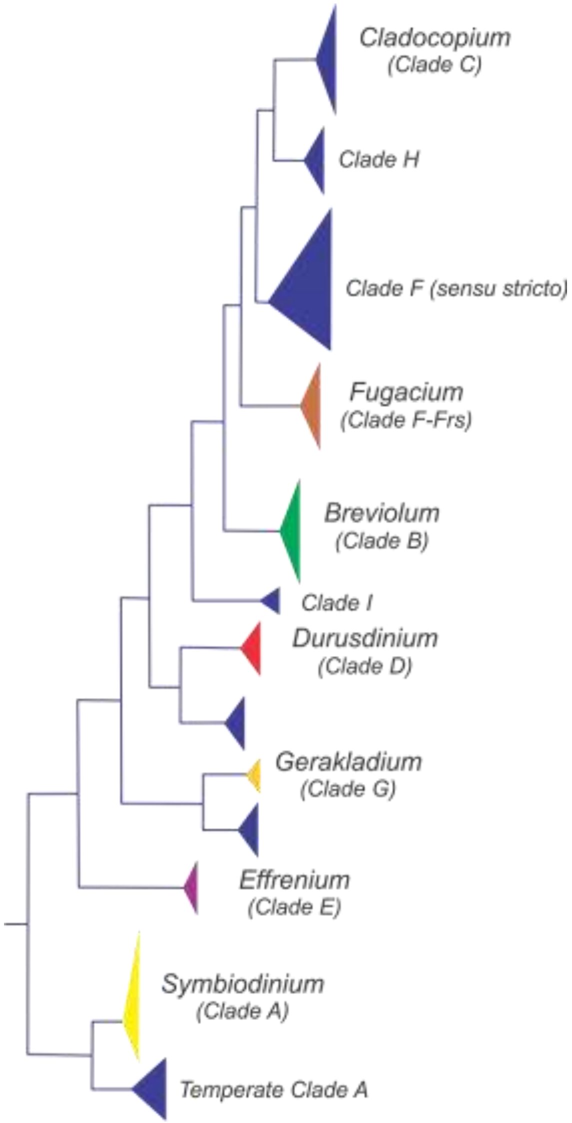
- ☐ Single-cell dinoflagellates
- ☐ Mutualistic endosymbionts
- ☐ Autotroph (Photosynthetic)
- ☐ Containing chloroplasts (chlorophyll a, c, peridinin and diadinoxanthin) and pyrenoid
- ☐ Coccoid in hosts; free living flagellated cell



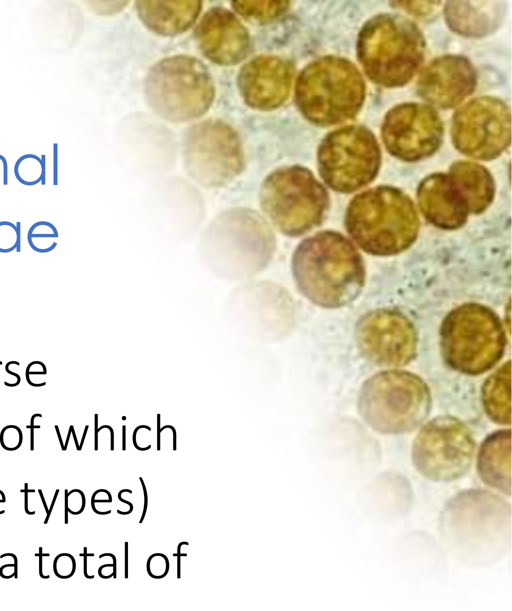
The symbiont is housed in a specialized compartment (peri-vacuolar membrane) of host cell, called symbiosome.

They give corals their vibrant colors.

Zooxanthellae will be expelled when the coral hosts are exposed to environmental stressors, and this caused the coral to bleach.



High species and functional diversity of zooxanthellae



Zooxanthellae is a highly diverse group, with divergent clades (of which comprise of multiple subclade types) that are now partitioned into a total of **seven** genera.

Different clades have different resistance to various environmental stresses.

Clade A – high/variable light
Clade D – thermal tolerance
Clade E- Free living-non symbiotic

LaJeunesse et al. (2018)

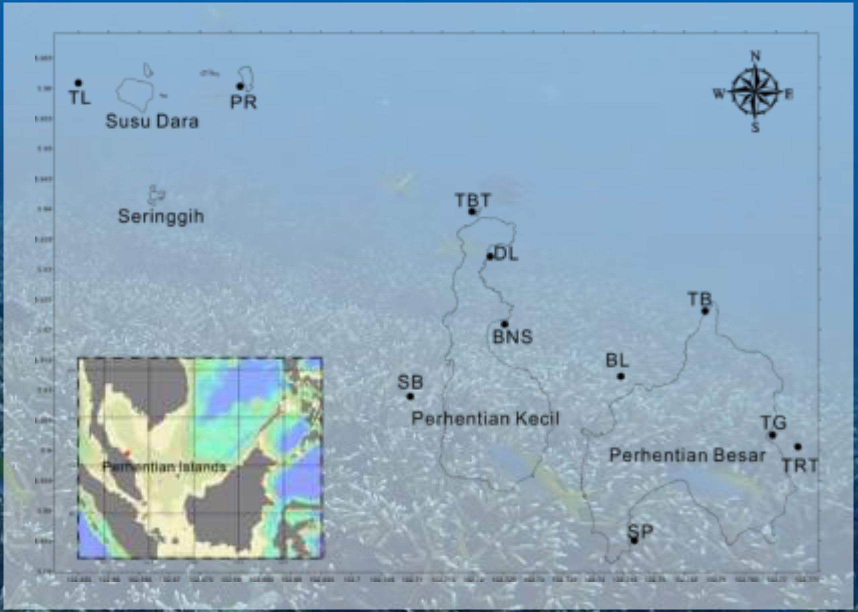
Goal:

