

Step-by-Step: Insights on key marine organisms associated with coral reef benthic cover using Stepwise Multivariate Regression

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Abstract. Coral reef ecosystems are severely threatened by human activities and climate change. However, the complexity of the ecosystem can complicate identification of significant ecological interactions between the organisms and the reef system. Thus, this study used a ten-year citizen science reef monitoring dataset by Reef Check Malaysia to identify key reef-associated organisms (fish and invertebrates) affected by the changes in benthic cover using Stepwise Multivariate Regression. Data collection started in 2013 to 2023, covering 148 sites from 23 islands of East Coast Peninsular Malaysia. The most dominant biotic benthic cover in coral reefs is the Hard Coral (mean $48.7 \pm 19.4\%$ median = 49.4%), followed by Rubble ($8.28 \pm 11.0\%$, 3.75%), and Nutrient Indicator Algae ($4.96 \pm 9.15\%$, 1.25%). Stepwise Multivariate Regressions showed that 6 out of 9 keystone fishes and 4 out of 8 keystone invertebrates were significantly correlated with each individual benthic cover. The regression models indicated that Butterflyfish, Grouper, Sweetlips, Snappers, *Diadema* Urchins are susceptible to the deterioration of coral reefs (reduction of Hard Coral and increase of Rubble and Nutrient Indicating Algae), while Sea Cucumber, Giant Clam and Pencil Urchin may flourish. Henceforth, the shortlisted reef-associated organisms can be an accessible and cost-effective targeted organisms for a quick and simple assessment of coral reef health.

1 Introduction

Coral reefs are very complex, sensitive and important ecosystems that provide invaluable ecosystem services to humans [1-3]. Due to the rich biological diversity of coral reefs, it can be challenging to elucidate the relationships among and between key components of the coral reef [1, 2]. Nevertheless, understanding the keystone or important relationships amongst the

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reef associated organisms and their dependency on the reef habitat is crucial in gauging and managing the health of the ecosystem [3, 4].

The significance of such studies cannot be overstated given the increasing rate of anthropogenic pressures, especially from climate change, are accelerating the deterioration of both healthy and degraded coral reef ecosystem [5, 6]. Furthermore, such disturbances are not localized and can spread globally if they are not rapidly addressed or mitigated soon [7]. There is a need of collaborative effort across different sectors is needed to monitor and report the ecological changes in coral reefs resulting from disturbances [8]. Thus, Reef Check Malaysia has been trying to fill the gap since 2007 with citizen science, by means to raise awareness of standardized reporting, data collection and data sharing with the involvement of the public [9]. In their annual reports, important reef associated categories were broken into 3 parts: benthic cover with biotic (living things) and abiotic (non-living things) categories, keystone fishing and invertebrate counts.

Hence, this research aimed to use the long-term monitoring programme of Reef Check Malaysia to identify the keystone reef associated organisms (Fish and Invertebrates) and their complex relationships with dominant reef benthic covers. The rapid modelling process could identify organisms that can be proposed as indicator species for cost-effective monitoring of local reef conditions.

2 Method and Materials

Surveys of coral reef benthic and associated organisms were conducted between 2013 to 2023 at six island archipelagos of East Coast Peninsular Malaysia (Figure 1), under the coordination of Reef Check Malaysia [9]. There were in total 23 islands monitored from the island archipelagos with 185 sites: Bidong/Yu (number of sites = 16), Kapas (5), Perhentian (10), Redang (19), Tenggol (7) and Tioman (128).

The monitoring methods adhered to the standard provided by Reef Check Malaysia [9] via either Point Intersect Transect (PIT) or Belt Transect. A 100 m transect line was placed along a constant depth (± 2 m) along the coral reefs. Surveyors would record the benthic categories found below the transect at every 1 cm (PIT method). The PIT would be conducted for a 20m sub-transect with a 5m gap between the next sub-transect. In total, there were 4 sub-transects per 100 m transect. For fish count, the types and numbers of fishes encountered within an approximately 5m (H) x5m (W) area would be recorded when surveyors swam along the 100m Belt transect. Finally, surveyors would record the categories and count the numbers of invertebrates found on the surface of 5m belt (2.5m to the left and right side) along the 100m transect. The categories of Benthic, Fish and Invertebrates were based on Reef Check Standards [9].

