

Assessing Fish Herbivore Functions on Benthic Composition in the Wakatobi Marine Park, Indonesia

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Postgraduate Certificate in Ecological Survey Techniques

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Photo taken by the author

Abstract

Herbivorous fish are important to the coral reef ecosystem, bridging primary producers and upper trophic consumers. Their complementary functions (browsing, grazing, scraping and excavating) can limit algae, preventing it from shading corals from photosynthesis and opening up spaces for coral recruitment.

However, the debate on top-down versus bottom-up control of algae has led to disagreement on herbivorous fish protective measures and enforcement within the Indonesia government. This research aimed to explore herbivore functions and link these to patterns in benthic community composition, so to explore the contribution of herbivorous fish. Surveys were carried out at the Kaledupa region, Wakatobi Marine National Park, Indonesia. Diver-Operated Stereo-Video Counting was employed to film 3 x 50m transects (at each of 3 depth zones) for four sites.

Algae percentage cover was found to be low at all sites, but grazers/detritivores group were found different and higher in the heavily fished site. Grazing functions seemed obvious. However, no significant correlation was found between herbivorous fish and algae. It is recommended that surveying be included on more sites. Notably, the functional length categories showed absence of fish ≤ 7.5 cm (fry stage) in 4 functional groups (except farmer), this was an alarming news of losing the species, hence, Wakatobi government is recommended to enforce protection on these length categories, by restricting the mesh size, prohibiting of certain type of gears and even having fishing closed seasons (Ahamadia et al., 2013; Comeros-Raynal et al., 2012).

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1. Introduction

Herbivorous fish functional contributions have long been recognized. They play a key role in controlling algae from dominating coral reefs systems (Lewis 1986, Lirman 2001, Williams and Polunin 2001, Mumby et al., 2006, 2007, Hughes et al., 2007) and directly facilitate reef-building taxa (for instance crustose coralline algae). However, many coral reefs worldwide are now undergoing non-selective overfishing, and because this reduces herbivore functions a great deal, it subsequently allows reefs to degrade from biodiverse coral to an inhospitable algal state (Bellwood et al., 2004; Bruno et al., 2009; Cheal et al., 2010; Hughes et al., 2010; Mcmanus and Polsenberg, 2004; Rogers and Miler, 2006).

In the Kaledupa region of Wakatobi Marine Park of Sulawesi, Indonesia (Figure 1), fishermen in the region have employed destructive, non-selective fishing methods (Figure 2) in attempting to compensate for the declining harvest of reef fish (Exton 2011). This negative loop caused local fishermen to fish more frequently, farther away and halved mesh size (Exton 2011). This subsequently caused fish being harvested before sexual maturity and became difficult to replenish (Operation Wallacea, unpublished data).

Subsequently, this might open up more opportunities for algae taking over coral from dominating reef systems (Edwards et al, 2014). This was a part of monitoring study looking into the state of herbivorous fish and benthic composition and to determine any correlation

between them, in order to recommend key herbivore species and related issues for the Wakatobi government as advice on setting policy and enforcement to conserve herbivorous fish (Pope, Lochmann & Young 2010).

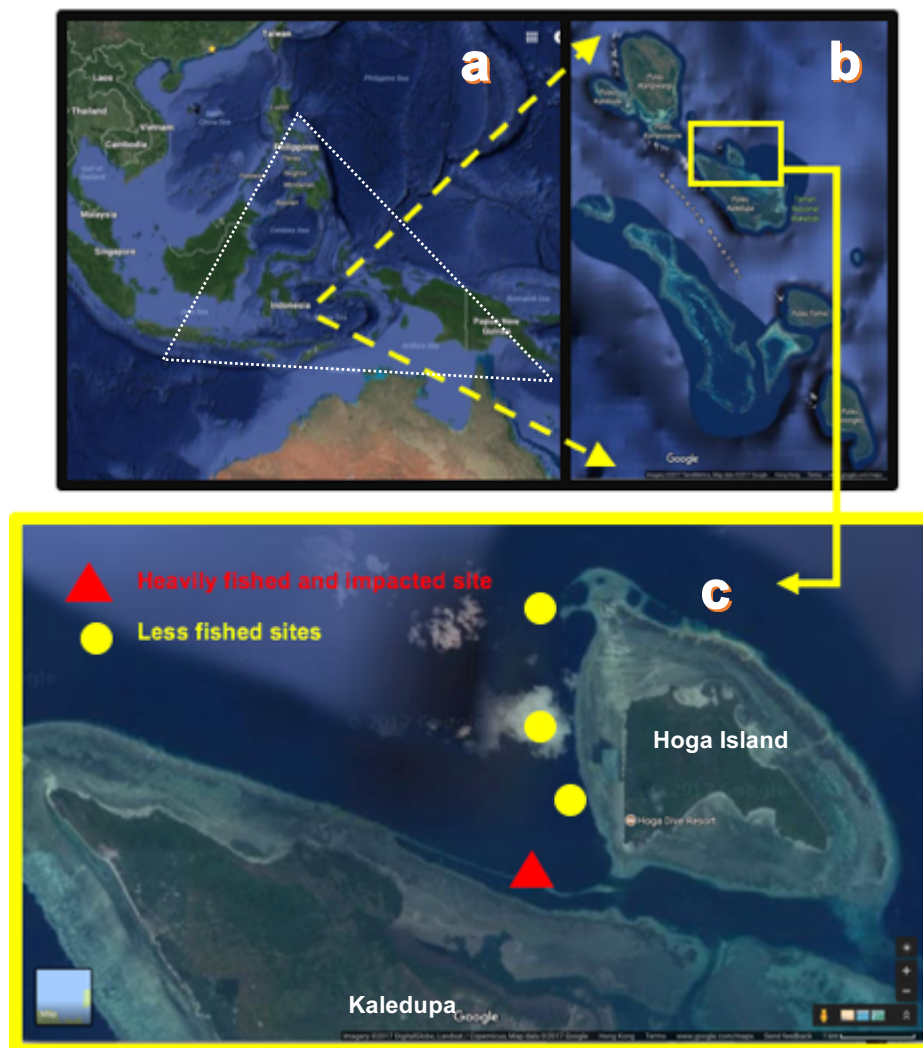


Figure 1. Study locations. a) Indonesia is inside the Coral Triangle b) Zoom in view of Wakatobi Marine Park, Southeast Sulawesi of Indonesia. C) 4 study sites are situated between Hoga Island and Kaledupa.

Adapted from Google map 2017.



Figure 2. Fish fences are widely used around study sites. They are highly unselective, designed to catch fish as the tide recedes. (Biodiversity science 2011)



a

Figure 3. Heavily fished and impacted sites – Samepla

a) Sandy substrate
b) Dead hard coral with macroalgae
Photo taken by author



b

1.1 Literature Review

Herbivorous fishes are an important part of the coral reef ecosystem. They transport nutrients from primary producers to upper trophic levels (Cornell University, 2017).

Herbivorous fish have complementary functions, like browsing, grazing, scraping and excavating (Table 1). Since not all herbivores consume the same types of algae, at the same rates or in the same way, functional diversity within the guild should be considered and has been shown to increase coral resilience following bleaching events (IUCN 2009). This is due to by their special teeth and jaw morphology (Figure 3) (Nanami 2016)- they easily crop down macroalgae stipe, remove holdfast, and graze turf algae (Figure 4). Like browsers functional group they feed on macroalgae, they could limit the extraordinarily fast growers, preventing them from shading corals from photosynthesis and open up spaces (Cheal, Emslie et al. 2013; Fox & Bellwood 2013) for slow growing hermatypic corals recruitment and reproduction, therefore herbivorous fish are important in helping to maintain the balance between macroalgae and corals. Some of them consume seaweeds that produce allelopathic chemicals that cause coral mortality (Cornell University, 2017).

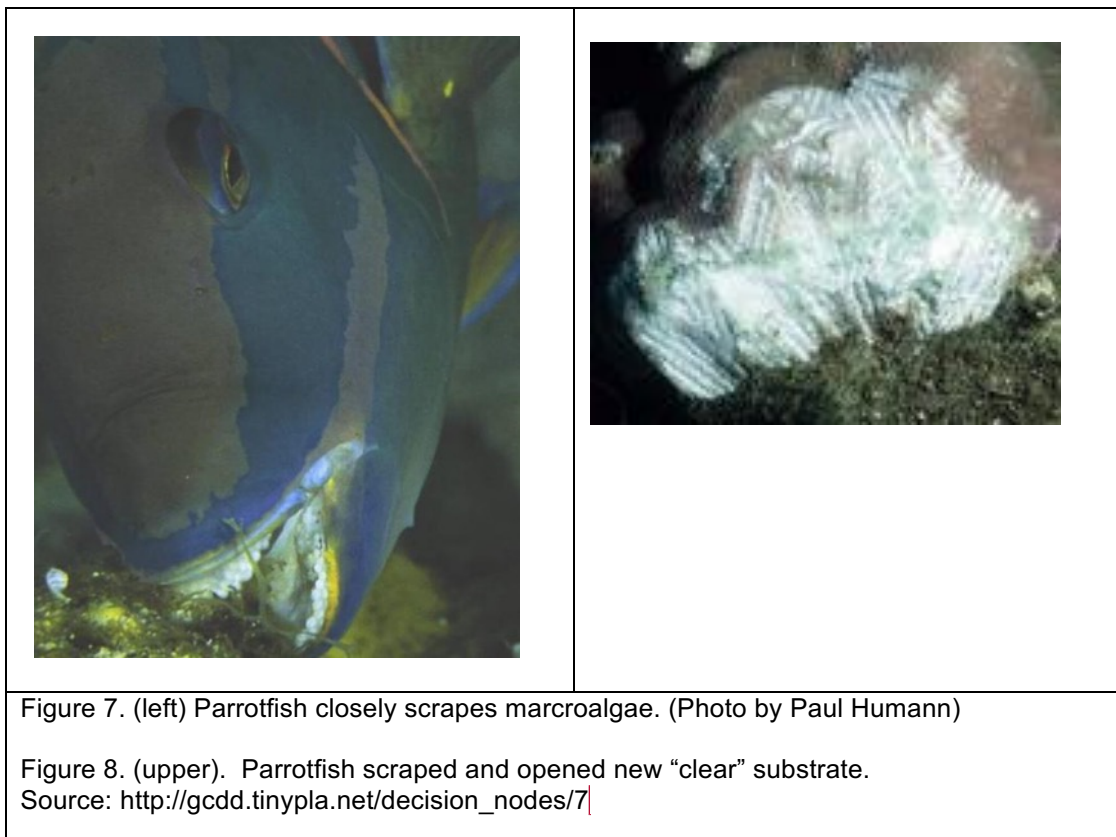
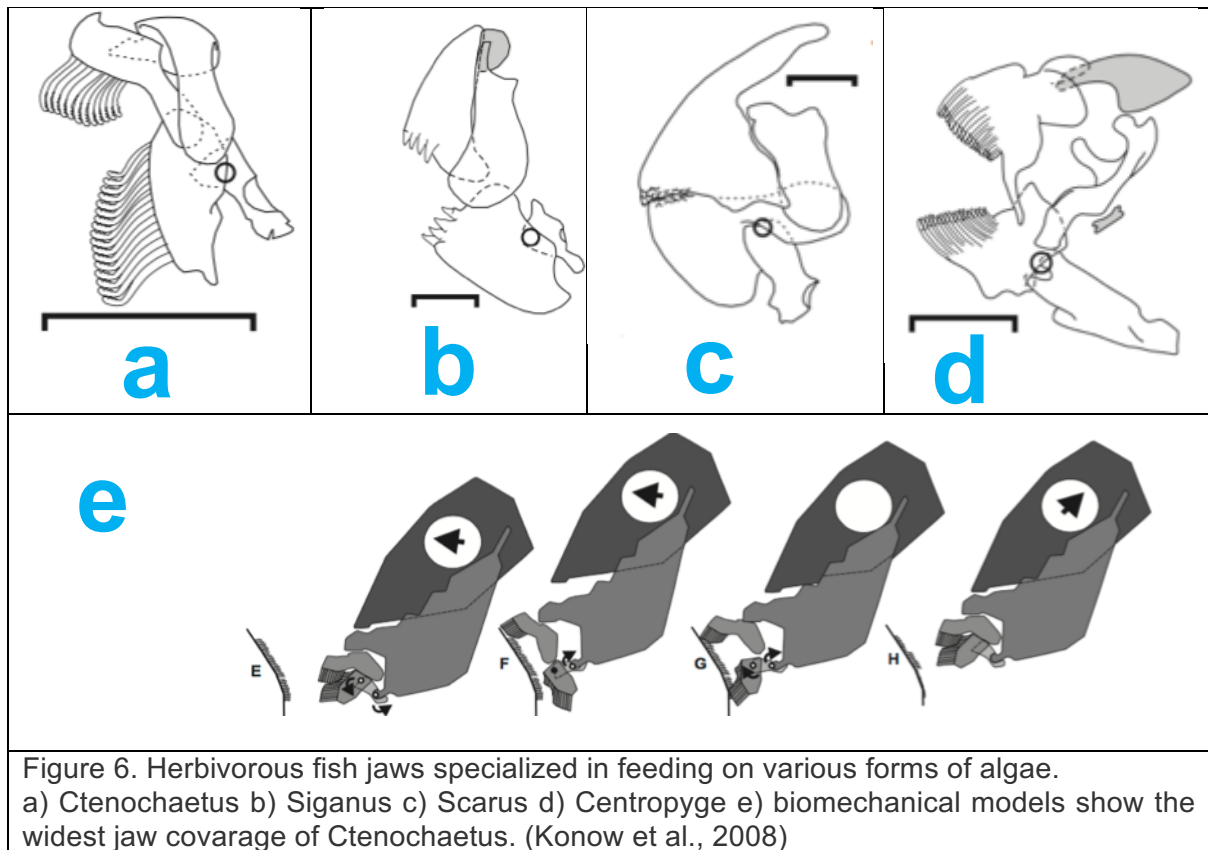


Figure 4. (left) Surgeon fish bites on algae

Figure 5. (below) turf algae occurred species between coral.

Photos taken by author





Hermatypic coral (hard coral) is usually called as ecosystem engineer because it in turn, creates shelters for fishes, increasing the complexity and subsequently the biodiversity of the reef ecosystem, and ultimately maintaining food security for humans.

But, if the reefs lack the appropriate number of herbivorous fish, their controlling functions would be weakened, which may lead to macroalgae overgrowth (Helfman 2009). Caribbean and Jamaican coral reefs are well-known cases; they alerted us to stop turning a rich and biodiverse ecosystem into a barren and infertile state (Aronson and Precht, 2001; Gardner et al., 2003; Hughes, 1994; Mumby et al., 2007; Mumby 2009).