Exploring the zooxanthellae diversity of Malaysia

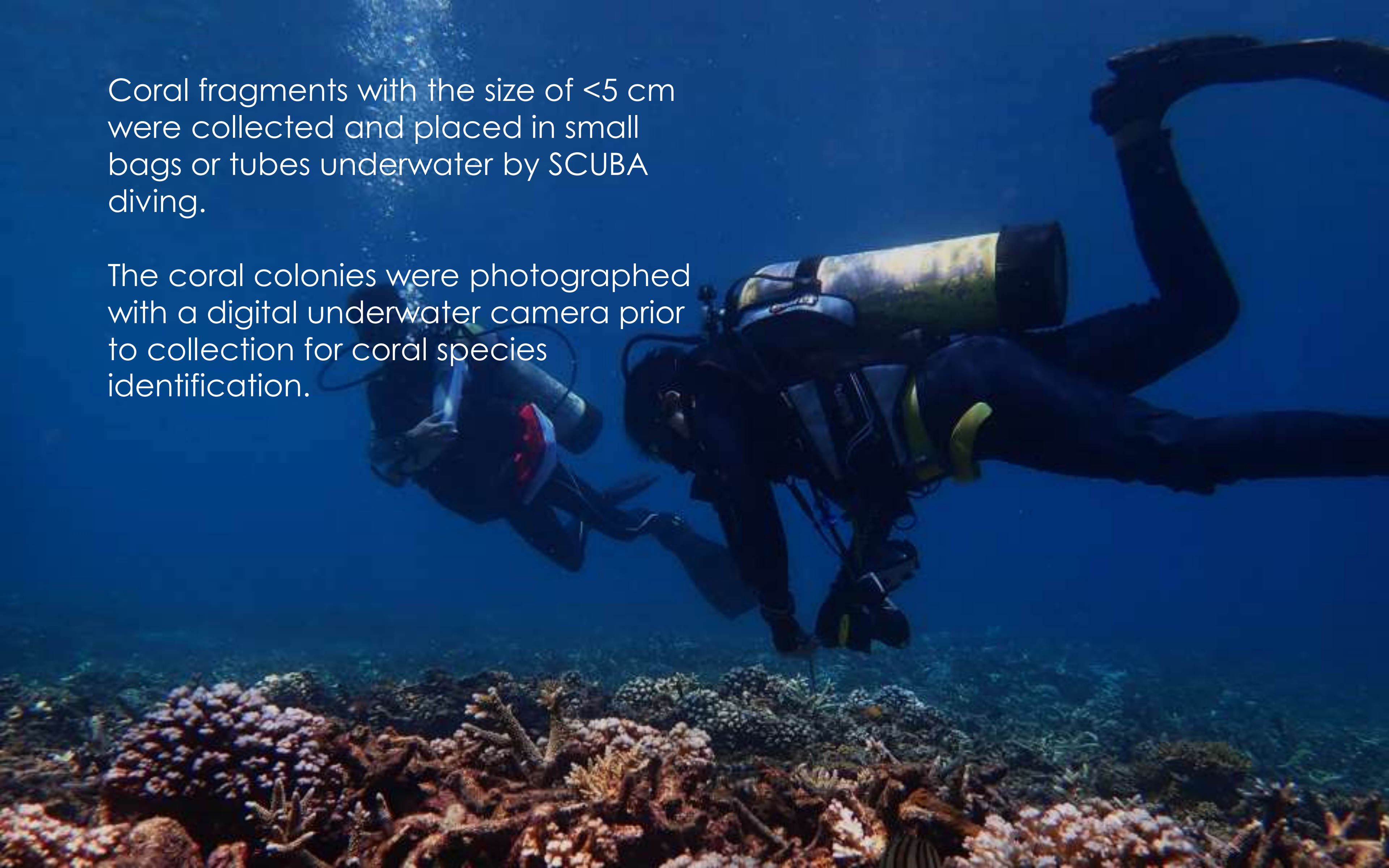
Assessing the symbiont composition in different corals is essential to understand the resilience and susceptibility of the reefs

To investigate the diversity of zooxanthellae in Malaysian common coral species,

To assess the coral resilience by comparing the zooxanthellae communities in the host species.



Perhentian Islands Marine Park

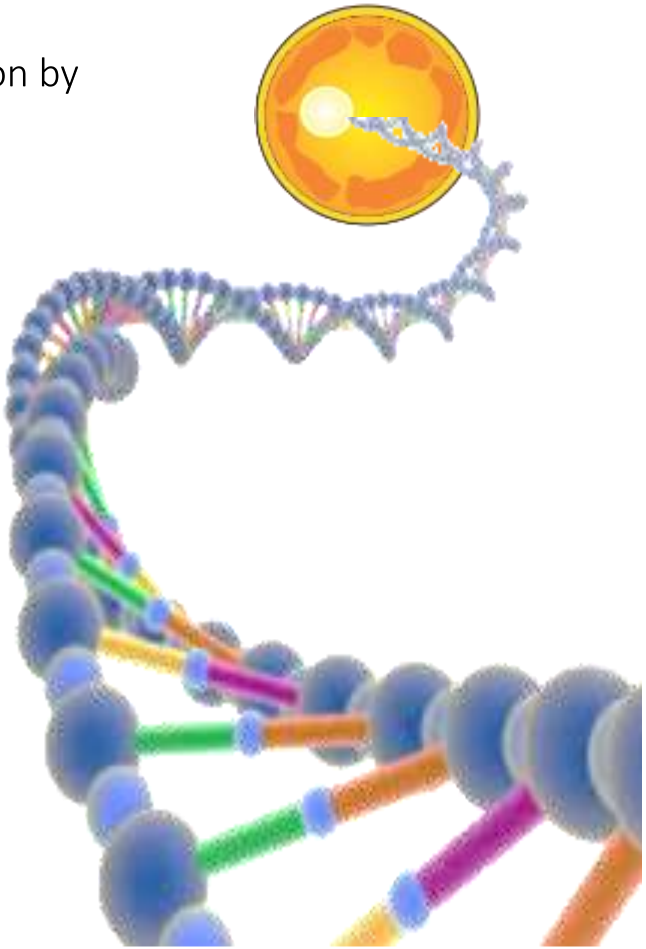


Coral fragments with the size of <5 cm were collected and placed in small bags or tubes underwater by SCUBA diving.

The coral colonies were photographed with a digital underwater camera prior to collection for coral species identification.

Molecular characterization

- Samples were processed prior to genomic DNA extraction by magnetic bead technique.
- **Gene amplification of the nuclear ribosomal gene (ITS2) was performed. The amplicons were purified and quantified prior to deep sequencing**



NGS deep sequencing

NGS deep sequencing was performed on ILUMINA MiSeq platform.