The survey and monitoring data were compiled into three datasets: Benthic (benthic cover percentage, %), Fish (count), and Invertebrates (count). In total, there were 1012 data points covering 185 sites from 23 islands. The sites were further grouped based on depth categories: < 5m (n=294), 5-7.5m (419), 7.5-10m (259), and > 10m (40).

The survey data were imported into the R Programming language [10] for further analyses, using *vegan* (Oksanen et al., 2012) and *StepReg* [11] packages. Due to the large variations of fish and invertebrate counts, Fish and Invertebrate datasets were log10 transformed (by adding a constant of 0.0000001 to remove the zero inflated data). To test the

variation in benthic and biotic (Fish, Invertebrates) composition based on factors (Year, Depth and Islands), Permutative ANOVA (PERMANOVA) [12] and subsequent post-hoc (pairwise.adonis) [13] tests were conducted. Similarity Percentage (SIMPER) analysis was conducted on the Benthic cover dataset to examine the drivers of differences among the Years.

This is followed by isolating important coral reef associated organisms (Fish and Invertebrates) which drive the changes among principal benthic cover of the reefs. Stepwise Multivariate Regression analyses with corrected Akaike Information Criterion (AICc) were performed on selected biotic benthic categories (HC, NIA, RB) with Fish and Invertebrate datasets to trim down significant keystone reef-associated organisms. Six lean-down regression model (linear) were constructed based on the stepwise regression model selection.

All statistics were conducted based on the alpha value of $\alpha < 0.05$.

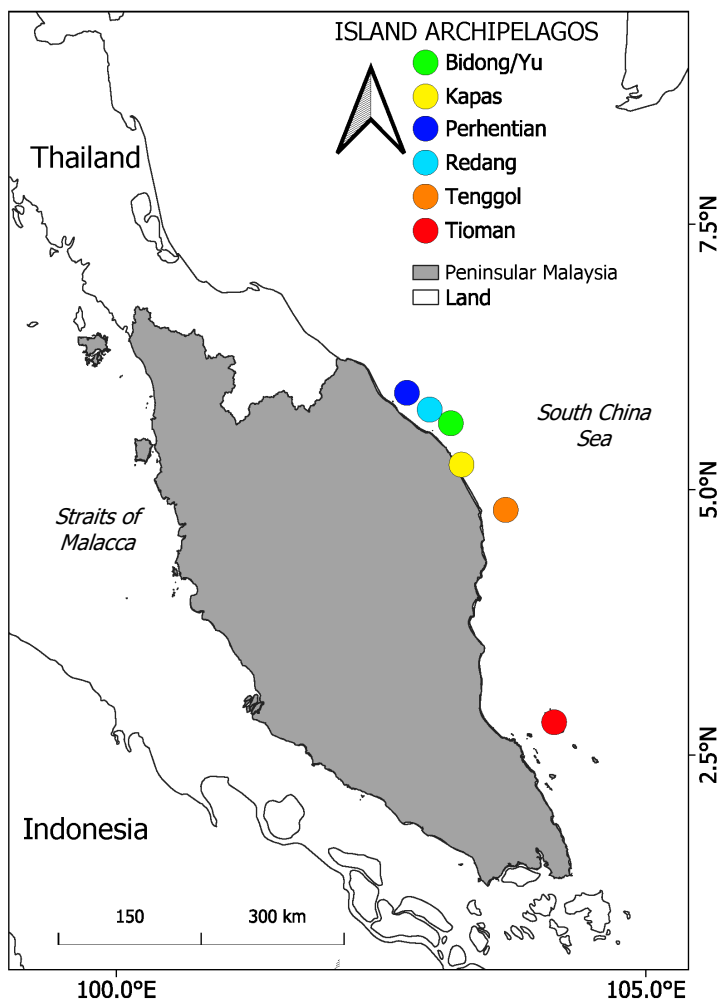


Figure 1. Location map of island archipelagos at the Peninsular Malaysia (coloured circle) monitored by Reef Check Malaysia between 2013-2018.