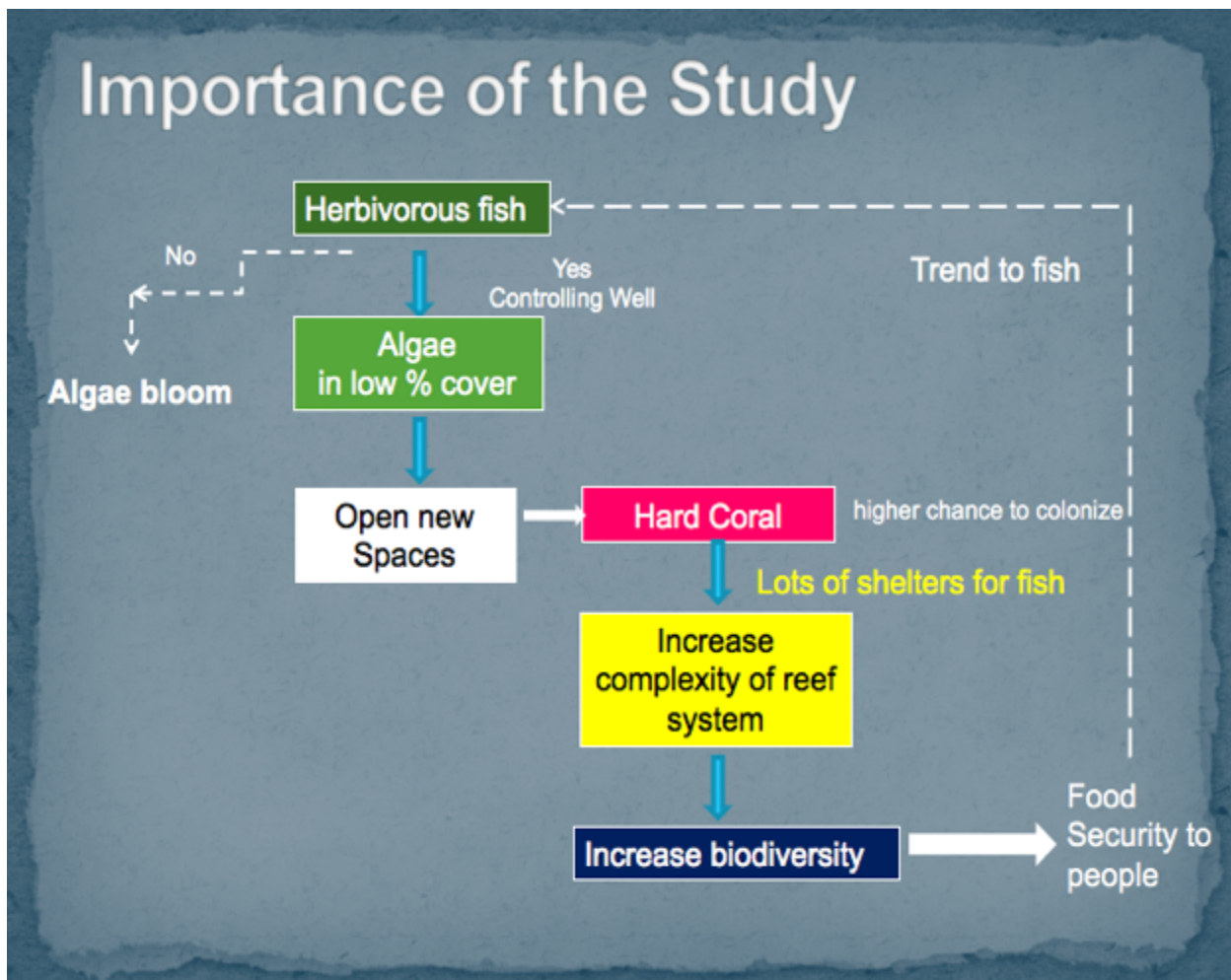


Figure 9. Consequence of with or without herbivorous functions.

However, it has been debated on macroalgal standing crops on reefs are controlled less from the top-down by herbivory but more from the bottom-up by nutrient flux (Lapointe 1997, 1999; Hughes et al. 1999; McCook 1999; Miller et al. 1999; Aronson and Precht 2000; Belliveau and Paul 2002; Jompa and McCook 2002; McClanahan et al. 2002; Diaz-Pulido and McCook 2003; Lapointe et al. 2004 ; Worm and Lotze 2006). Nonetheless, there are reef systems where both top-down and bottom-up factors are simultaneously important (Smith et al. 2001, 2010; Stimson et al. 2001), a pattern that is also evident in meta-analyses (Gruner et al. 2008). Coral-seaweed-herbivore triangle illustrated the dynamics in between (Hixon 2015).

This disagreement on herbivorous fish functions leads to dissensus on working out protective measures and enforcement among the Wakatobi government. Without governance, fishermen not only continue to catch any species and any amount of fish for

their major food source, but also set up more efficient but non-selective fish fences capturing diurnal migratory fishes between coral reef and mangroves (Opwall 2017). Additionally, fishing for export worldwide (fish biomass yield) increased steadily over the past 10 years (Opwall 2017). There is a risk of overfishing, including herbivorous fishes.

Bellwood et al. documented strong evidence of fishing effects on functional roles of parrotfishes (Scaridae) in the Pacific and Indian Oceans, suggesting that fishing can systematically affect at least some functional characteristics of the herbivore community. Therefore, there is an urgent need to assess the current status of herbivore functional groups and benthic situation.

Table 1: Herbivorous Fish Functional Groups and Roles, respective Indo-pacific fish names provided. (IUCN 2009; Reef Resilience 2017)

Functional Group	Role	Family Name
Scrapers / small excavators	- take non-excavating bites - remove algae, sediment, other material by closely chop or scape the reef surface - leave shallow scrape marks on the reef substratum - provide areas of clean substratum for coral recruitment	Majority of parrotfishes (<i>Hipposcarus</i> and <i>Scarus</i> species)
Large excavators/bioeroders	- remove dead coral and expose hard reef matrix for coral recruitment - differ from scrapers by taking deeper excavating bites and remove greater quantities of substrate with each bites - major agents of bioerosion on reef	- all large individuals of excavating species (> 35cm standard length) - all <i>Chlorurus</i> species
Grazers/detritivores	- Intensely graze on epilithic algal turfs, which can also limit the establishment and even remove macroalgae before it becomes established. - don't scrape and excavate the reef substratum.	- Most rabbitfish (exc. <i>Signaus canaliculatus</i> and <i>S. lineatus</i>) - Most butterflyfish - Small angelfishes - Many surgeonfish (all <i>Zebbrasoma</i>, <i>Acanthurus</i> species exc. Plankton-feeders) <i>Acanthurus lineatus</i> are predominately territorial, but also consume algal turf outside their territories (Grag 1996)
Browsers	- Consistently feed on macroalgae, select individual algal components, remove only algae and associated epiphytic material - Important role in reducing coral from shading by macroalgae and thus in reversing coral-algal phase shift	- Surgeonfish (<i>Acanthuridae</i>), Unicornfishes (<i>N. unicomis</i>) - batfish (<i>Platax teira</i>) - rudderfishes - rabbitfish - parrotfishes <i>Calotomus</i>, <i>Leptoscarus</i>
Farmers	- graze on turf algae - but also allow turf algae to grow - expel other herbivorous fish grazing	territorial damselfish

1.2 Aims

This research aimed to explore the status of reef fish herbivores species, functional biomass, and then link these to patterns in benthic community composition, specially exploring variations in percentage cover of hard corals and algae among heavily fished and less fished study sites.

1.3 Research Questions

1. What are the temporal variances in species richness and functional biomass of herbivorous fish species over the past 5 years?
2. What are the current variations of species richness, functional biomass and length categories among heavily fished and less fished sites?

As some functions and its effect of size may be more important than others for regulating the specific ecosystem status, hence this study is going to investigate it (Bonaldo & Bellwood 2008; Micheli & Halpern 2005).

3. What are the key herbivorous fish species?
4. What is the temporal variance in abundance (percentage cover) of benthic community (algae, coral, mobile substrate) over the past 5 years?
5. What is the current variations in abundance (percentage cover), composition and predominance of the benthic community among heavily fished and less fished sites?
6. Is there any statistical significant correlation between herbivorous functional groups and benthic community?

2. Methods

2.1 Ethical Review

The activities for this study were conducted under permission from the Wakatobi National Park Authority offered to Operation Wallacea. Only visual censuses of fish and benthic communities were conducted during this study; no fauna or flora were collected. Hence, ethical review was not required.

2.2 Study Sites

This study took place in the Indo-Pacific region, which has an exceptional record of diverse fish species (3000), almost 3 times more than the next richest marine region, the Western Atlantic. The Coral Triangle of Wakatobi Marine National Park (Indonesia) itself boasts one of the world's most diverse ecosystems (Comeros-Raynal et al., 2012); 750 coral reef species and 942 fish species.

Wakatobi Marine National Park, located within the Tukangbesi Archipelago, Southeast in Indonesia, is inside the world-famous "Coral Triangle", recognized as a global center of marine biodiversity, and a priority for conservation. There is a tropical climate, temperature ranging from 21°C to 35 °C (mean 27°C), with dry (November to March) and wet (April to October) seasons, and total rainfall is about 2,000mm/year (Whitten, Henderson and Mustafa 1987).

This study was conducted in July 2017 on the fringing reef (Figure 6) around the Island of Hoga (Figure 7), in a remote island group within Wakatobi Marine National Park (Figure 1) of about 200,000 ha, off Southeast Sulawesi in Indonesia (Elliot et al., 2001).

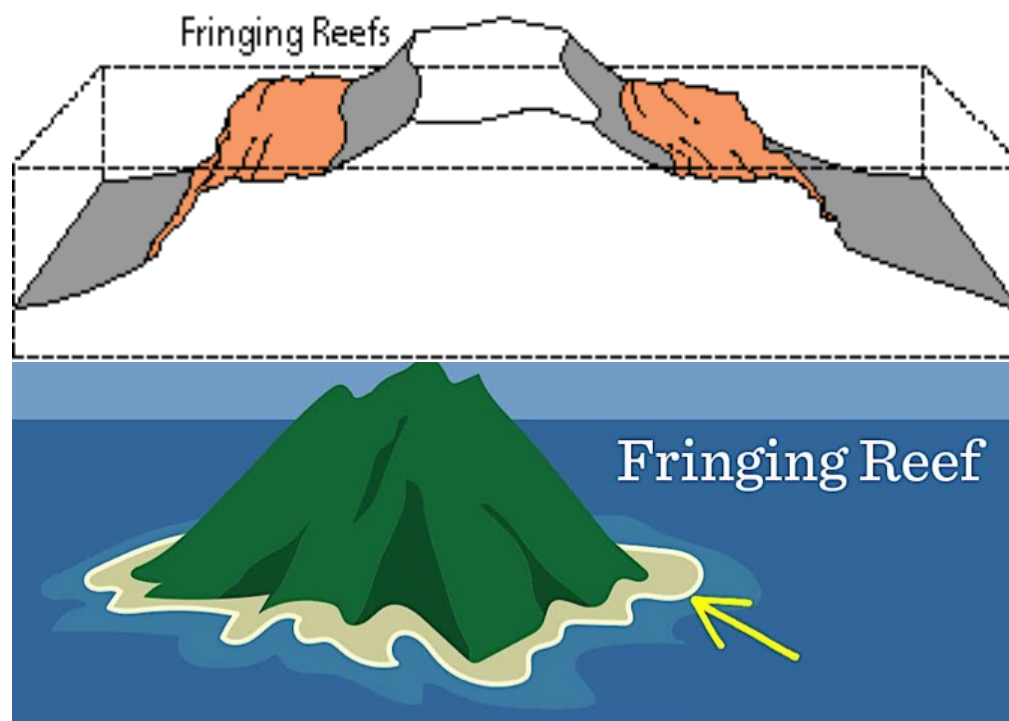


Figure 10. (upper) Brown color zone shows fringing reef. (lower) fringing reef diagram in aerial angle. Source: (upper)

<https://sludge.wordpress.com/2006/11/02/coral-reefs-and-oil-spills-a-guided-tour/>

(lower) <http://thinga.com/boom/articles/reef-brief>



Figure 11. Aerial view of Hoga Island fringing reef, they develop in shallow waters along the coast of tropical islands, which are attached, laying adjacent to the shore and continent. Source: <http://www.indonesia-tourism.com/forum/showthread.php?1913-Let-s-Get-Diving-To-Hoga-Island-Souteast-Sulawesi>

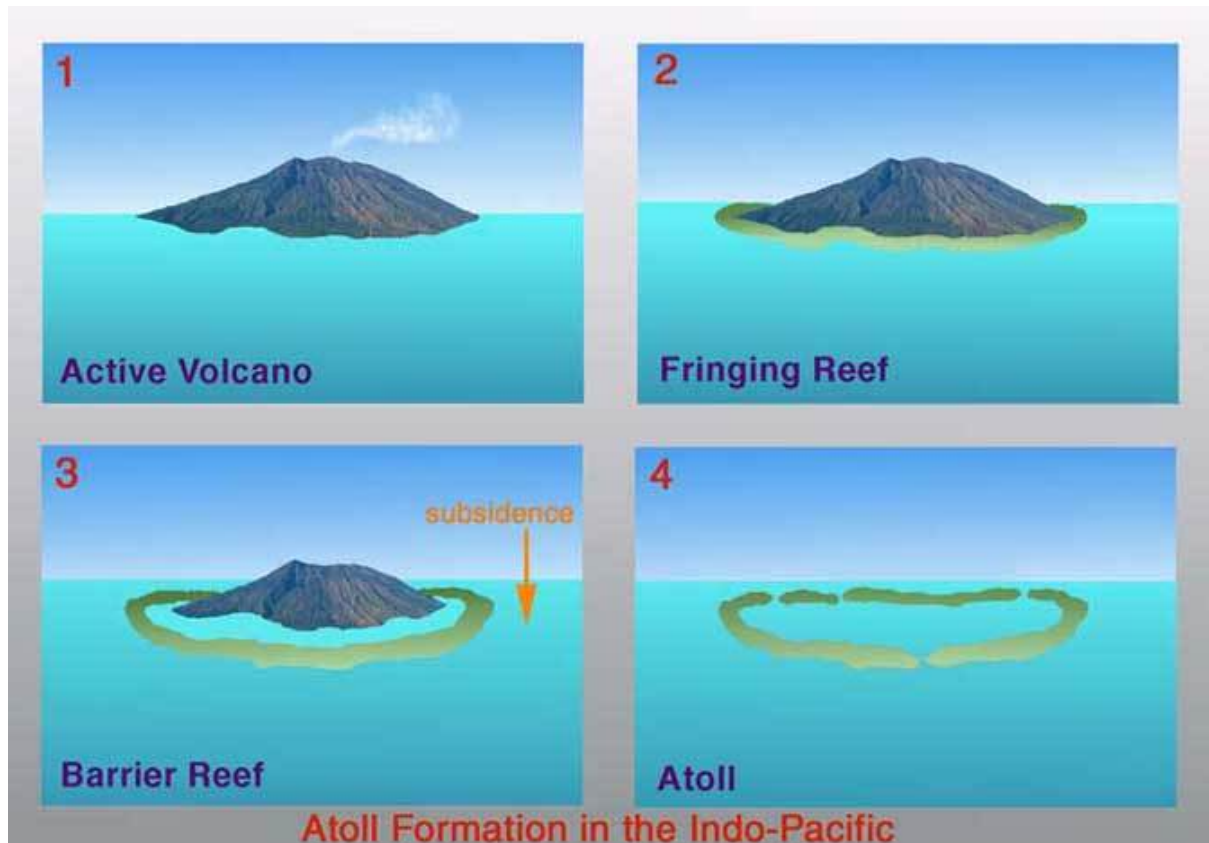


Figure 12. Long-term reef growth process. Source: <https://www.pmfias.com/coral-reef-fringing-reefs-barrier-reefs-atolls/>

2.2.1 Fringing Reef

The study reefs are a type of fringing reef (Figure 7). They develop in shallow waters along the coast of tropical islands, which are attached, laying adjacent to the shore and continent like this study region adjacent to Hoga Island and Kaledupa. These are the common types of reef (Coral Reef Alliance 2017) also found in the Caribbean and Red Sea. Charles Darwin (1809-1882) suggested that fringing reefs are the first kind of reefs to form around a landmass in a long-term reef growth process (Figure 9).