Gene amplifications

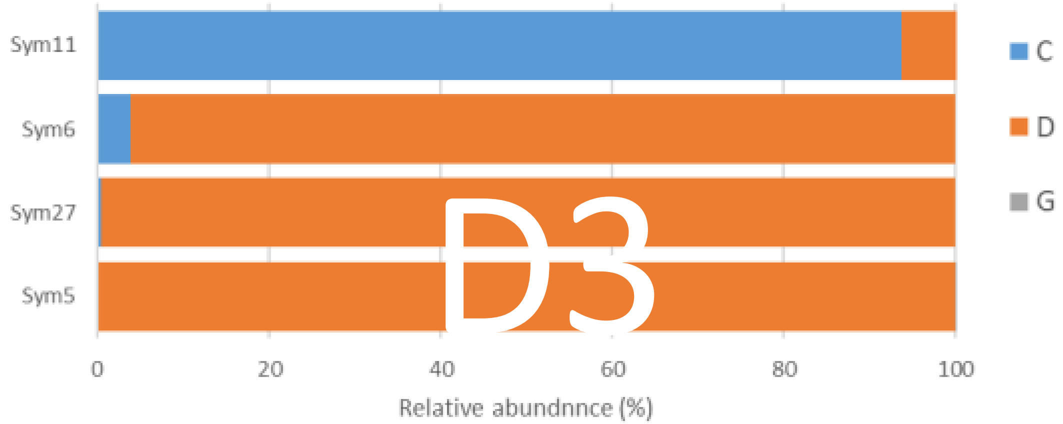
NGS deep sequencing

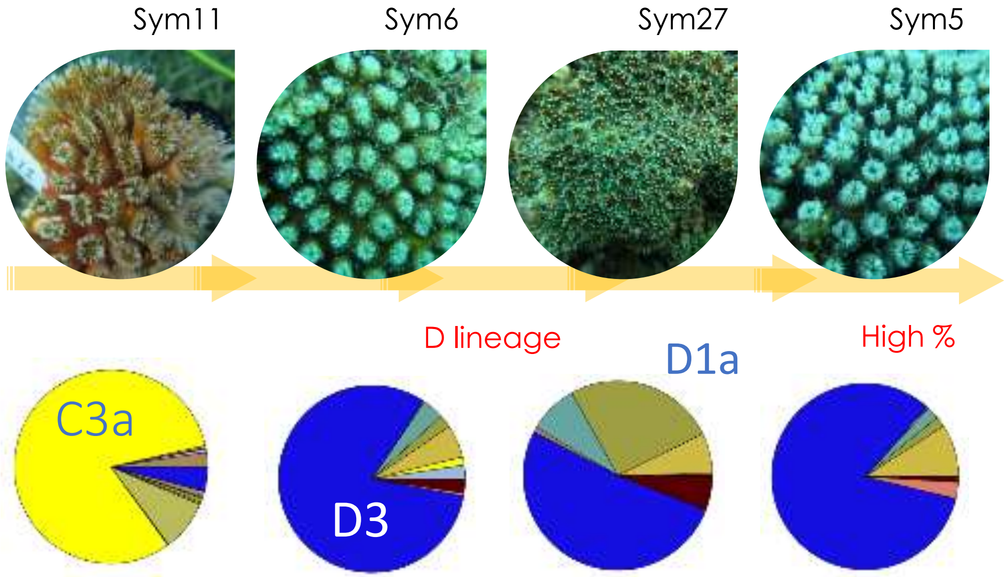
DNA extraction

Zooxanthellae diversity

- In total, 24 coral samples were analyzed.
- 1,232,181 reads were obtained with median length of 292 bp.
- 900,055 reads were retained with an average 37,503 sequences per sample after sequences filtering.
- Two dominant genetic clades, viz. Clades **C** and **D** were identified from these coral samples.
- A total of **134 subclades C and 14 subclades D** were confirmed.

Zooxanthellae diversity in *Galaxea* species



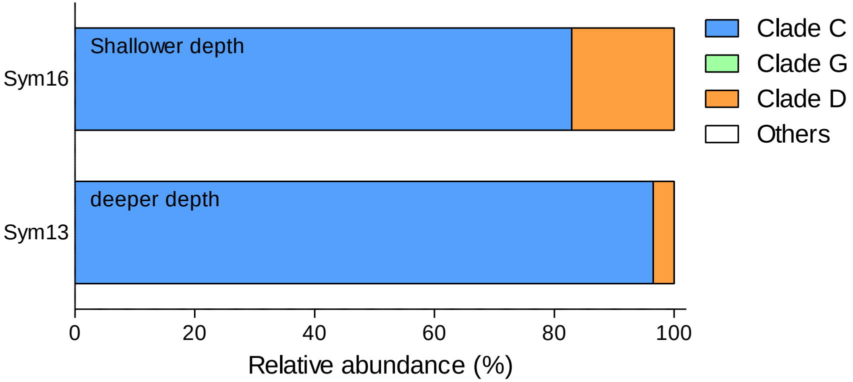


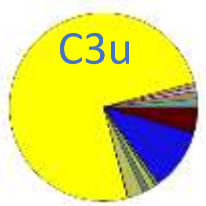
The high abundances and varying distributions of D lineage in the zooxanthellae communities of ***Galaxea* species** might indicated recent bleaching of this coral species, since Clade D is often associated with recently bleached and/or recovering corals (Baker et al., 2004), but can be outcompeted following recovery (Thornhill et al., 2006).

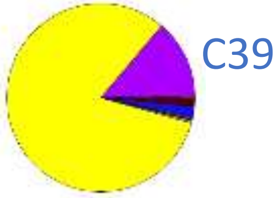
D1a = *D. trenchii*

Zooxanthellae diversity in Tubinaria species

Specimens from shallower depth exhibit at least 10% higher proportion of D lineage compared to the deeper counterpart.





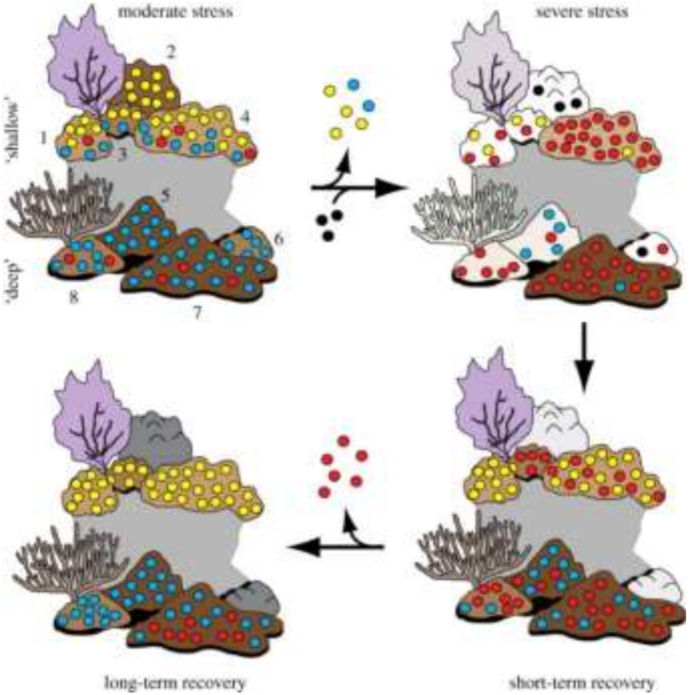


It is interesting to note that the deeper *Tubinaria* hosted only C39 lineage.
This lineage has been shown to be prevalent and **restricted to the Red Sea and the Sea of Oman** as potential type that display increased thermotolerance (Zigeler et al., 2017).

Thermal stress-tolerance ecotypes

Opportunistic symbionts may impart rapid adaptation to environmental change by their hosts

A reconstruction of how thermal stress influenced symbiont populations among shallow and deep colonies of *Montastraea annularis* during and after the 2005 Caribbean bleaching event.



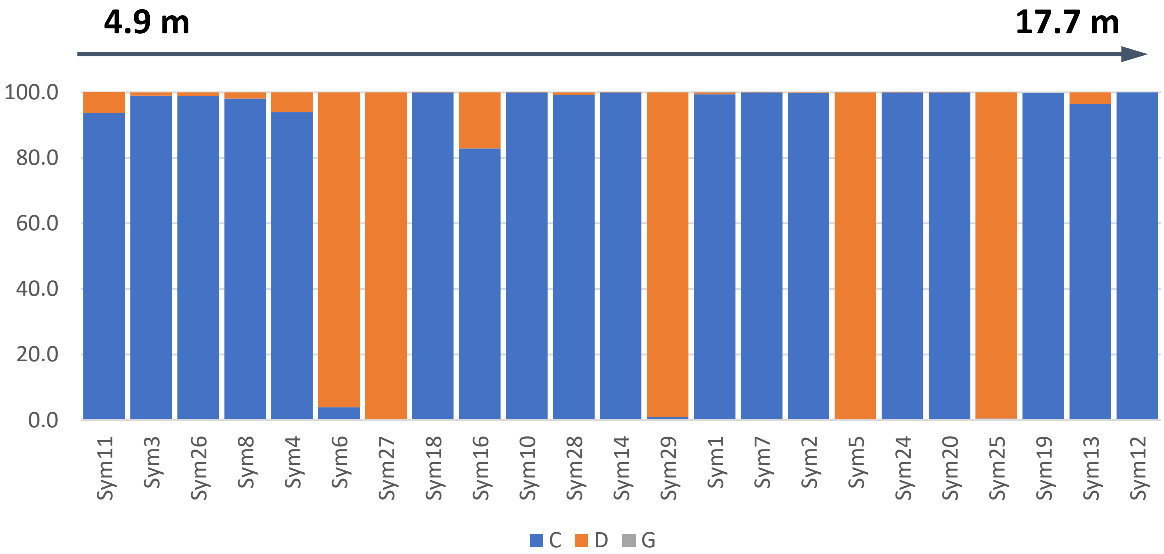
LaJeunesse et al. (2009)

Opportunistic species may proliferate during periods of physiological stress and displace the normal resident symbiont and remain abundant in the host's tissues for months to years before being replaced by the original symbiont.