3 Results

For the Benthic percentage cover, the most dominant biotic benthic cover in coral reefs was the Hard Coral (mean $48.7 \pm 19.4\%$ median = 49.4%), Rubble ($8.28 \pm 11.0\%$, 3.75%), followed by Nutrient Indicator Algae ($4.96 \pm 9.15\%$, 1.25%). The abiotic cover of the coral reefs was dominated by Rock ($21.3 \pm 12.1\%$, 20%) and Sand ($5.81 \pm 7.18\%$, 3.13%). For the Fish count, only Butterflyfish (5.91 ± 4.18 , 5) and Parrotfish (2.95 ± 3.59 , 1.75) were recorded with more than one individual per reef. Lastly, Invertebrates count showed that *Diadema* urchin (40.7 ± 71 , 3.75) was the most common invertebrates in the study period.

Based on the PERMANOVA analysis (Table 1), significant differences in Benthic cover, Fish count and Invertebrates count were seen among Years (PERMANOVA: $df = 10$, $R^2 > 0.029$, $p < 0.001$), Depth ($df = 3$, $R^2 > 0.006$, $p < 0.002$), and Islands ($df = 22$, $R^2 > 0.142$, $p < 0.001$). For two-way interaction of factors, Benthic cover showed no significant difference in composition changes, i.e., consistent across Depths throughout the Years ($df = 10$, $R^2 = 0.006$, $p = 0.873$); while Invertebrate counts showed significant composition changes for Depth ($df = 10$, $R^2 = 0.006$, $p = 0.829$) and Islands ($df = 105$, $R^2 = 0.059$, $p = 1.000$) throughout the Years. The three-way interaction of factors on composition of Benthic, Fish and Invertebrates showed no significant differences. This showed that there were significant variations of Benthic, Fish, and Invertebrate compositions within each factor, but consistent in factors interactions, that would cover various reef associated organisms' interactions.

For changes in individual components of benthic cover (Figure 2A, SIMPER), the dominant benthic biotic cover, Hard Coral, had the highest coverage at 2015 (median = 55%), but reduced to 40.313% by 2020, and recovered back to 50% by 2023. Nutrient Indicator Algae on the other hand, showed the lowest coverage in 2014 (median = 0%), but reached peak at around 2020 (9.375 %) and had been declining to almost normal in 2023. Rubble cover indicated the highest record in 2017 (median = 7.188%) but had been decreasing to its lowest until 2022 (3.125%), and later saw an increase to 5 % (median) in 2023. Both Fish and Invertebrates datasets changes by year was mainly driven by the dominant organisms (Butterflyfish, Parrotfish, *Diadema* urchin).

Hard Coral (HC), Rubble (RB) and Nutrient Indicator Algae (NIA) were selected out from Benthic cover as important biotic (living) coral reef component. Stepwise multivariate regressions showed that six out of nine keystone fishes and four out of eight keystone invertebrates were significantly correlated with each individual benthic cover. The regression models (Table 2) showed that Butterflyfish, Grouper, Sweetlips, Snappers, *Diadema* Urchins were susceptible to the deterioration of coral reefs (reduction of HC and increase of RB and NIA). On the other hand, Parrotfish, Moray Eels, Sea Cucumber, Giant Clam and Pencil Urchin showed higher densities with increasing levels of either Nutrient Indicator Algae or Rubble.