Four study sites were chosen with contrasting reef health from two adjacent independent reefs separated on opposite sides of a channel. The first study site, Sampela, (S1; 05°29.3S, 123°45.15E) is located on a degraded reef east of Kaledupa. Sampela is the closest to the local village, and is impacted by destructive fishing, reef gleaning, mining, high turbidity and high sedimentation (Crabbe and Smith, 2005; Happkyla et al., 2007; 2009). About 1km away from Sampela, two other study sites, Pak Kasim and Buoy 3, (B3; 05°28.23S, 123°45.19E) were chosen to allow reasonable distance (English et al., 1997). These sites, located west of Hoga Island, are considered a relatively healthy reef, and are subject to similar environmental conditions, have similar physical characteristics (English et al., 1997), slope and mild wave exposure and the same tidal regime. And the fourth study site, the Ridge, is situated north of Hoga Island, and it faces stronger waves and a higher nutrient load. (Operation Wallacea unpublished data).

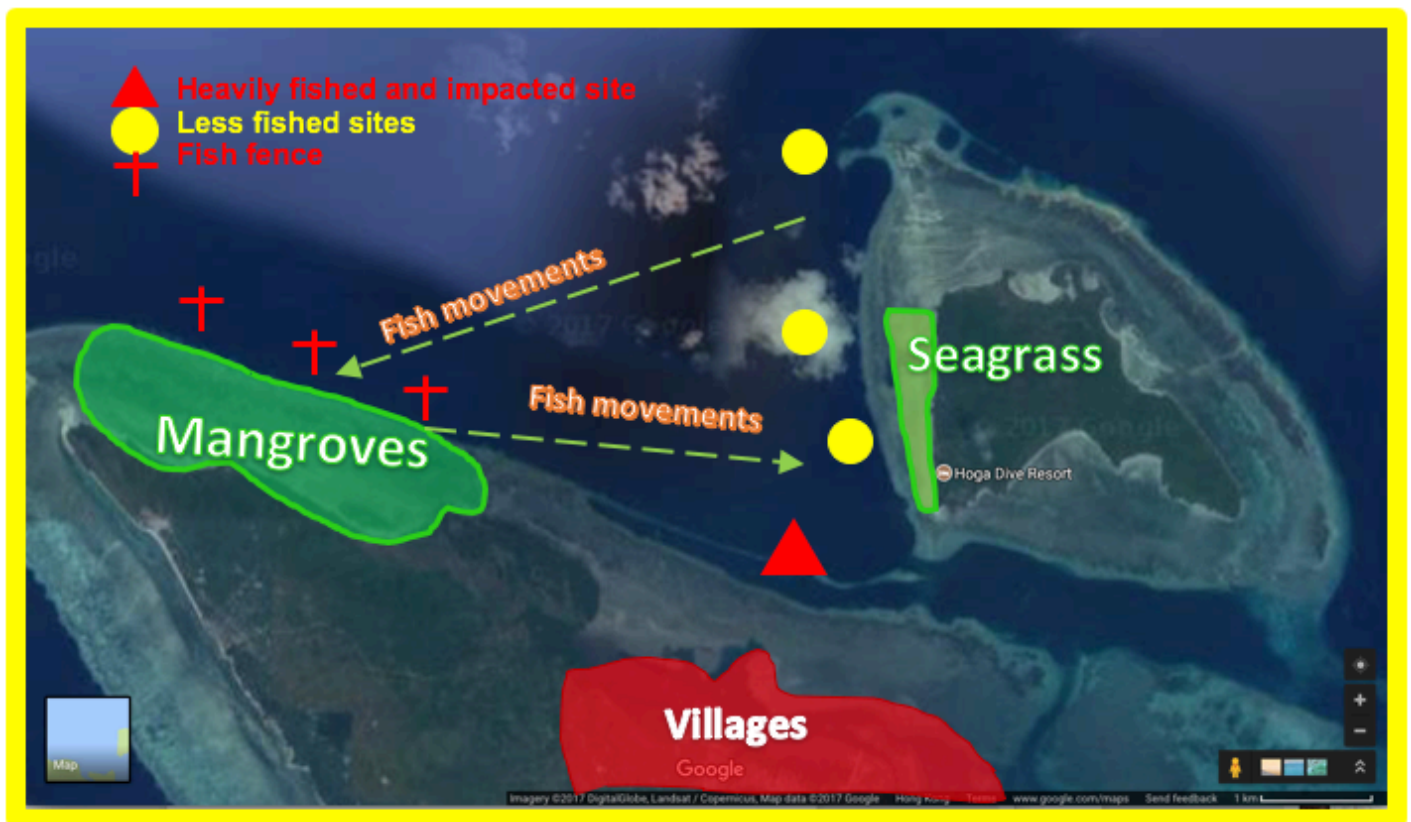


Figure 13. Characteristics of study locations. Fish move daily on and on between seagrass, coral reef and mangroves. Fishermen have set up fish fence to catch those moving fish.

2.2.2 Depth Zones

Water depth is a limiting factor of algae and coral occurrence (Morris 2005). As light becomes attenuated when interacting with the water column, different wavelengths of light penetrate the water to a varying degree, for example red light is attenuated rapidly and does not penetrate further than 5 m in clear water (Figure 5). Hence, in this study, water depths were considered to represent varying habitats (Ahmadia, Wilson & Green 2013). This study has investigated 3 habitats along depth contours, i) reef flat (ca. 2m), ii) reef crest (ca. 5m), iii) reef slope (ca. 12m) (Figure): A total of 3 transects were laid on each habitat except for the Ridge flat habitat exists.

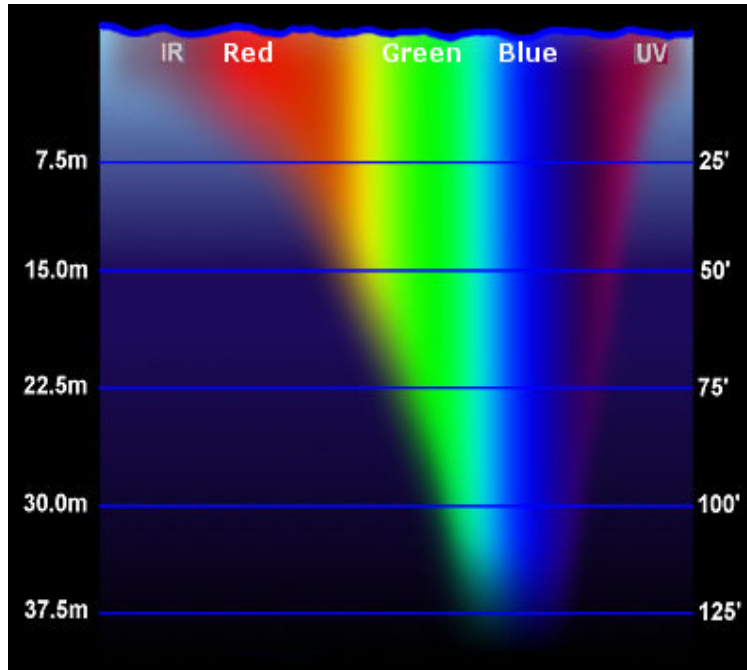


Figure 14. Patterns of light penetration into coral reef. Source: Tom Morris, Fullerton College.

Source: <http://www.seos-project.eu/modules/coralreefs/coralreefs-c05-n04.html> Accessed on: 23 Aug 2017

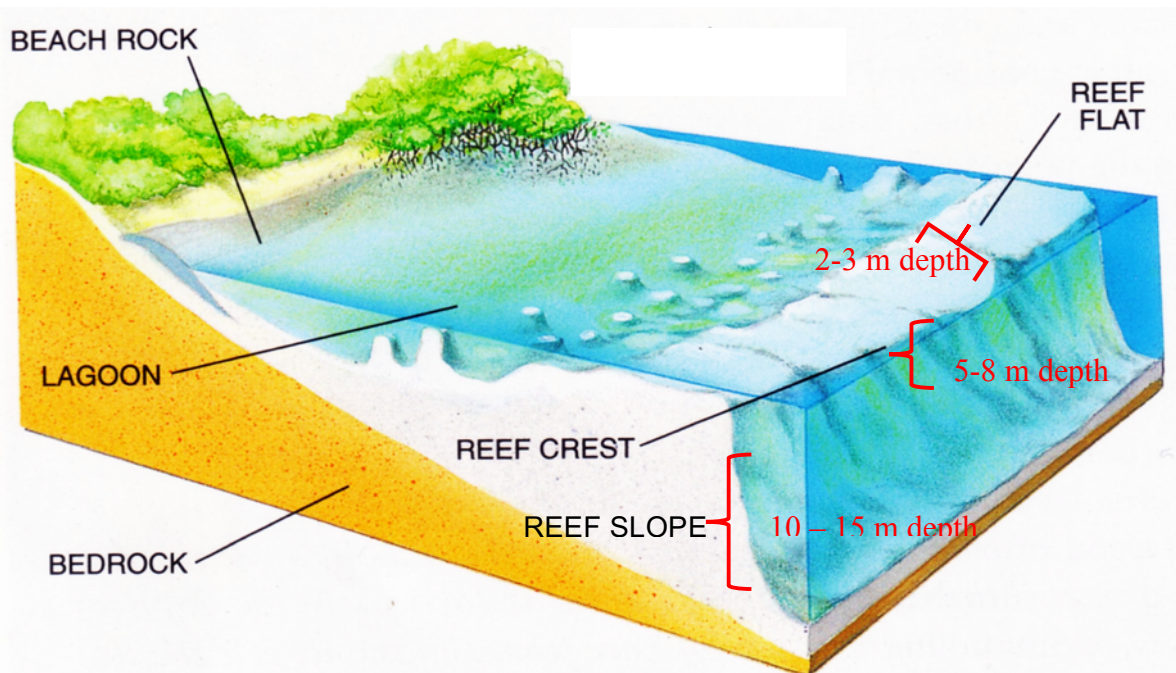


Figure 15. Fringing Reef Habitats Diagram (Reef flat, reef crest and reef slope) Source: <http://amazingbiotech.blogspot.hk/2014/02/coral-reefs.html>

2.3 Survey Techniques

This study had deployed underwater visual census by diver-operated stereo-video counting for both herbivorous fish and benthic composition. As research questions required only censual data, no biological information or dissection was needed; hence a non-invasive/non-destructive approach was adopted (Sutherland 2006).

The study sites have high water clarity and are suitable for underwater visual census type survey. The survey collected data down to a maximum depth of 12 m, and as Sutherland (2006) suggested that for depths deeper than 1-1.5m, scuba should be employed. Plus, a maximum depth of 12m is within the 15m limit for scuba-diving survey (JNCC, 2001). These conditions make this scuba-diving survey feasible.

2.3.1 Diver-Operated Stereo-Video Count

Due to the fact that naked-eye counting has the disadvantage of observer errors and biases, especially for fast moving and schooling fish, with difficulties of under or overestimates of count/size; too much attention on specific fish and data loss due to diver hesitation and recording delays (Labrosse et al., 2002; Sutherland 2006)) or re-censusing of fish that left and subsequently re-entered the transect area (JNCC Monitoring handbook 2001); transects were filmed using diver-operated stereo-video system, composed of two high-definition video cameras at fixed angles to allow footage of fish to be filmed simultaneously from two different positions (Figure 12 and 13) (Andradi-Brown et al., 2016; Yulianto et al., 2015). By using the EventMeasure software v3.51 (<http://www.seagis.com.au>), fish abundance, species and fork length were calculated without duplication and human error (Fig 8).

Using the dual images, it is possible to accurately calculate the fork length of fish (distance from tip of snout to end of the center of caudal fin rays), and then convert this into biomass estimates using standardized length-weight relationships (Andradi-Brown et al., 2016).



Figure 16. Stereo-video fish length measurement. Source: <http://www.methodsinecologyandevolution.org>

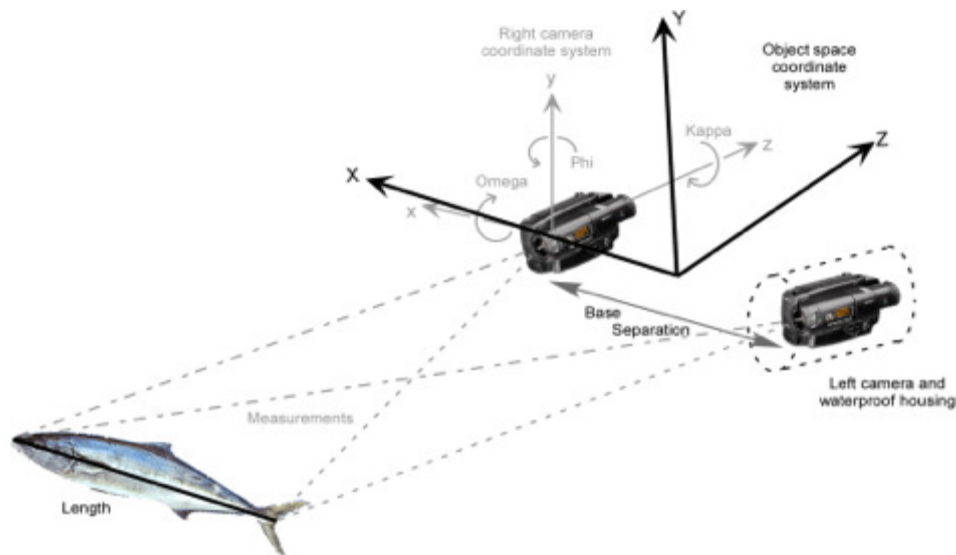


Figure 17. Fish fork length estimates. (Graph by Nada Boutros)

It has been determined that a 50mx5m transect fish visual census, with three replicates of total 750m² at each depth provide appropriate variability under resource and time constraint situations (English et al., 1997, IUCN 2009, Ahamadia et al., 2013).