= tolerate stressful environments + accelerate recovery

Zooxanthellae communities in the coral species of Perhentian Islands did not show significant trend in depth distribution.

The abundances of thermally tolerant D lineage in various coral species was not associated with depth distribution, presumably shallower depth experienced wider ranges of thermal fluctuations and exposed to highest temperatures.

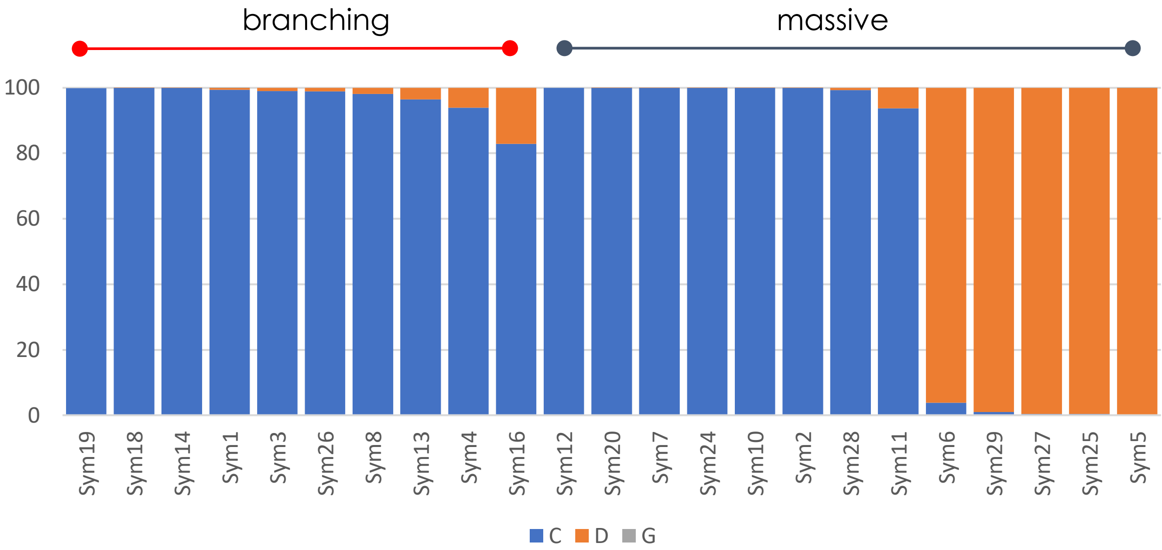


The zooxanthellae communities differed significantly among various coral species.

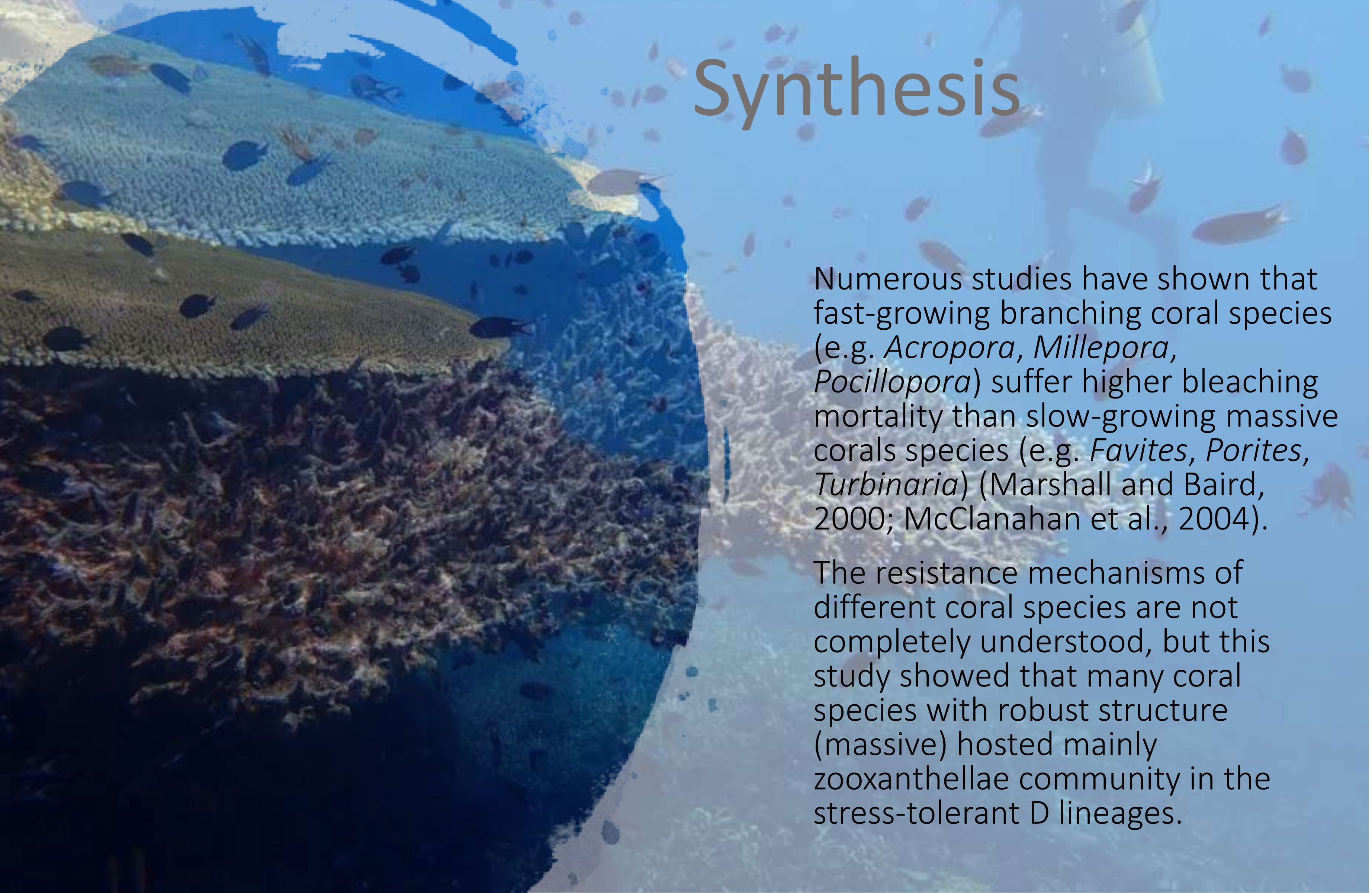
This difference appears to be driven by higher relative abundances of lineages C3u, C115, C18, and C30_type 1.

For example, the most abundant C lineage in *Leptoseris* sp. Sym8 was C30_type 1, with up to 97% of relative abundance, while in *Echinopora* sp. Sym10, a relatively higher proportion of C3u was detected. This supported previous findings of **host-specific associations** of the endosymbionts (Finney et al., 2010).