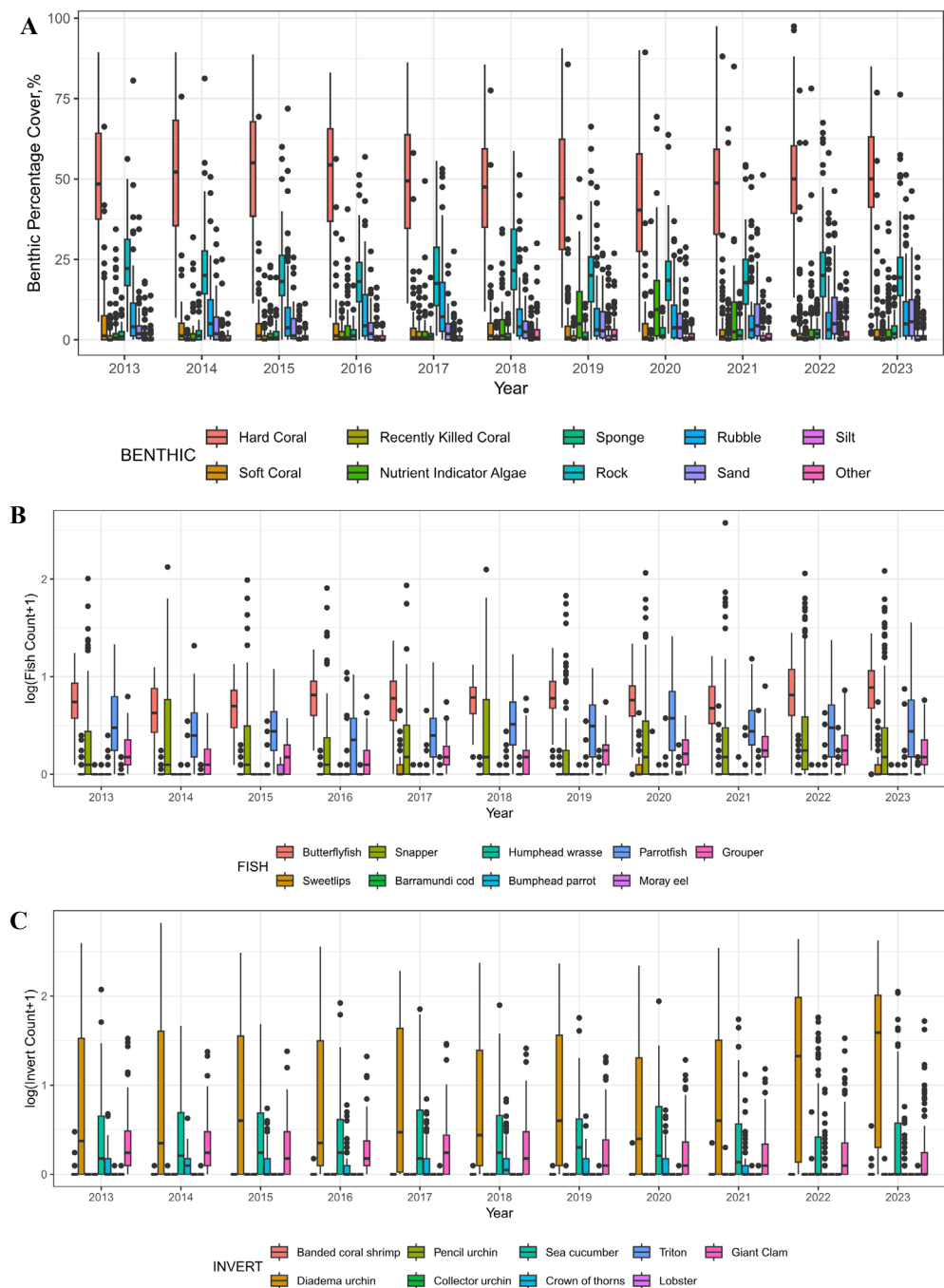


Figure 2. Boxplot of major categories for coral reef associated (A) Benthic cover, (B) Fish ($\log_{10} [\text{count}]+1$ transformation), and (C) Invertebrate ($\log_{10} [\text{count}]+1$ transformation), between 2013 to 2023.

Table 1. Permutative ANOVA (PERMANOVA) output of Benthic, Fish and Invertebrates datasets amongst and within the factors Year, Depth, Islands.