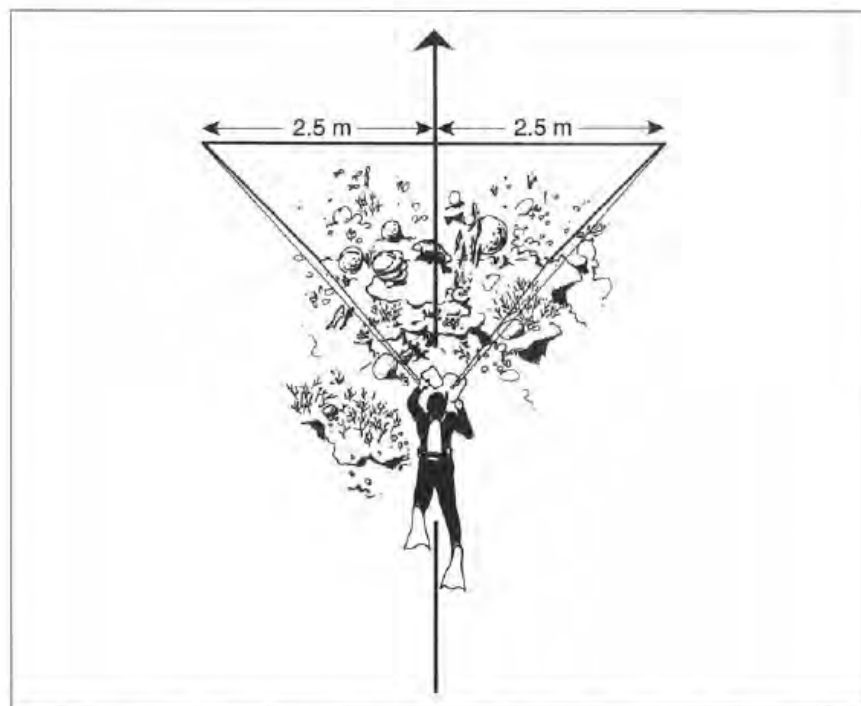


Figure 18. The diver swims along and filming the 50m long transect with 2.5m x 2.5 m wide on both sides, the total transect area is 250m². (Source: English, Wilkinson and Baker 1997)

2.3.2 Fish Survey Procedures

- Survey carried out during day time between 8:00am – 4:00p.m to avoid the diurnal-nocturnal changeover periods of fish (Carpenter et al., 1981; IUCN 2009).
- Permanent bolts had been previously installed by Operation Wallacea in the reef for permanent transects, so to collect benthic and fish data from the same transects.
- Monitoring has been conducted every summer, providing longitudinal data.
- Fish transects followed the depth contour but replicates were not overlapped, and they were allowed separation by 10 – 20m (English et al., 1997).
- GPS coordinates were recorded for each study site.
- The diver waited 5 mins after swim into the water to allow the fish to resume normal behavior (Carpenter et al, 1981; Ahmadi, Wilson and Green 2013)).
- Each transect was filmed as a complete 50x5m belt (English et al., 1997; IUCN 2009).
- The diver filmed the whole transect in 3 min.

After filming the transect, footage was analyzed on land (Figure 19), Fish outside 2.5m either side and 5m in front of the camera system were excluded (Andradi-Brown et al., 2016), and fish were classified down to species-level, if possible, otherwise to genus or to family level (Unsworth et al., 2007). Species scientific names, family name, abundance, length, biomass, respective sites and habitats (depth zone) were recorded in Excel sheet (Appendix 4). And the species were assigned to functional groups with reference to their feeding types: browser, scrapers/small excavators, large excavators/bioeroders, grazers/detritivores, and farmer (territorial demersalfish) (IUCN 2009; www.fishbase.org).



Figure 19. Screen shot of software Eventmeasure used to mark fish scientific names and measure their fork lengths.

2.3.3 Benthic Survey Procedure

Afterwards, benthic videos were filmed by the video camera. By filming the benthic substrate, divers were not restrained by maximum gas supply of their cylinder.

- Benthic survey shared the same transects of fish.
- At each site, laid 3 (replicates) transect of 50m length at each of three depths. (English et al., 1997; Wilson & Green 2009; IUCN 2009)
- The replicate transects were located haphazardly at each depth and were not overlapped (English et al., 1997).
- The transect lines should be stretched tightly and follow the depth contour (English et al., 1997).
- Camera pointing vertically down is held approximately 0.3m above the benthos, keeping the transect tape within the frame (Andradi-Brown et al., 2016).
- Percentage covers were surveyed by continuous counts along the 50m transect (Pratchett et al), which resulted a total of 201 points (English et al., 1997).

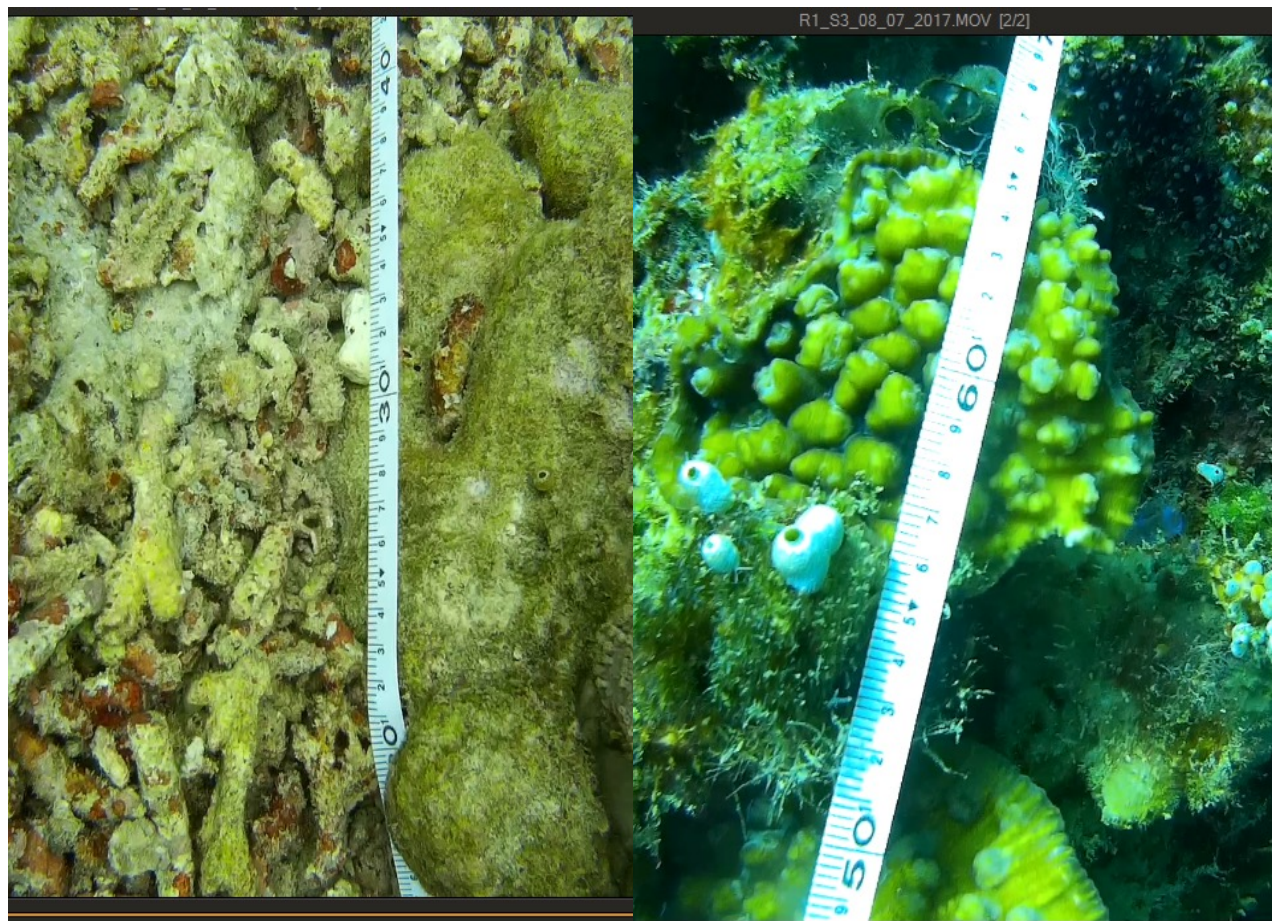
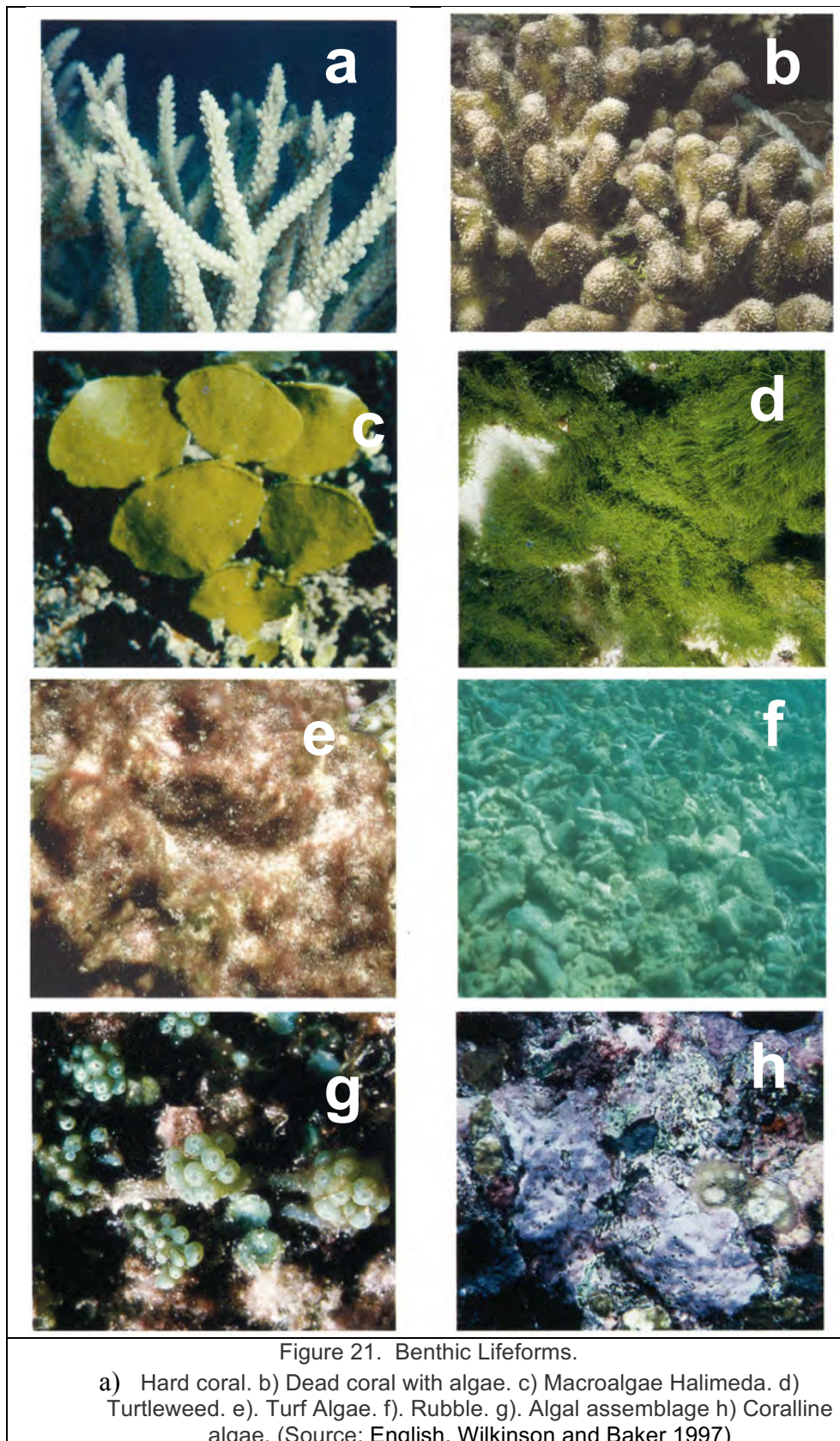


Figure 20. (left) Rubble and turf algae. (right). Turf algae and hard coral. Video analyzed on the right side of the 50m tape.

After filming the transect, the footage was analyzed on land, and benthic composition was recorded (Appendix 2) as live scleractinian (stony) corals, dead coral, turf algae, macroalgae, coralline algae, rock, and mobile substrate (i.e. unsuitable for coral recolonization, they are sand, silt, rubble and water) (Figure 21 and Appendix 1).

2.4 Quality Assurance

The study sites have an incredible diversity of life, so to assure quality of identifications (English et al., 1997; IUCN 2009), it was compulsory to take a 1 week lectures and in-water practical course on reef ecology and fish species identification and 80% marks were required before footage analysis.



2.5 Data Analysis

Fish size estimates were converted to biomass using a known length-weight relationship for each species using the formula described in Kulbicki et al., (2005):

$$W = aL^b$$

Where:

W=weight of fish in grams (g); L=fork length (FL) of the fish in cm; and *a* and *b* are constants calculated for each species or genus. Biomass constants (*a* and *b*) for each species were based on www.fishbase.org. For each census, biomass was converted to total biomass per unit area mean biomass (per ha) for belt transects.

Where:

biomass (kg⁵ per ha) = [(total biomass per sampling unit in g ÷ 1000) ÷ area of the sampling unit in m²] x 10,000.

In order to investigate fish population dynamics, size (length) categorizations were used (Pope, Kevin L.; Lochmann, Steve E.; and Young 2010), with a 2.5cm interval for fishes less than 10 cm in size, and 5 cm size categories for fishes 10cm or greater in size. (i.e <5cm, 5 to <7.5cm, 7.5 to 10cm, 10 to <15cm, 15 to <20cm etc (IUCN 2009; Ahmadia, Wilson & Green 2013)). Besides, variability was shown by including error bars in the figures (Ahmadia, Wilson & Green 2013).

The primary data would be used with the secondary data (2013-2016) provided by Operation Wallacea to project a trend for the future.

Both fish biomass and benthic percentage cover data were to fulfill normality and homogeneity, hence the former one was log-transformed (when needed) and the later one was arcsine square root transformed (when needed) and were then tested by Shapiro-Wilk normality test and Bartlett Test.

In order to examine the variation of means of benthic types and the biomass of herbivorous fishes, these dependent variables were compared among fixed variables (sites and habitats), using a series of two-way ANOVA. To perform it, assumptions were fulfilled: all data of dependent variables were in continuous level, benthic covers were in percent cover. Independent variables were in two categories: sites (heavily fished, lightly fished) and habitats (reef flat, crest and slope). Also, data were expected in a normal distribution; but only algae, hard coral and mobile substrate were normally distributed after checking this with Shapiro-Wilk test, so, the rest were transformed, like rock cover was arcsine squareroot transformed. And they also fulfilled homogeneity of variances. (Appendix 9-12)

However, as not all functional groups occurred in all habitats and sites, there were zeros on raw data. Therefore, one-way ANOVA & Tukey post hoc tested were used instead. And sites were set at fixed factor.

To carry out one-way ANOVA, assumptions were to fulfill: all data of dependent variables were in continuous level, like benthic covers were in percentage, biomass was in kg/ha. Independent variables were in one categories, sites (heavily fished, lightly fished). Also, data were expected in a normal distribution; however, they were not normally distributed after checking with Shapiro-Wilk test, so, total herbivorous fish and grazing biomass were log transformed and they also fulfilled homogeneity of variances. (Appendix 7-8)

ANOVA (post hoc test - Tukey's range test) were carried out to determine where the difference occurred (Ahmadi, Wilson and Green 2013; Unsworth et al., 2007).

In addition, Principal component analyses (PCA) were used to investigate main component in the benthic community composition and herbivorous fish assemblages among sites and habitats. The analyses were based on the covariance matrix of the mean proportion of each substratum category, and the mean biomass of each herbivorous species in each habitat within each site, respectively. The biomass of each herbivorous species was square-root transformed.

Shannon 's diversity index (Penczak et al., 1994) was employed to find out how the level of fishing activities impacted across habitat quality gradients. Evenness index (Gerald et al., 2001) was used to find out abundance level of herbivorous fish species of distribution throughout sites and habitat types. Dominance Index was employed to further explore key fishes for future conservation plan.