Corals with complex structure (branching) hosted mainly zooxanthellae community in the C lineages while D lineages are associated with some robust corals (massive)



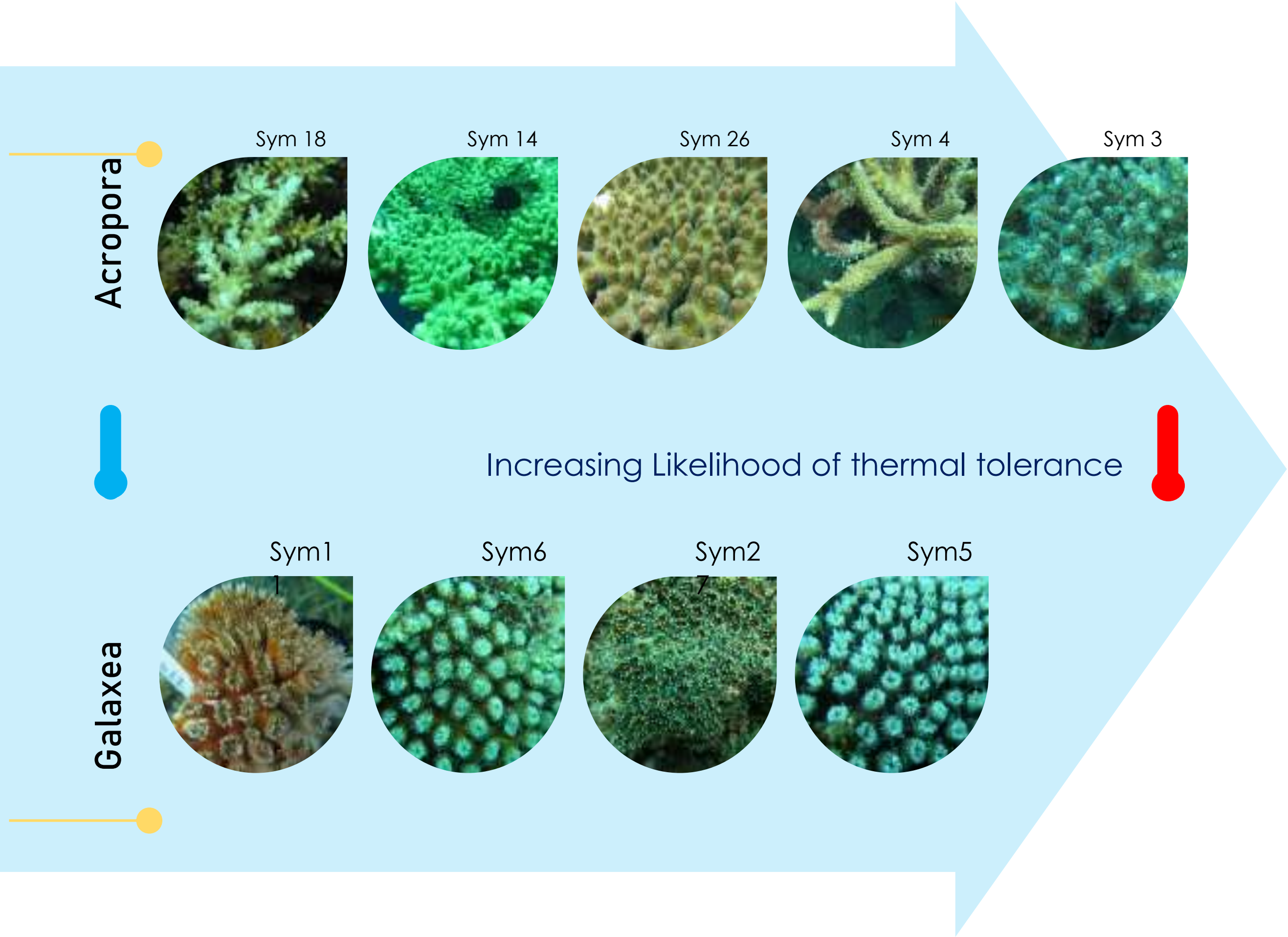
Synthesis



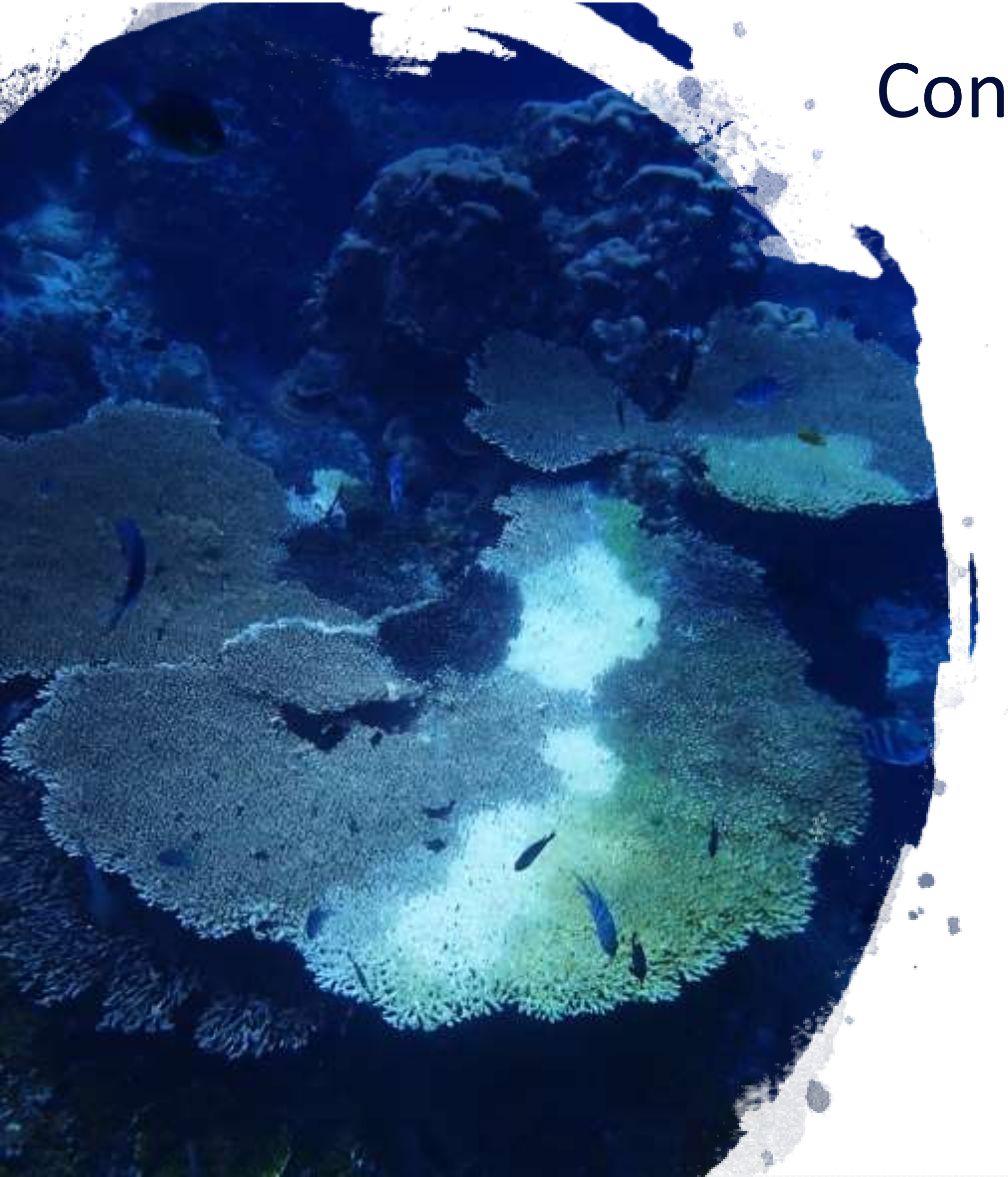
Numerous studies have shown that fast-growing branching coral species (e.g. *Acropora*, *Millepora*, *Pocillopora*) suffer higher bleaching mortality than slow-growing massive corals species (e.g. *Favites*, *Porites*, *Turbinaria*) (Marshall and Baird, 2000; McClanahan et al., 2004).