FACTORS	Benthic				Fish				Invertebrates				
	Df	ΣX ²	R ²	F	p	ΣX ²	R ²	F	p	ΣX ²	R ²	F	p
Year	10	3.232	0.037	4.528	0.001	4.958	0.029	3.613	0.001	10.78	0.032	4.494	0.001
Depth	3	1.106	0.013	15.497	0.001	1.032	0.006	7.519	0.002	5.03	0.015	20.966	0.001
Islands	22	12.531	0.142	7.981	0.001	25.746	0.149	8.528	0.001	88.78	0.262	16.825	0.001
Year:Depth	10	0.544	0.006	0.763	0.873	1.911	0.011	1.392	0.024	1.96	0.006	0.819	0.829
Year:Islands	105	9.014	0.102	1.203	0.005	21.243	0.123	1.474	0.001	19.94	0.059	0.792	1.000
Depth:Islands	20	3.989	0.045	2.795	0.001	4.739	0.027	1.727	0.001	14.57	0.043	3.038	0.001
Year:Depth:Islands	100	4.938	0.056	0.692	1.000	11.375	0.066	0.829	0.993	19.65	0.058	0.819	1.000
Residual	741	53.027	0.600			101.965	0.590			178.21	0.526		
Total	1011	88.381	1.000			172.969	1.000			338.92	1.000		

Table 2. Stepwise Multivariate Regression of Benthic Covers (Hard Corals: HC, Nutrient Indicating Algae: NIA, Rubble: RB) with Reef-Associated Organisms (Fish and Invertebrates). All values had significant regression values with the individual benthic covers.

Benthic Covers	Reef-Associated Organisms	Stepwise Multivariate Regression Model (p < 0.05)
HC	Fish	1.309Bf - 0.662Pf + 2.574Gp - 2.574 Me + 40.902
HC	Invertebrates	-0.288SC - 0.335GC + 0.019DU + 49.814
RB	Fish	0.392Pf - 0.242Bf + 5.541Me - 2.157Sw - 0.036Sn + 8.736
RB	Invertebrates	0.392SC + 30.813PU + 0.256GC + 6.104
NIA	Fish	-0.333Bf + 0.227Pf + 6.307
NIA	Invertebrates	-0.024DU + 5.946

Fish: Bf = Butterflyfish, Pf = Parrotfish, Gp = Grouper, Me = Moray eel, Sw = Sweetlips, Sn = Snapper
 Invertebrates: SC = Sea Cucumber, GC = Giant Clam, DU = *Diadema* Urchin, PU = Pencil Urchin

4 Discussions

The main Benthic biotic components that exhibited changes with Year, Islands, and Depth at the coral reefs of East Coast Peninsular Malaysia were Hard Coral, Nutrient Indicator Algae, and Rubble. To reduce the complexity of comparisons, only biotic benthic cover categories were used in the Stepwise study to observe biological interactions. When examining the reef-associated organisms, the commonly recorded Butterflyfish and Parrotfish appear to be dominant driver of changes for all three targeted Benthic cover. On the other hand, *Diadema* urchin showed association with Hard Coral and Nutrient Indicator Algae, but not with Rubble.

Hard Coral is the main component of coral reefs, as they are the foundation of the coral reef ecosystem [3, 4, 14]. For the surveyed reefs, the Hard Coral cover of most sites were in Moderate (25-50%) to Good (50-75%) condition, with some reached as low as 1.25% (Bad) to 97.5% (Excellent) coverage [15]. This showed that the Reef Check Malaysia sampling effort was useful to assess the condition of the reefs. Thus, supporting the variation of coral reef cover in the study area, suited to establish the driver and influencers of major reef associated organisms [16].

Moreover, timeline data showed that Hard Coral cover was reduced probably due to major disturbance events, ecosystem shifts of dominant benthic cover would occur. Previous studies [17, 18] indicated during such shifts, other biotic organisms would replace the open space left by Hard Coral, such as algae, zoantharians, soft corals, and live rocks such as rubbles. Some of these shifts were merely a transitional stage of coral reef recovery [19], while others serve as permanent predominant benthic cover after the reduction of Hard Coral cover [20]. This could in turn affect the resilience of the reef ecosystem.