Relationships between benthic categories and herbivore biomass were examined using Pearson correlation analysis.

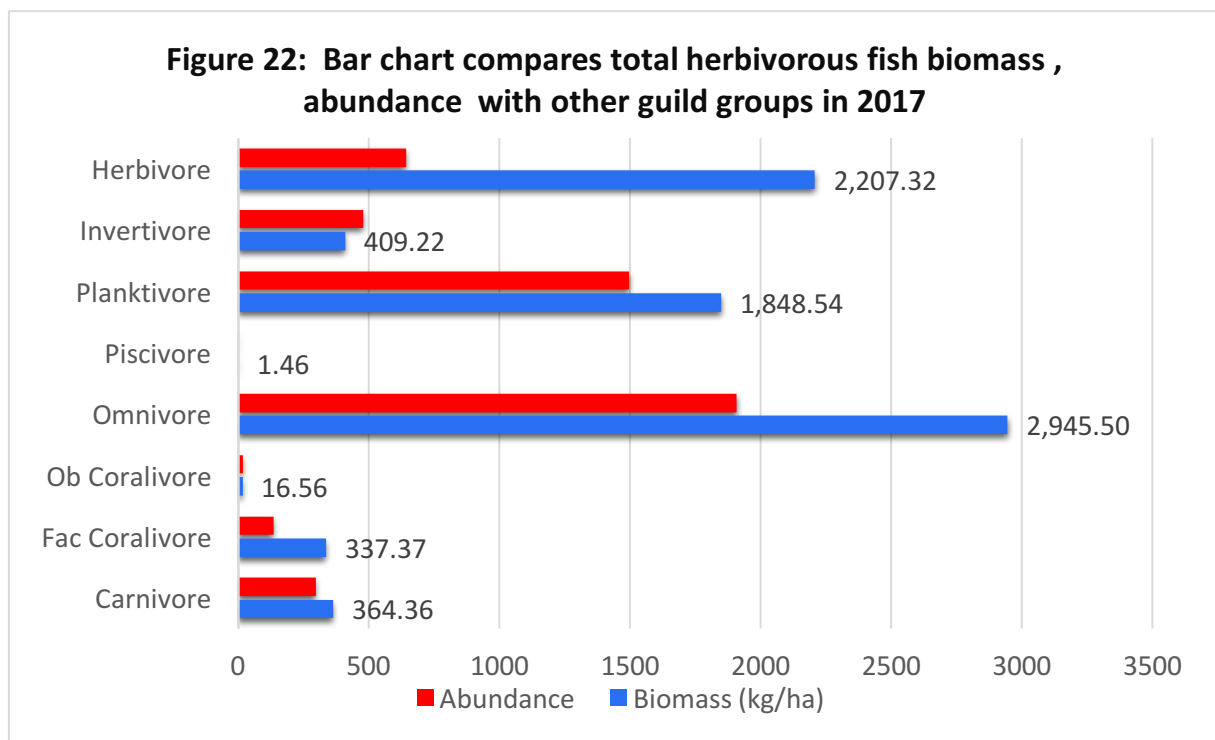
3. Results

3.1 Herbivorous Fish

This part looks into herbivorous fish species richness, biomass, functional length categories with variations among different habitat quality.

3.1.1 What are the current variations of fish herbivores richness and biomass?

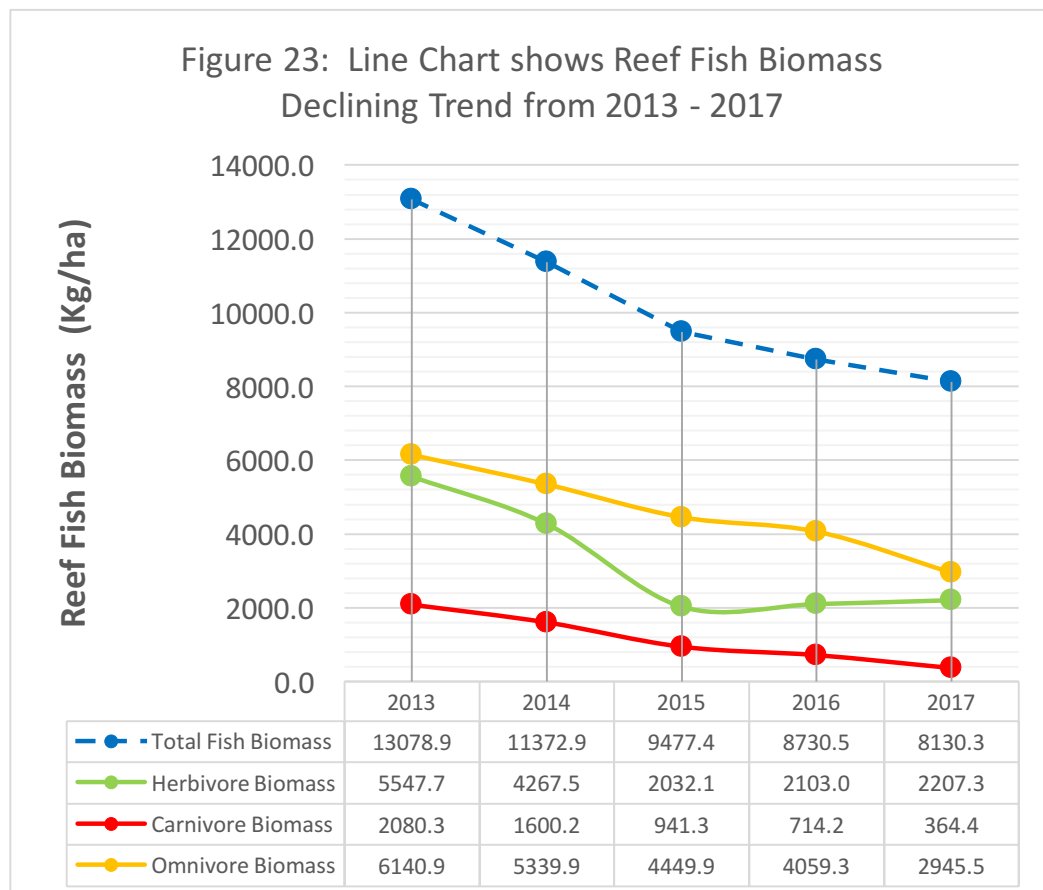
This year 23 species, 643 individuals and 2207.32 kg/ha of herbivorous fish were recorded in 4 study sites. In terms of biomass, herbivores were the second largest guild group (27.1%; Figure 22).



3.1.2 What are the temporal variances of reef fish biomass over the past 5 years?

The line chart below illustrates the changes of biomass of reef fish at the Wakatobi Marine Park between 2013 and 2017. Compared to last year, herbivore fish biomass has grown 4.95%.

However, in the past 5 years herbivore fish biomass fell 60.21%, carnivore 82.48%, omnivore 52.0% and the total fish biomass plummeted by 37.84%. 2013-2015 contributed to the largest decreases, and then going stabilized for herbivore, but slightly decreased for the rest of the groups (Figure 23). This is an obvious declining trend.



3.1.3 How do the herbivore diversity, abundance and dominance differ among sites?

The magnitude of anthropogenic impacts on the herbivorous fish diversity, abundance and dominance was obvious in the heavily fished and impacted site Sampela, which displayed the lowest Shannon-Wiener diversity and evenness Index values (0.488 and 0.191; 0.315 and 0.381), but highest to the less impacted site Ridge (1.2645 and 1.843; 0.885 and 0.789). The rest of the sites had closer values to the Ridge (Table 2).

The dominance index was negatively correlated with Shannon-Winner diversity index.

Except for reef crest on Buoy and Ridge, *Ctenochaetus striatus* was the most dominant species across the many habitats and sites (41.7 to 89.8%; Table 2). Fishing impacts on herbivorous fish diversity, abundance and dominance was obvious.

Table 2: Strength of Anthropogenic Impacts on Herbivorous Fish reflected by Shannon-Wiener Index, Dominance Index and Dominant Species

	Site	Habitat	Shannon-Wiener Index	Evenness Index	Dominance Index	Dominant Species	
						Name	Percentage
Heavily fished and Impacted	Sampela	Flat	0.4551	0.315	0.810	<i>Ctenochaetus striatus</i>	89.8%
	Sampela	Crest	1.153	0.352	0.488	<i>Ctenochaetus striatus</i>	68.2%
	Sampela	Slope	1.232	0.381	0.473	<i>Ctenochaetus striatus</i>	67.2%
Less Impacted	Pak Kasim	Flat	1.689	0.492	0.260	<i>Ctenochaetus striatus</i>	41.7%
	Pak Kasim	Crest	0.574	0.444	0.716	<i>Ctenochaetus striatus</i>	83.7%
	Pak Kasim	Slope	1.500	0.498	0.360	<i>Ctenochaetus striatus</i>	57.1%
	Buoy	Flat	1.784	0.397	0.284	<i>Ctenochaetus striatus</i>	48.9%
	Buoy	Crest	1.383	0.664	0.304	<i>Chrysiptera rex</i>	45.2%
	Buoy	Slope	0	1	1	<i>Ctenochaetus striatus</i>	100.0%
	Ridge	Crest	1.843	0.790	0.190	<i>Zebrasoma scopas</i>	32.0%
	Ridge	Slope	1.265	0.886	0.319	<i>Ctenochaetus striatus</i>	47.4%

3.1.4 What is the current functional diversity?

To look closer at the functional groups, the dominant group was the grazers/detritivores groups, which accounted for 1891.55 kg/ha (77.6%) of total biomass with 11 species and 498 individuals (Table 3), as opposed to large scraping taxa which only had 71.11 kg/ha (3.2%) with 1 species, and 64.65kg/ha (2.9%) with 3 species of scraping taxa, and 3.2%, 4 species for farming taxa.

It is important to note that the browsers (1 species), only one individual of *Naso lituratus* was found. According to IUCN red list, all the species found were either fell in least concerned or not assessed groups.

Table 3: Functional Groups Details on no. of species, group biomass, species and it respective abundance (IUCN 2009)

Functional Groups	No. of species	Group Biomass (kg/ha)	Species Name (* - IUCN red list, least concern; ** not assessed)	Frequency
Browser	1	7.04 (0.3%)	<i>Naso lituratus</i> *	1
Grazers/detritivores	11	1891.55 (85.7%)	<i>Acanthurus grammoptilus</i> *	3
			<i>Acanthurus pyroferus</i> *	27
			<i>Ctenochaetus striatus</i> *	363
			<i>Dischistodus chrysopoecilus</i>	1
			<i>Scarus dimidiatus</i> *	2
			<i>Scarus russeli</i> **	3
			<i>Scolopsis sp1</i>	2
			<i>Siganus doliatus</i> *	2
			<i>Siganus vulpinus</i> *	6
			<i>Zebrasoma scopas</i> *	61
			<i>Zebrasoma veliferum</i> *	1
Scrapers/ small excavators	3	64.65 (2.9%)	<i>Hipposcarus longiceps</i> *	3
			<i>Scarus sp1</i>	14
			<i>Scarus psittacus</i> *	3
Large Scrapers/ excavators	3	172.96 (7.8%)	<i>Cetoscarus bicolor</i> *	4
			<i>Chlorurus bleekeri</i> *	11
			<i>Chlorurus sordidus</i> *	19
Farmer	4	71.11 (3.2%)	<i>Chrysiptera sp1</i>	40
			<i>Chrysiptera rex</i> **	55
			<i>Dischistodus melanotus</i> **	19
			<i>Pomacentrus bankanensis</i> **	2
Total	22			541

3.1.5 What are the current functional length categories?

To dig deep in investigating functional health, population structures were examined by size categories. In the chart of all functional groups (Figure 18a), a bell-shaped distribution of population structure was found and the largest portion of length group was 10 to <15cm.

Grazers/detritivores (Figure 18b) have the largest sample size, and its chart was characterized by high and narrow peaks of distribution, with the mode of 10 to <15cm length categories (67.03%) which contributed the majority of frequency for figure 18a, however, declines found in growth (<7.5 cm), plus very few in larger length categories.

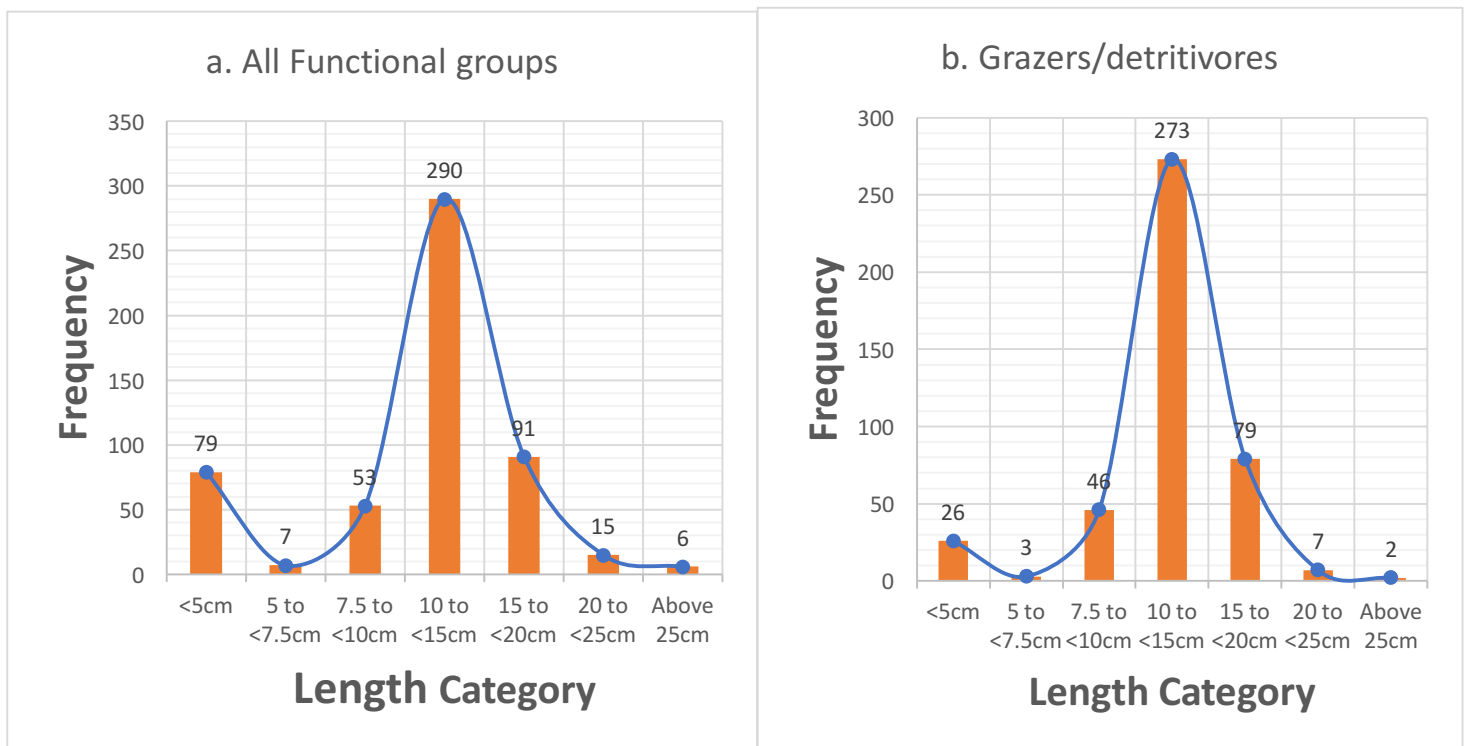


Figure 24. Frequency distribution of length category among herbivore functional group. a) all functional groups, b) Grazers/detritivores.