The resistance mechanisms of different coral species are not completely understood, but this study showed that many coral species with robust structure (massive) hosted mainly zooxanthellae community in the stress-tolerant D lineages.

Likelihood of thermal tolerance



Based on the results of the relative abundances of the thermally tolerant D lineage in our coral species, we proposed that zooxanthellae diversity to be considered as one of the suite of coral resilience indicators



Conclusion

Our understanding on the diversity of zooxanthellae in term of species- and functionality in Malaysian coral reefs has been severely limited by the lack of comprehensive description on their diversity.

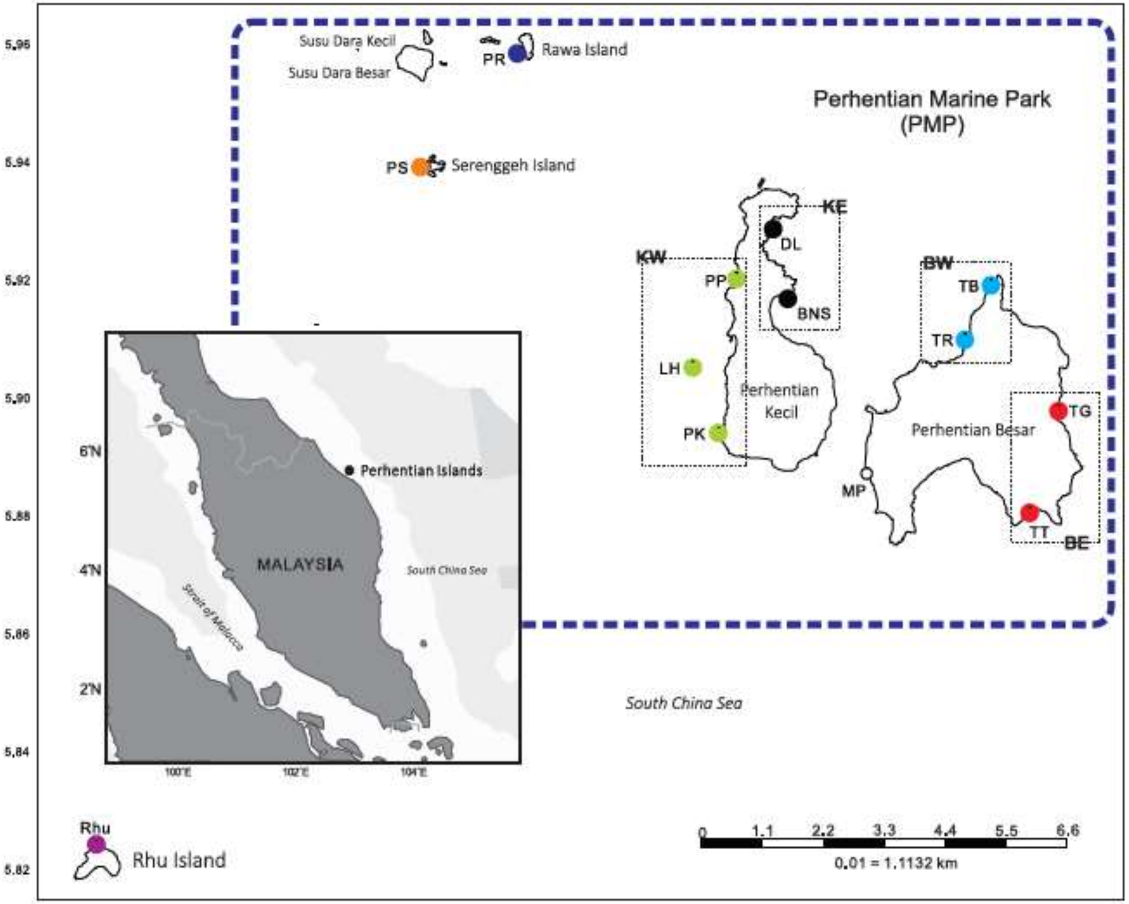
This study, by utilizing next-generation sequencing of ITS2 genetic marker, provides insights into the interspecific diversity of zooxanthellae and its community in various coral species of the Perhentian Island Marine Park.

The results obtained provide a comprehensive inventory of zooxanthellae diversity as a baseline assessment to study coral resilience in our coral reef ecosystem.

Part IV

Distribution, abundance and population genetic diversity of the giant clams, *Tridacna squamosa* and *T. maxima* (Cardiidae) in the Perhentian Islands Marine Park, Malaysia

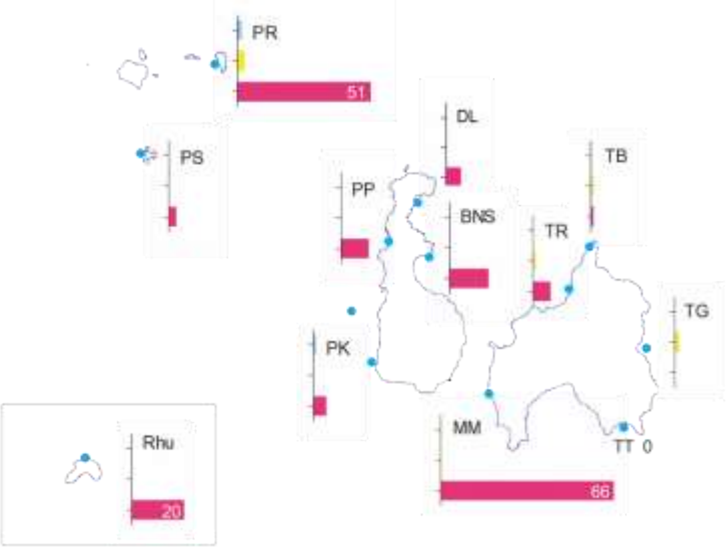
Chui Pin Leaw,
Li Keat Lee, Zhen Fei Lim, Kieng Soon Hii, Haifeng Gu, Mei Lin Neo, Po Teen Lim


5.96
5.94
5.92
5.90
5.88
5.86
5.84
5.82
Susu Dara Kecil
Susu Dara Besar
PR
Rawa Island
Perhentian Marine Park
(PMP)
PS
Serenggeh Island
KE
DL
KW
PP
BNS
BW
TB
TR
LH
Perhentian
Kecil
TG
Perhentian Besar
PK
MP
TT
BE
6'N
Perhentian Islands
MALAYSIA
South China Sea
4'N
Strait of Malacca
2'N
100'E
102'E
104'E
South China Sea
Rhu
Rhu Island
0
1.1
2.2
3.3
4.4
5.5
6.6
0.01 = 1.1132 km