Nutrient Indicating Algae was one of the most common ecosystem shift colonists post disturbance events, natural or human caused, in coral reefs [20]. The algae do not just compete with the keystone coral for open spaces; they further over grow and choke the corals from resources, making them hard to be removed without aid after colonization. Furthermore, they also indicate underlying cause of reef deterioration [20, 21]. Thus, showing a less than desirable condition for the coral reef recovery when algae are present in high densities.

Another sign of coral reef ecosystem shift is the presence of large number of live rocks or coral rubbles (Rubble). This metric is more reliable in determining the long-term disturbances as compared to another principal Benthic cover, Recently Killed Coral [22]. Furthermore, coral rubbles have also been shown to loosely aggregate, thus making it hard for settling new corals to survive on the unstable substrate[23]. Hence, other more resilient covers, such as Nutrient Indicator Algae, can dominate the benthic cover in the long-term. Thus, Rubbles are indicator of coral reef recovery success post disturbances.

Parrotfish and Butterflyfish are the key reef Fish that displayed strong associations with changes in principal Benthic cover (Hard Coral, Nutrient Indicator Algae, and Rubbles). Parrotfish are mostly herbivores and are crucial in coral reefs as algae grazers [24]. This suggests that Parrotfish are keystone species in controlling the cover of Nutrient Indicator Algae, which spatially competes with the Hard Coral in reefs [25]. On the other hand, the complexity of Hard Corals provides conducive environment for reef associated fishes, such as Butterflyfish, to thrive. These are keystone reef associated organisms which often found high in abundance in complex reef, thus making them often the indicator species for healthy reefs [26].

For Invertebrates, the organisms with the most consistent association with principal Benthic cover is the *Diadema* urchin. These urchins are known to be herbivorous and grazing on turf algae, keeping the algae cover in check [27]. Thus, the results showed that the *Diadema* urchin had inverse correlation with Nutrient Indicator Algae, with turf algae was part of it [27, 28]. Nevertheless, it should note that high density of *Diadema* urchin might indicate a disruption of coral reef ecosystem equilibrium. Thus, it is important to keep *Diadema* populations in check.

This exercise has showed that of among keystone reef associated organisms surveyed by Reef Check protocols [9], the identified organisms were either help in controlling the coral reef equilibrium, which are useful as indicator of healthy reefs. Thus, the wider public, especially diver communities, could adopt the survey of these organisms as initial and quick assessment of reef health. While this approach may not be the most accurate indicator of reef health condition, a preliminary report could help for reporting purposes to the authorities. Furthermore, the simplified three keystone species (Parrotfish, Butterflyfish and *Diadema* urchin) are common reef associated organisms which the public can easily identify. This in turn can make the effort for public reef monitoring streamlined and simplified.

The results of the principal benthic cover with other reef associated organisms are crucial in determine the resilience of the sensitive coral reef ecosystem [29]. Recent climate change related events, such as heatwave induced mass bleaching, and unpredictable weather causing typhoons, have threaten the health of coral reefs around Malaysia [30, 31]. Studies [26, 27,28] have shown that the presence of those keystone groups of organisms may help in increasing the resilience of the coral reefs; this study has provided empirical evidence to support their importance. However, organisms such as Parrotfish, Groupers, *Diadema* urchin, Sea cucumber, Sweetlips and Snappers are commercially important marine commodities [32]. Hence, local authorities have been monitoring the exploitation of these groups, but improvements can be done for the system. This study has shown that Parrotfish, Butterflyfish, and *Diadema* urchins should be prioritized as the top coral reef resilience indicators.

5 Conclusion

These results demonstrate the possibility to show multivariate interaction of keystone associated coral reef organisms with major benthic cover of coral reefs in Malaysia. Further analyses with more comprehensive machine learning approach should be undertaken with analytics such as Canonical Correlation Analyses to elucidate the complex spatio temporal interactions that will help to inform better spatial management of these valuable ecosystems.

Future studies could revisit this dataset to identify patterns with individual localities or islands. This could help in identifying the ecological importance of the identified keystone reef-associated organisms in this study. Thus, could also expand to other non-listed organisms in coral reefs.

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