Large scrapers/excavators (Figure 18c) and scrapers/small excavators (Figure 18d) had much smaller sample sizes, but the mode was also 10 to <15cm length, so they had decline in growth. Especially notable is that lengths below 7.5 cm were totally absented from all transects.

Farmers (Figure 18e) had a small high and narrow right-skewed distribution of length categories, with the highest frequency on the shortest range of length group (<5cm), it had low frequency of length group from 5 cm onwards. As noted previously, in browser, only 1 individual was spotted (Figure 18f).

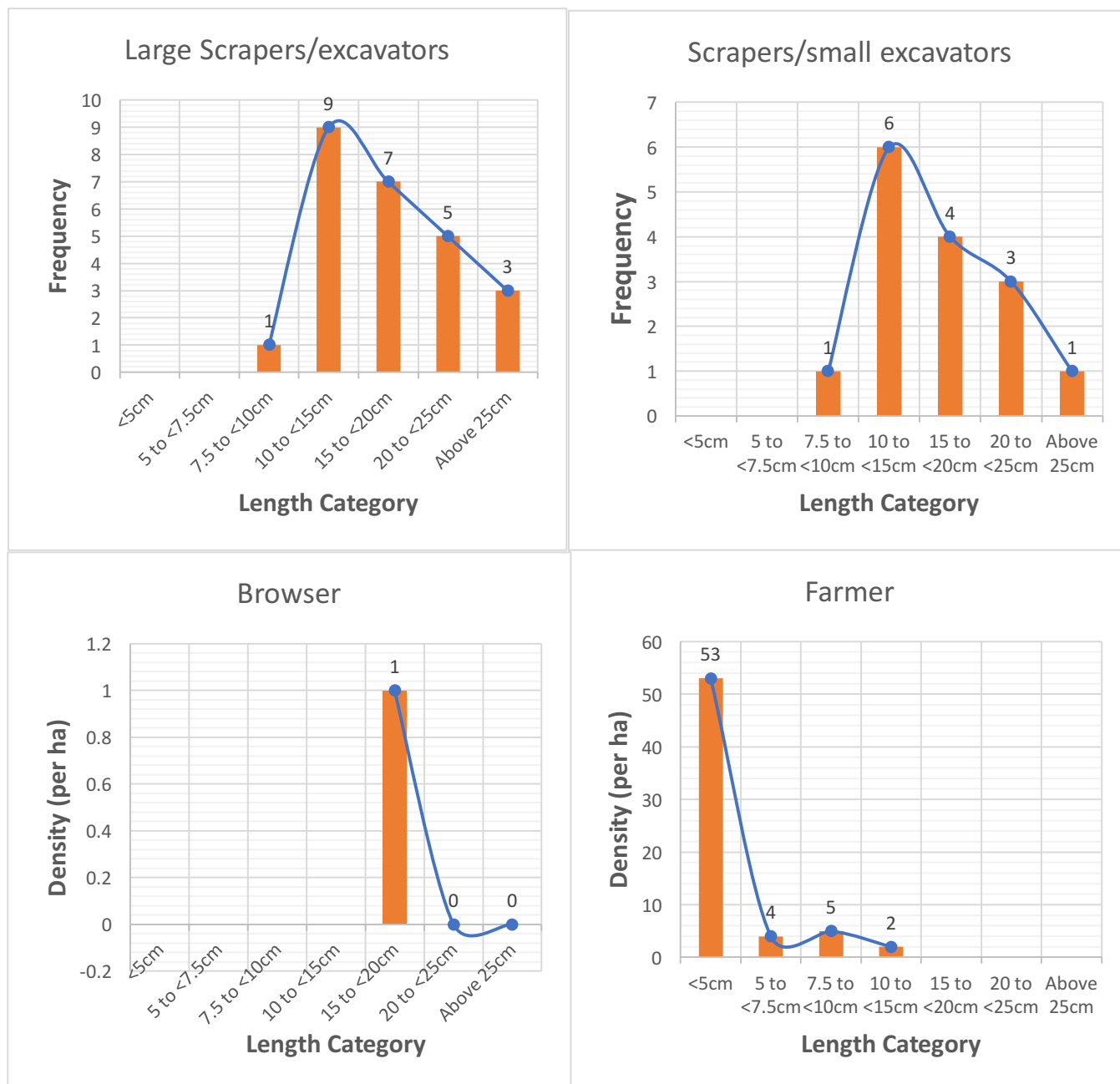


Figure 24. Frequency distribution of length category among herbivore functional group. a) all functional groups, b) Grazers/detritivores.

3.1.5 How do functional groups varied among sites?

There was a significant difference in biomass of overall herbivorous fish among all study sites (One way ANOVA, $F_{3,29}=4.116$, $p<0.05$). After completing the Tukey post hoc test, Sampela had a significantly higher biomass of herbivorous fish than in Buoy (Table 5A).

Grazers was significantly different among all study sites (One way ANOVA, $F_{3,29}=4.614$, $p<0.05$). After doing the Tukey post hoc test, Sampela had a significantly higher biomass of grazer than Buoy and Ridge (Table 5B). Figure 25 showed not all function occurred at all sites and habitats.

Table 4: One-Way ANOVA's Herbivorous Fish Communities among Sites on Wakatobi Marine National Park, SE Sulawesi, Indonesia.
Significant results were given in bold.

Source of Variation	DF	Sum of Square	Mean of Square	F value	P
A) Total herbivorous fish biomass					
Site	3	15.22	5.072	4.116	0.0151
Error	29	35.74	1.232		<0.05
B) Grazer					
Site	3	16.42	5.474	4.614	0.0093
Error	29	34.41	1.187		<0.05

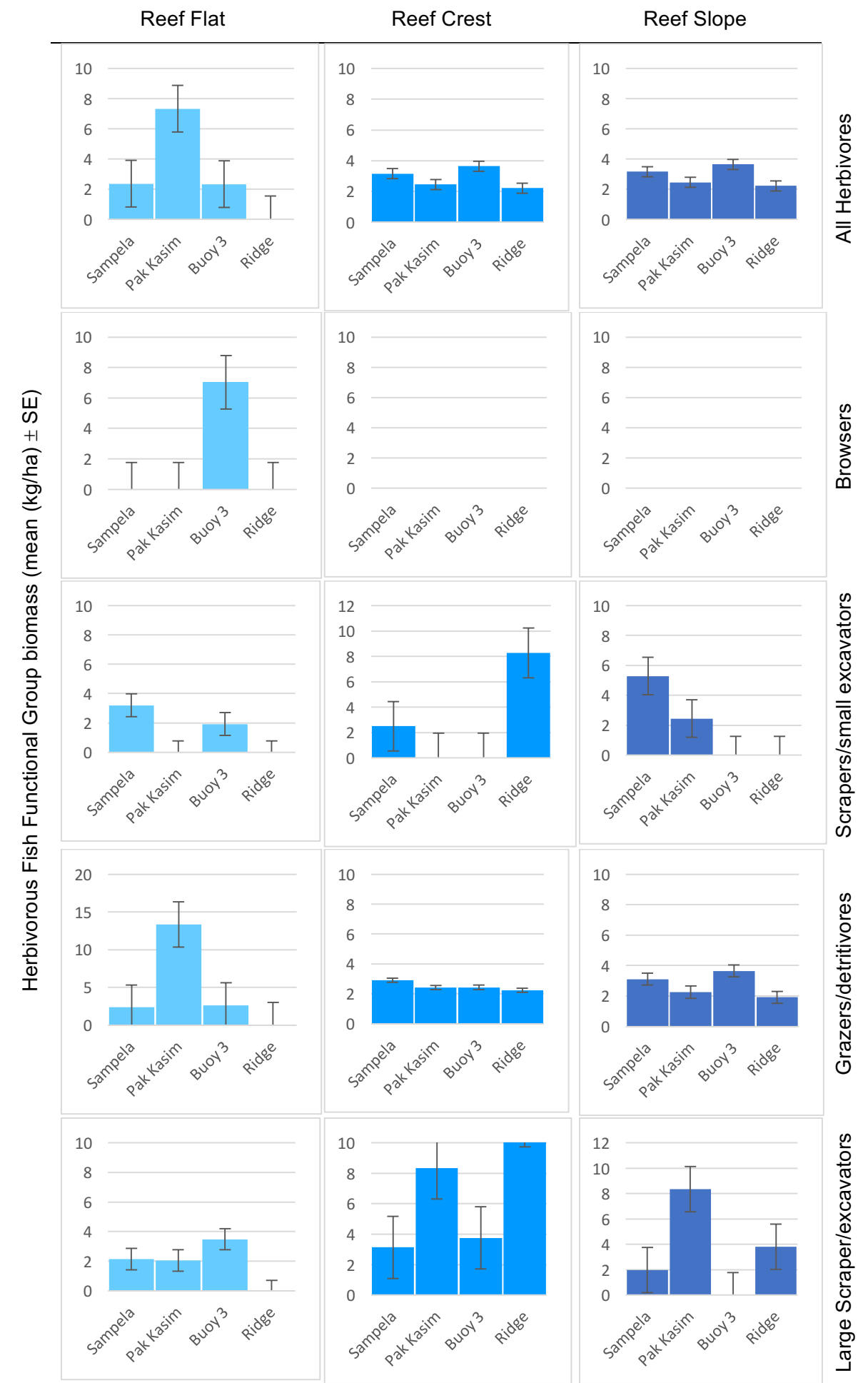
Table 5: Tukey post hoc test of total herbivorous fish biomass and Grazers/Detritivores

A) Total herbivorous fish biomass

Site (i)	Site (j)	Difference in mean	p	
Pak Ksim	Buoy	1.3148215	0.0789483	
Ridge	Buoy	0.7384728	0.5935586	
Sampela	Buoy	1.7414948	0.0120832	<0.05
Ridge	Pak Kasim	-0.5763487	0.7589646	
Sampela	Pak Kasim	0.4266733	0.8467241	
Sampela	Ridge	1.0030220	0.3348017	

B) Grazers/Detritivores

Site (i)	Site (j)	Difference in mean	p	
Pak Ksim	Buoy	1.19167162	0.1165846	
Ridge	Buoy	-0.04449914	0.9998289	
Sampela	Buoy	1.55417555	0.0250397	<0.05
Ridge	Pak Kasim	-1.23617076	0.1605639	
Sampela	Pak Kasim	0.36250393	0.8939073	
Sampela	Ridge	1.59867470	0.0437264	<0.05



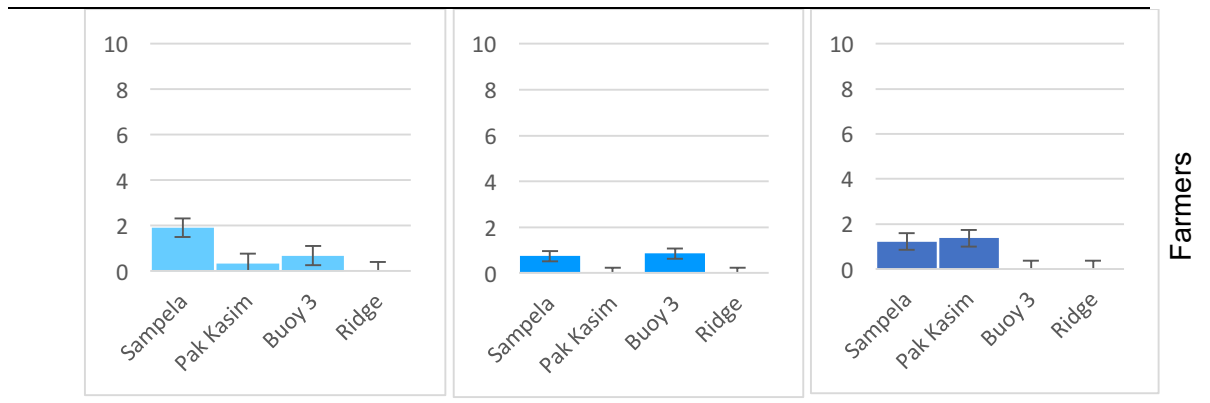


Figure 25: Variation in the biomass of functional groups of herbivorous reef fishes in Hoga Island. The mean biomass (kg/ha SE) of all herbivores, browsers, scrapers/small excavators, large scrapers/excavators and grazers and farmers.

3.2 Benthic Cover

3.2.1 What is the temporal variance in abundance of benthic community over the past 5 years?

Algae maintained an average cover of 5-9% from 2013 to 2017 except in 2014. Hard coral also kept an average cover of 17-19%. For Mobile substrate, it maintained an average cover of 32-36% (Figure 26). 5 years of PCA plots clustered together showed little variations (Figure 27).

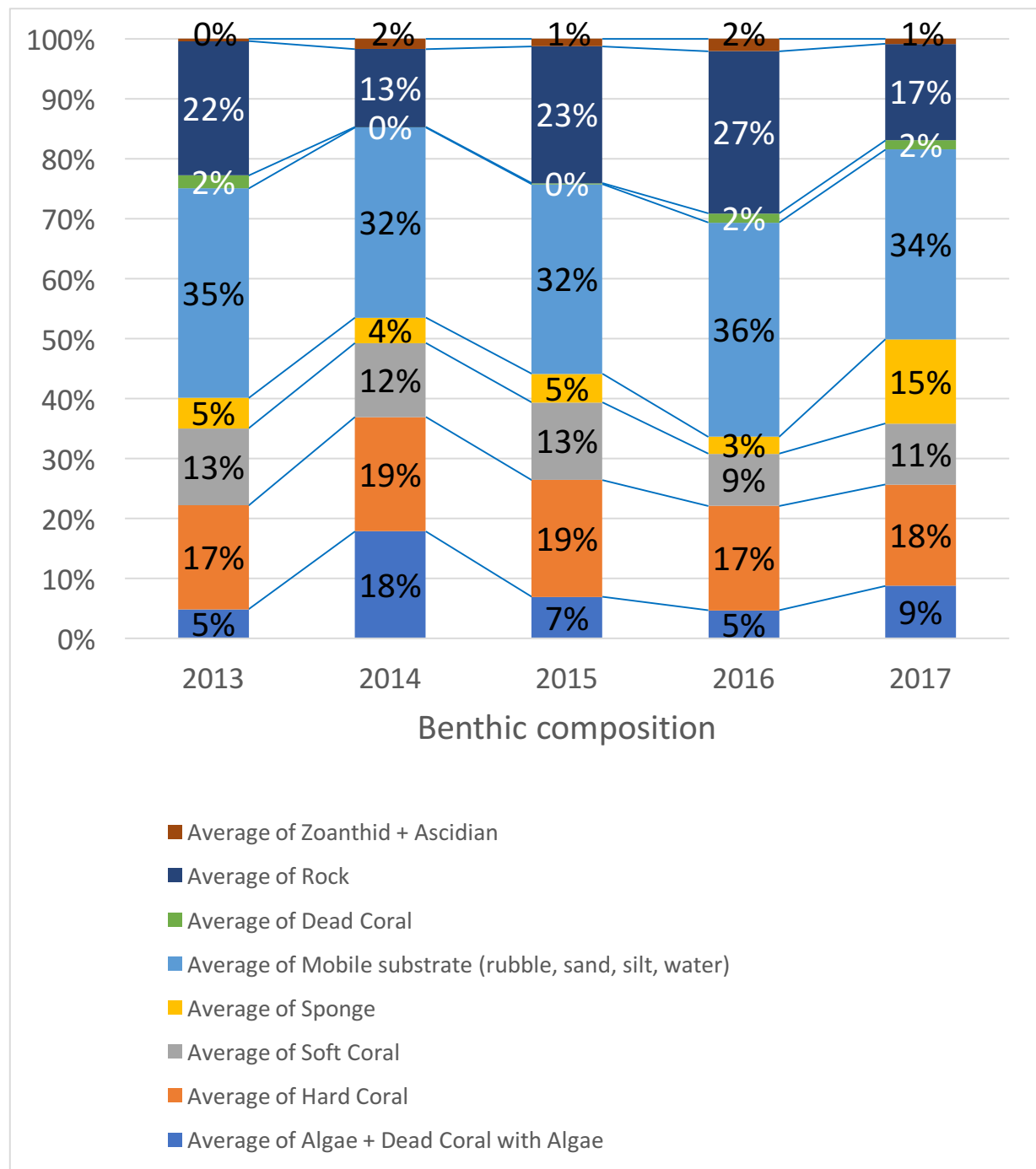


Figure 26. Variations of benthic composition between 2013 -2017.