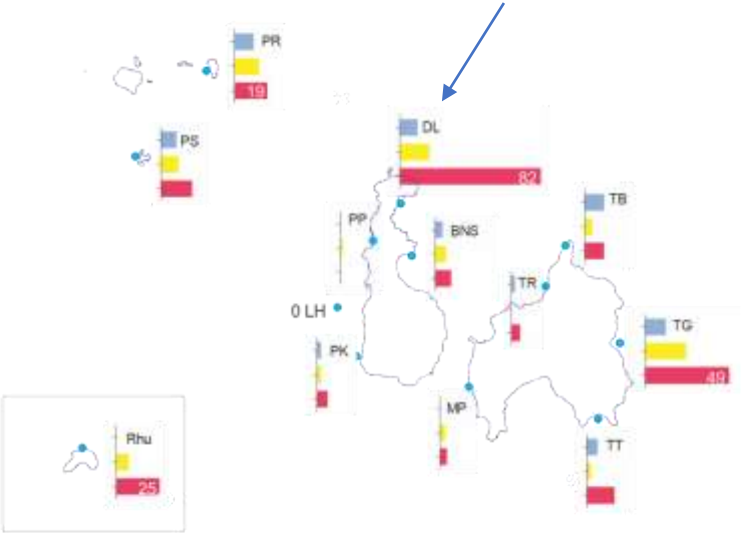
Laying Transect

ABUNDANCES OF T. SQUAMOSA and T. MAXIMA

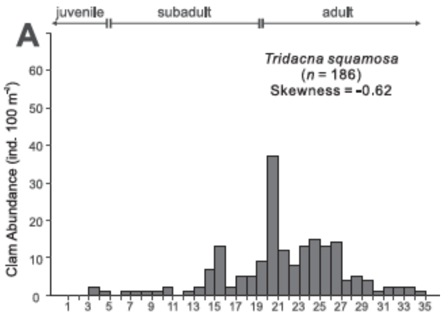
in three different size classes



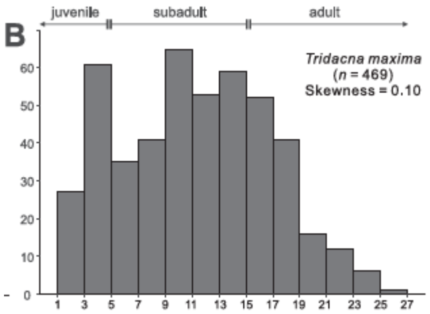
juveniles



1.62 ind. 100 m^{-2}

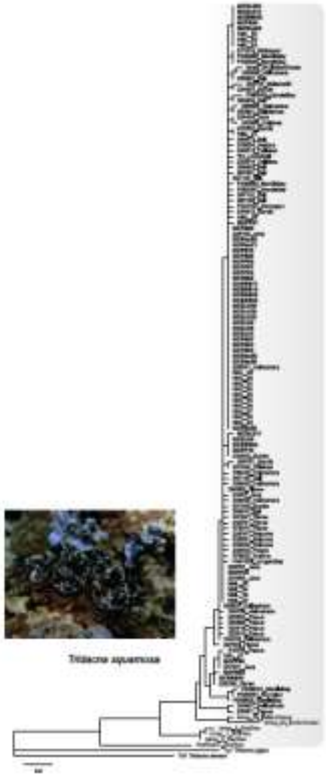


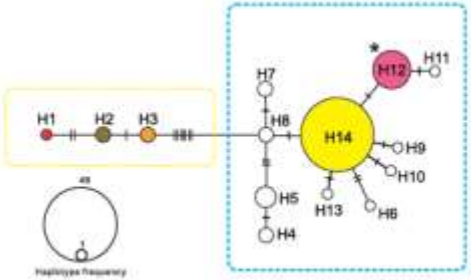
4.08 ind. 100 m^{-2}

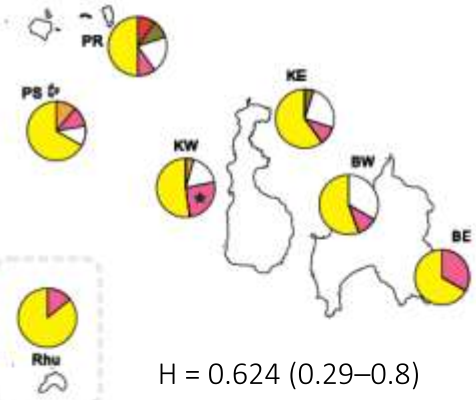


Lee et al., Marine Biodiversity, submitted

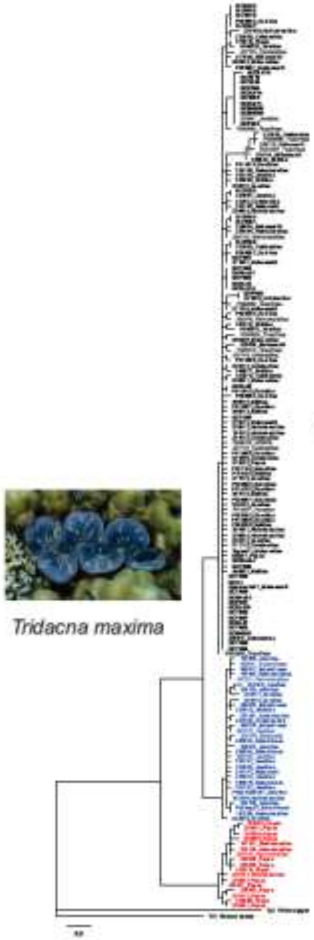
mt COI marker

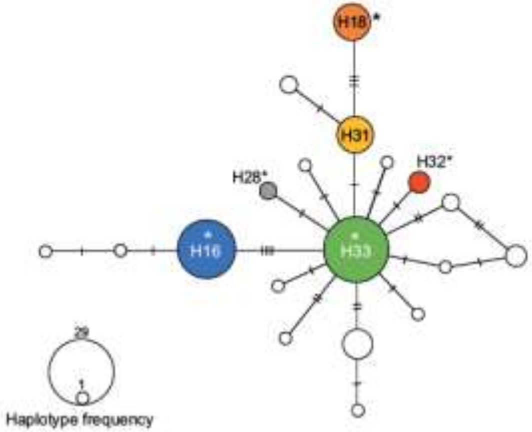

Tridacna squamosa



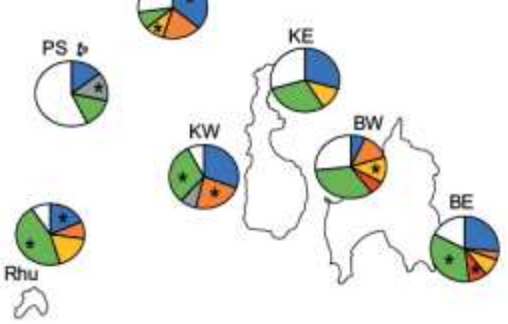


H = 0.624 (0.29–0.8)
π = 3.5% (1.4–21.2%)

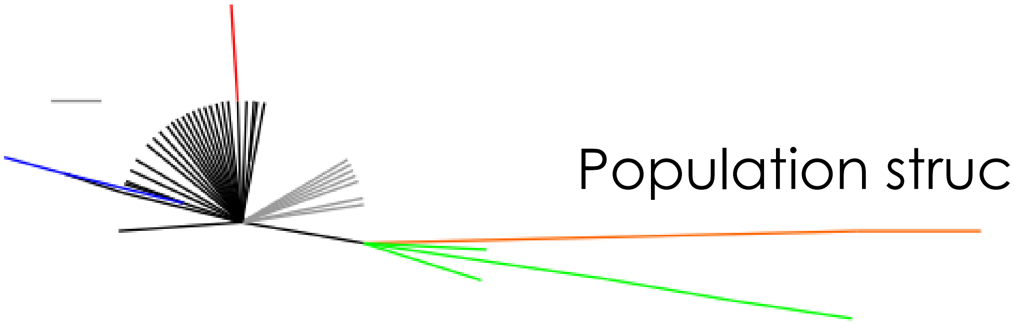

Tridacna maxima

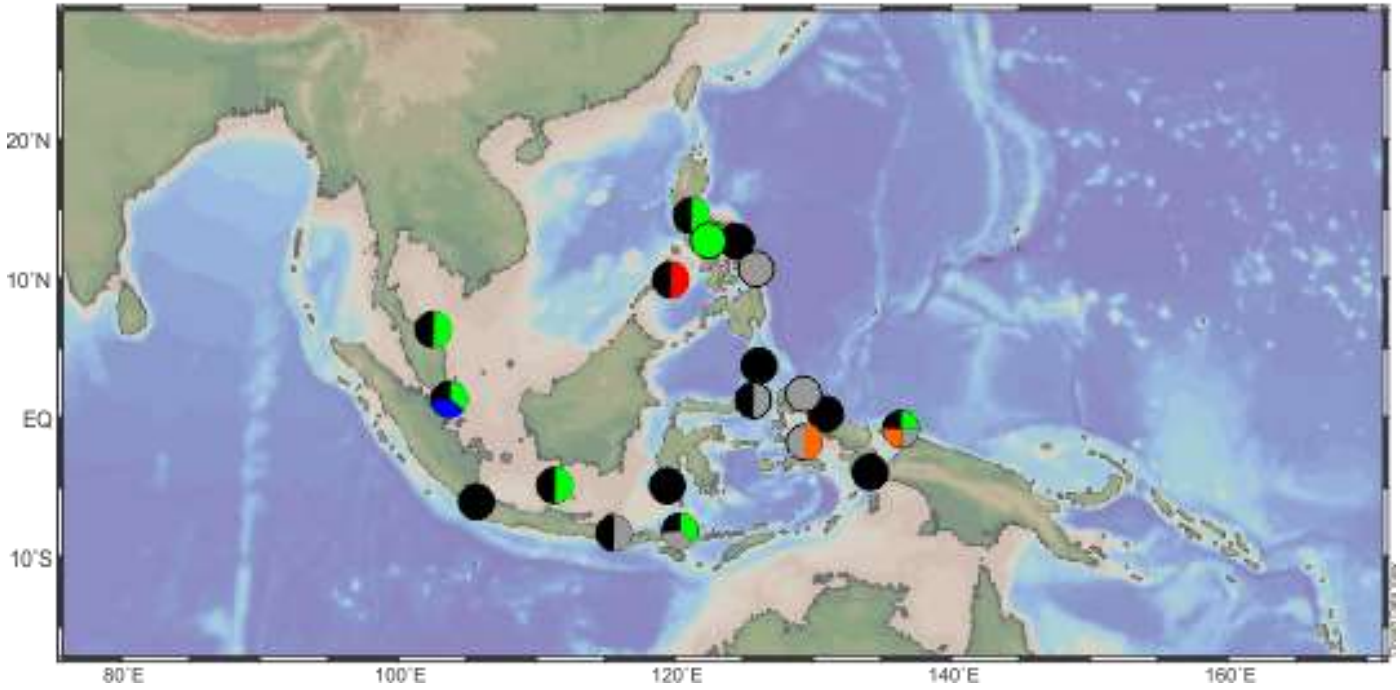


H = 0.842 (0.67–0.93)
π = 6.2% (8.9–17.9%)



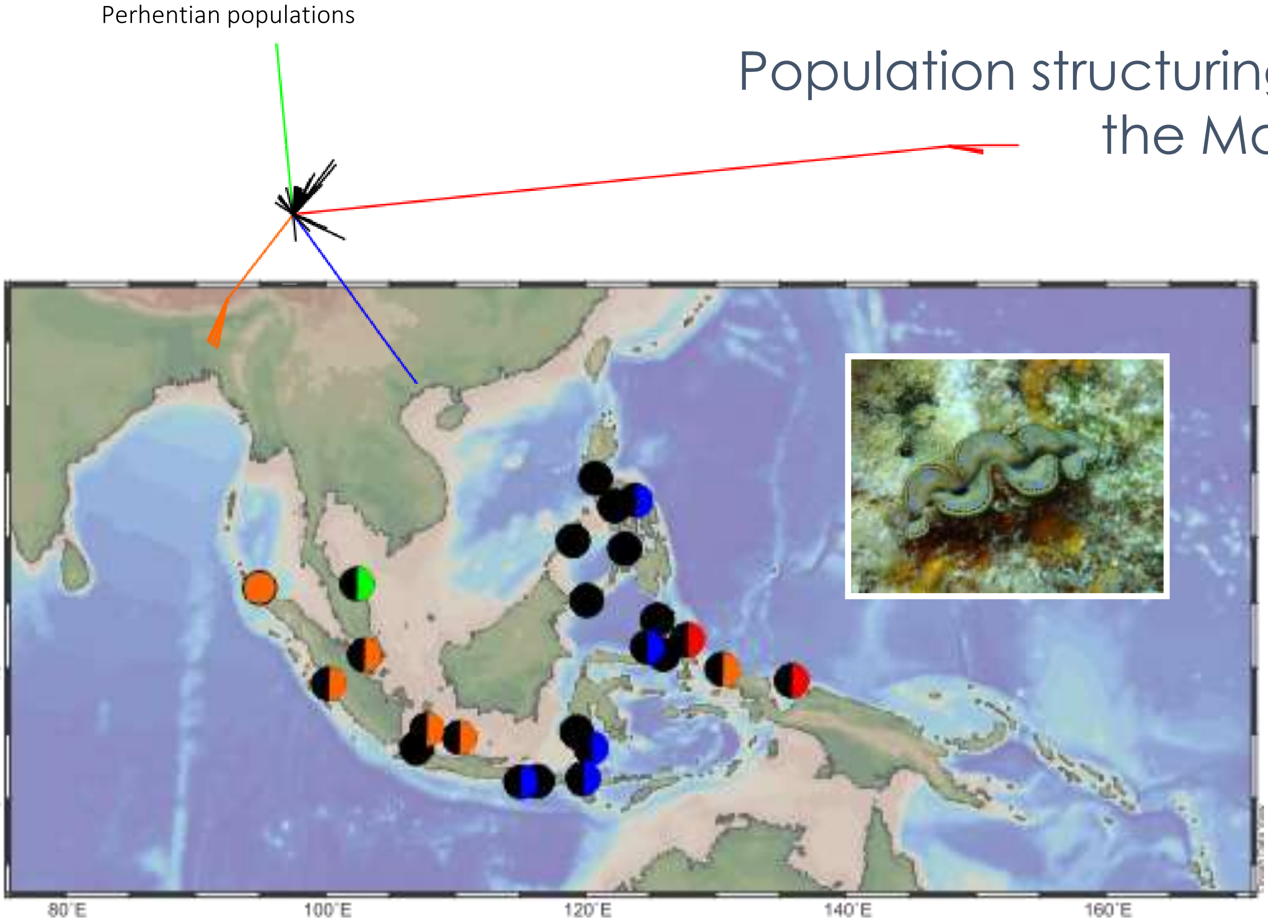
Population structuring of *T. squamosa* in the Malay Archipelago





Perhentian population shared a degree of genetic homogeneity with other populations from Philippines, Singapore and Indonesia.

Population structuring of *T. maxima* in the Malay Archipelago



Perhentian populations showed some degrees of genetic heterogeneity.

acknowledgements

This work was funded by Dept. of Marine Park Malaysia [GA003-2017] and MOE FRGS [FP058-2019A]

Conclusion

1. An update data inventory on the distribution and abundance of two giant clams in Perhentian Marine Park.
2. Insights into the population genetic connectivity of the two species of *Tridacna* of Malaysia.

Concluding remarks

- Molecular methods are high precision and powerful tools in assessing ecosystem health status of marine ecosystem (for short-term and long term) changes.
- These methods are useful, not only to assess ecosystem health and changes, but also an enabler wide geographical region observation and comparison.
- Standardization of methods (qualitatively and quantitatively) and better curated database will be critical to ensure the success of molecular approaches in biological observation and monitoring.