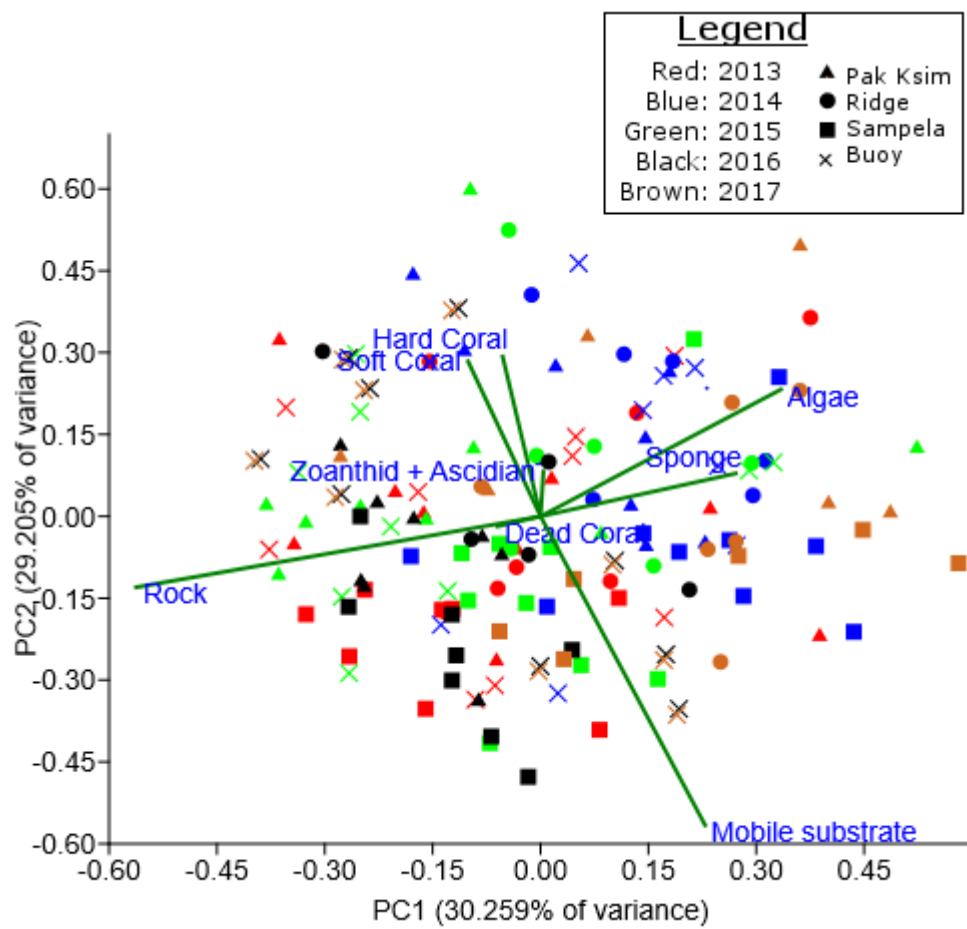


Figure 27: PCA graph showed principal component of benthic types from 2013-2017

3.2.2 Difference of benthic composition across habitat quality gradient

Hard coral was significantly less in heavily fished Sampela than the rest of less fished sites (Two-way ANOVA, $F_{3,16}=18.062$, $p<0.05$). The interaction between site and habitat was significant (Two-way ANOVA, $F_{3,16}=13.9541$, $p<0.05$). This indicated that relationship between site and percentage cover of hard coral depended on the habitat (Appendix 10).

There is no significant difference in percentage cover of algae among all sites (Two-way ANOVA, $F_{3,16}=1.601$, $p>0.05$), plus algae had no interaction between site and habitat (Two-way ANOVA, $F_{3,16}=0.5614$, $p>0.05$). The rest of benthic types had no significant difference (Appendix 9).

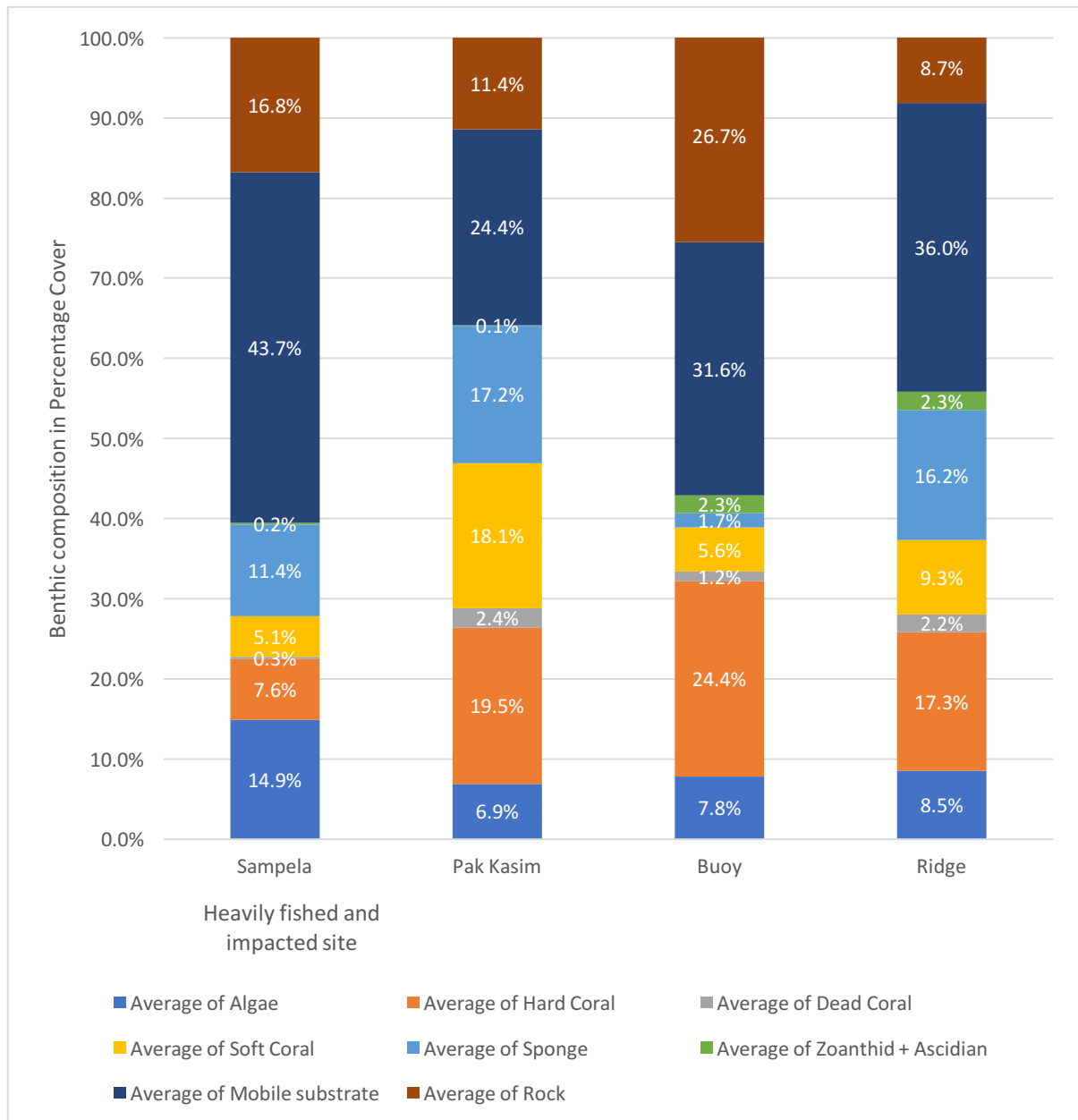


Figure 28: PCA graph showed principal component of benthic lifeforms in 4 study sites.

Table 6: 1-Way ANOVA’s Benthic Communities among Habitats and Sites on Wakatobi Marine National Park, SE Sulawesi, Indonesia. Significant results are given in bold.						
Source of Variation	DF	Sum of Square	Mean of Square	F value	P	
1) Algae Cover						
Site	3	0.0229	0.0076	1.601	0.22673	>0.05
Habitat	1	0.0010	0.0010	0.210	0.65279	>0.05
Site x Habitat	3	0.0080	0.0027	0.5614	0.64821	>0.05
Error	16	0.0759	0.0047			
2) Hard Coral Cover						
Site	3	0.1030	0.0343	18.062	2.17x10 ⁻⁵	<0.05
Habitat	1	1.17x10 ⁻⁵	1.17x10 ⁻⁵	0.006159	0.9384	>0.05
Site x Habitat	3	0.0796	0.0265	13.9541	9.91x10 ⁻⁵	<0.05
Error	16	0.0304	0.0019			

Table 7: Further Tukey post hoc test on hard coral and rock cover.

A) Hard Coral Cover

ID	Mean	Variance	SE
Buoy_Crest	0.341692	0.000240	0.01550
Buoy_Slope	0.174307	0.003049	0.05482
PK_Crest	0.209751	0.004383	0.06621
PK_Slope	0.179606	0.003181	0.05640
Ridge_Crest	0.0982921	0.001826	0.04273
Ridge_Slope	0.247624	0.001195	0.03457
Sampela_Crest	0.0542881	0.004414	0.02101
Sampela_Slope	0.0968997	0.000930	0.0305

3.3 Correlation between herbivore functional groups and benthic community

Pearson correlation showed insignificant correlations between all functional groups and algae (Appendix 13) .

3.3.1 How do PCA tell in combining two plots to analyze?

By comparing functional groups and sites PCA ordination plots, the heavily fished and impacted site -Sampela was characterized by high grazer/detritivores biomass for the mobile substrate (-0.175 along PC1).

For less fished sites, Pak Kasim was distinguished by grazer (3.684 along PC1) for rock (0.224 along PC1) and hard coral cover (0.404 along PC1) as primary contributors. And Buoy was characterized the browsers for rock cover (4.166 along PC1). Finally, the Ridge was characterized by the scrapers/excavators and large scrapers for the algae assemblage (0.178 along PC1) and macroalgal (-0.095 along PC1) respectively (Figure 29 and 30).

Figure 29:

PCA graph showed principal component of functional group among sites and habitat.

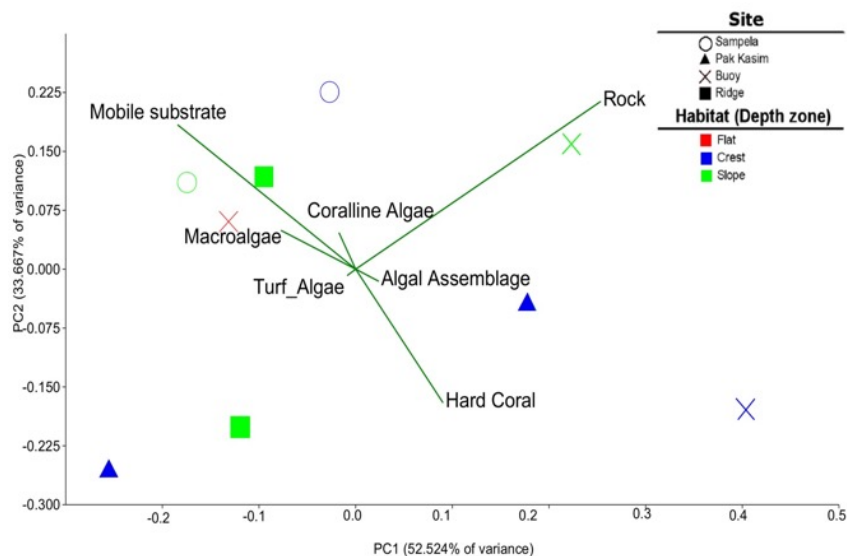
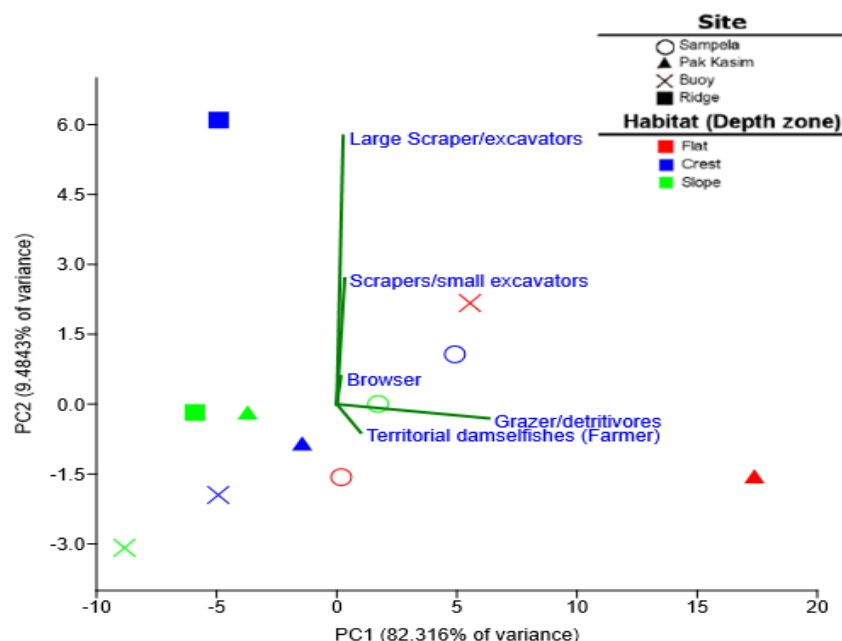


Figure 30:

PCA graph showed principal component of benthic composition among sites and habitat.



4. Discussion

4.1 Correlation between herbivore functional group and algae cover

Algae cover percentage is low this year (ranging from 6.9% to 14.9 %). There was no coral-algal phase shift seen.

Overall, herbivore fish was the second largest reef fish group across all study sites. By comparing means of sites, one-way ANOVA showed that the grazers/detritivores group has a notable difference in Sampela, however, their food had no difference among sites, which suggests that they were functioning in keeping algae under control.

However, the Spearman's rank correlation showed no significant correlations between them, which may be due to the very little variations of algae percentage cover among sites. This does not necessarily mean grazing was not functioning, because the biomass of herbivorous fish was large.

One potential explanation of the insignificant correlation is the differing scale of the studies, as this work conducted within only one region (with four sites), while the Caribbean study was conducted across a number of distant regions (Sandin et al., 2008), and even more in American Samonan Archipelago (240 study sites), so with many more data points which may resulted significantly (Heenan & Williams 2013).

Another explanation is the limitation of continuous count technique. Since algae usually grow in clusters, if points are missed, then the cover around the tape would be missed out. However, quadrats were suggested to have higher counts than continuous counts/point intercept (Jokiel et al., 2015). A study site in Makassar of Indonesia had gotten a significant result with similar surveying techniques, but the only difference was using quadrant percentage cover count; however, they selectively chose algae dominant sites to survey (Husain et al., 2013).

One final explanation is it really does not have significant correlation, as herbivore biomass declined continuously for the past 5 years, while the algae percentage cover had remained steady, plus the almost lost of browsers, but no macroalgae overgrown, hence, other factors may contribute the low algae cover stats (Adam et al., 2016), however, it does need further investigation to prove it, like collecting more sites data and environmental variables of tide, reef habitats (i.e. back reef; Russ 1984; Cheal, Emslie et al. 2013).

4.2 Limitation of the Research

This survey was constrained to a single season and only involved daytime sampling, fish communities' changes markedly over day and night (Sutherland 2006). This did not account for diel or seasonal changes, especially during Dec - Feb, that is the season of algal peak growth (citation), so how herbivorous fish and algae interacted, and how algae and hard coral interacted during growth season is unknown. Hence, this might not fully reveal the variation changes over seasons.

Also, nearby fishing activities – which may affect the fish data collected, like illegal fishing, areas of reef damage should be recorded which may deviate data collected (Ahmadia, Wilson and Green 2013). Finally, due to resource constraints, environmental variables (i.e wave, visibility, pH) should have been recorded.

4.3 Herbivory Functional Group

4.3.1 Functional group

A pattern of anthropogenic downsizing of herbivores is evident in our study where there is significantly lower biomass of all feeding guilds of herbivorous fishes, aside from the smallest-bodied taxa, in areas accessible to fishing.

The fishes in the grazer/detritivores group (mostly Acanthurids) feed almost exclusively on organic matter/detritus or turf algae. Species in this mid-sized functional group tend to have the highest bite rates and probably are major contributors to the cropping of turf algae, often the first benthic functional group to increase in abundance following coral mortality (Werner et al. 1983, Helfman et al., 2009). Thus, the large impacts will probably happen affecting reef health to certain degree in fished locations if fishing activities continue.

Large scraper/excavator species are responsible for bioerosion and extracting holdfast of macroalgae. These fish were spotted very low in number at surveying time, although they were witnessed schooling other times, which suggests that they were underrepresented in the survey data. In order to get more accurate data, it may be necessary to increase replicates from different sites and habitat complexity, since their occurrences may be affected by tides or wave exposures (Werner et al. 1983, Helfman et al., 2009). The intensity of herbivory by schooling fishes often varies unimodal with depth: low in very shallow water due to wave stress and other factors limiting accessibility by fishes, high at moderate depths, and low in deeper water where coral growth that provides shelter for fishes' declines. The browser and large scraper/excavator functional group were the most susceptible to fishing, these two groups certainly need conservation, as their functional roles are irreplaceable.

Therefore, the government should restrict the use of non-selective fishing equipment such as fish fences, and establish a fishing closed seasons for fish replenishment.

4.3.2 Size Category

Length categorizes showed a lack of short length groups below 7.5cm. It suggests that without fry stage, there is a threat of losing that species.

Investigations suggest that mature fish lay larger eggs, but younger fish are smaller in size and which consequently produce fewer and often smaller eggs, which lessens the chance that any will make it past egg and larval predators and starvation (Werner et al. 1983, Helfman et al., 2009).

This leads to an issue of shifting in the overall size structure from large to small within-species reductions or among-species shifts in relative abundance (Ochavillo et al., 2009). Both have important consequences for the emergent foraging capacity of the herbivore guild, as there are size-dependent effects, within and among species and functional groups, on algal consumption and feeding impacts. As, larger individuals generally consume more algae and expose larger areas of substratum. A shift to smaller-bodied fishes may also result in a loss of key ecological functions provided by the largest species, such as bioerosion and coral predation by large scraper/excavators. (Edwards, Friedlander et al. 2014)

4.4.3 Key Species

The highest biomass was contributed by *Ctenochaetus striatus*, so it was the dominant species. Its role is to feed on turf algae and detritus. This species therefore has a disproportionately large effect on its environment relative to its abundance. Without this keystone species, the ecosystem would be dramatically different or cease to exist altogether.

Additionally, this species has very low numbers of small size individuals, which means protecting the adults is necessary so that there will be sufficient reproduction. Therefore, the government is suggested to set a high priority on protecting the adult from fishermen.

5. Conclusion

Although the correlation was found insignificant between herbivorous fish and algae, grazer/detritivores were clearly higher in Sampela responding degraded, heavily fished and impacted situation.

As mentioned in the introduction, fish herbivore is important transporting nutrient to upper trophic consumers, if they are at risk, the upper carnivore will be at risk. This study showed important findings of the absence of fry stage on all functional groups. The government is recommended to enforce protection urgently on herbivore fry stage (Edwards et al., 2014), by setting restriction of mesh size (releasing ≤ 7.5 cm fish), forbidden of a certain type of gear (Ahamadia et al., 2013; Comeros-Raynal et al., 2012) and even having fishing closed season.

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Appendices

Appendix 1. Benthic Lifeform Categories		
(English, Wilkinson and Baker 1997)		
Categories		Remarks
Algae	Algae Assemblage	Consists of more than one species
	Coralline Algae	
	Halimeda	
	Macroalgae	Weedy/fleshy green, brown, reds
	Turf Algae	Lush filamentous algae, often found inside damselfish territories
Dead Coral with Algae		This coral is standing, skeletal structure can still be seen
Hard Coral	Acropora	
Dead Coral		Recently dead, white to dirty white
Mobile Substrate	Sand	
(not good for coral colony)	Rubble	Unconsolidated coral fragments
	Silt	
	Water	Fissures deeper than 50 cm
	Rock	
Soft Coral		Soft bodied corals
Sponges		
Zoanthids		e.g. <i>Platythoa</i> , <i>Protopalythoa</i>
Others		Ascidians, anenomes, gorgonians, giant clams. Etc.

Appendix 2. Benthic Survey Data Recording Sheet

Date:	Start time:
Site:	End Time:
Transect number:	Surveyor Name:

use the box on the right to record the environmental conditions when the survey was conducted	Water depth (m):	
	Remarks:	

Transect Distance		Benthic Type	Morphology	Further Information	Remarks
Start	end				
0 m					
					
	50 m				

Appendix 3. Screen shot of Benthic Survey Recorded spreadsheet

Benthic Survey Data Recording Sheet							
Date:	20-Jun	Start time:	11:28				
Site:	Ridge	End time:	11:31				
Transect no.:	1	Water Depth:	5m				
For example:		HC - hard coral	SC - Soft coral	DC - Dead Coral	Rubble		
Countinous count (cm)	Length	Benthic Type	Morphory	Further Information	Summary	cm	% cover
0	23	hard coral	branching	Acropora	Algae	240	5.5%
23	42	sponge			Ascidian,	0	0.0%
65	30	algae			Hard Coral,	1215	27.9%
95	15	hard coral	encrusting	Pavona	Dead Coral (Corallites visible),	104	2.4%
110	103	sponge			Dead Coral with Algae,	0	0.0%
213	117	rubble			Rock (no corallites visible),	46	1.1%
330	40	sponge			Rubble,	623	14.3%
370	130	hard coral	branching	Acropora	Sand,	0	0.0%
500	5	sponge			Silt,	90	2.1%
505	15	sponge			Soft Coral,	248	5.7%
520	90	water			Sponge,	1703	39.1%
610	46	rock			Water,	90	2.1%
656	21	hard coral	branching	Acropora	Zoanthid	0	0.0%
677	13	hard coral	branching	Stylophora	Total	4359	100.0%
690	8	rubber					
698	2	hard coral	branching	Stylophora			
700	43	rubber					
743	7	hard coral					
750	150	sponge					
900	140	sponge					
1040	60	hard coral	Foliosa	unknown			
1100	130	unknown					
1230	70	sponge					

Appendix 4. Fish Survey Data Recording Sheet

Date:	Start time:
Site:	End Time:
Transect number:	Surveyor Name:

use the box on the right to record the environmental conditions when the survey was conducted	Water depth (m):	
	Remarks:	

Instructions : Use the table below to record individual fishes species, fork length and count. Species identification will be performed by Event Measure software. Length will be measured from the tip of fish snout to the end of the middle caudal fin rays. Biomass will be derived from the length: weight relationship of each species of fish.

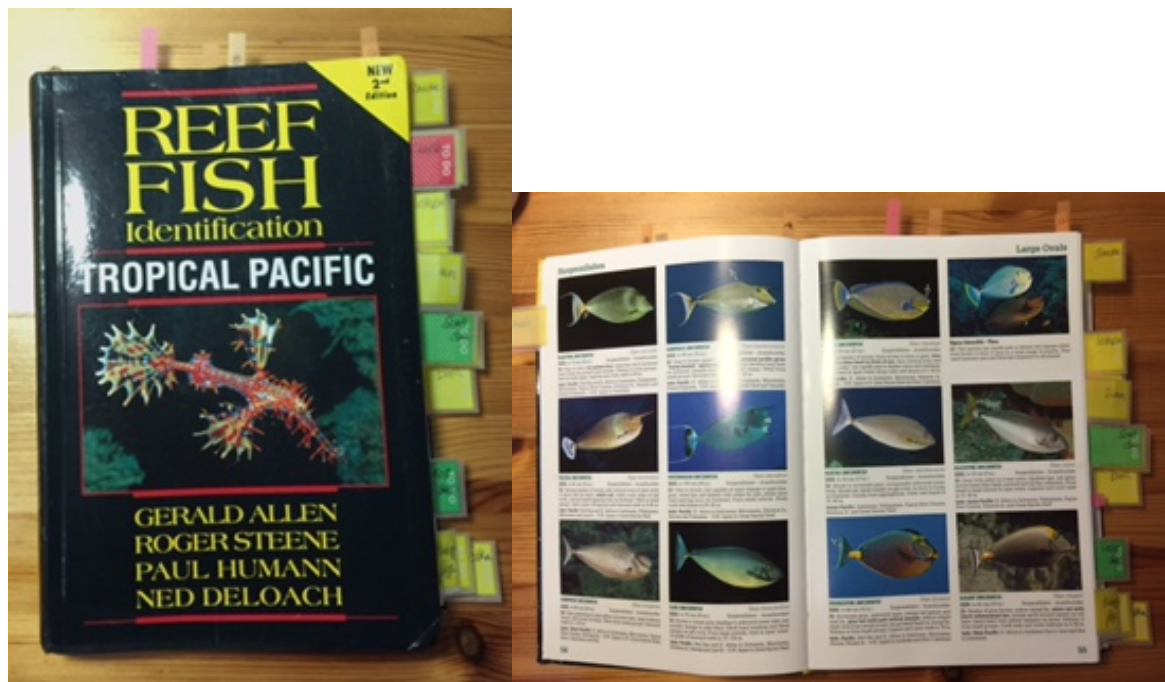
Genus	Species	Frequency	Guild	Length (mm)	A	B	Biomass (g)	Remarks

Notes:

Appendix 5. Screen shot of Fish Survey Recording Spreadsheet

Flat 1											
Family	Genus	Species	Full name	Number	Guild	Length (mm)	Length (cm)	A	B	Biomass (g)	
Acanthuridae	Acanthurus	pyroferus	Acanthurus pyroferus	1	Herbivore	108.298	10.8298	0.0213	3.081	32.81294177	
Acanthuridae	Acanthurus	pyroferus	Acanthurus pyroferus	1	Herbivore	140.617	14.0617	0.0213	3.081	73.36419427	
Scaridae	Chlorurus	sordidus	Chlorurus sordidus	1	Herbivore	126.917	12.6917	0.0145	3.07	35.41390206	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	138.22	13.822	0.0233	3.06	72.02824618	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	143.509	14.3509	0.0233	3.06	80.79901825	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	97.135	9.7135	0.0233	3.06	24.475169	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	120.505	12.0505	0.0233	3.06	47.34034583	
Pomacentridae	Chrysiptera	rex	Chrysiptera rex	1	Herbivore	48.22	4.822	0.151	2.99	16.6658049	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	103.88	10.388	0.0233	3.06	30.05686359	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	586.026	58.6026	0.0233	3.06	5986.618563	
Pomacentridae	Dischistodus	melanotus	Dischistodus melanotus	1	Herbivore	97.658	9.7658	0.0537	2.89	38.92511483	
Scaridae	Cetoscarus	bicolor	Cetoscarus bicolor	1	Herbivore	173.887	17.3887	0.0194	3	102.0006817	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	135.652	13.5652	0.0233	3.06	68.01114661	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	115.386	11.5386	0.0233	3.06	41.45190201	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	128.963	12.8963	0.0233	3.06	58.26117119	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	122.385	12.2385	0.0233	3.06	49.63684727	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	137.451	13.7451	0.0233	3.06	70.80900744	
Acanthuridae	Ctenochaetus	striatus	Ctenochaetus striatus	1	Herbivore	137.148	13.7148	0.0233	3.06	70.33244675	

Appendix 6. Screen shot of Reef Fish Identification Guide Book



Appendix 7. One Way ANOVA's Herbivorous Fish Communities among sites

Justification of using one way ANOVA

As not all kinds of functional fish exist in 3 habitats of each site, there will be lots of zeros on fish raw data. Therefore, two-way ANOVA test cannot be conducted in this situation. Because of this, one-way ANOVA & Tukey post hoc test (if needed) are used. Site will be the fixed factor.

Assumptions

To carry out one-way ANOVA, three assumptions must be met:

Assumption 1: all samples must be independent to each other. Met. Site was independent.

Assumption 2: Data must be normally distributed. Met. Data are normally distributed after $\log(x+1)$ transformation.

```
> Herb_fish <- read.csv(file='Herb_fish_v2.csv',header=T)
> Herb_fish
  Site      Biomass
1  Sampela  7.818898
2  Sampela 84.131330
3  Sampela 40.371604
4  Sampela 58.905418
5  Sampela 143.665918
6  Sampela 88.902593
7  Sampela 52.022278
8  Sampela 93.730825
9  Sampela 46.848892
10     PK 159.146875
11     PK 80.161602
12     PK 23.516809
13     PK 52.422861
14     PK 33.184760
15     PK 24.558812
16     PK 43.022286
17     PK 13.750251
18     PK 11.942260
19  Buoy 60.914362
20  Buoy 55.403929
21  Buoy 62.609837
22  Buoy  2.814021
23  Buoy  1.433281
24  Buoy 20.566547
25  Buoy  4.748136
26  Buoy  0.000000
27  Buoy  2.550014
28  Ridge 31.979830
29  Ridge 37.330064
30  Ridge 59.179640
31  Ridge  4.694060
32  Ridge 33.117487
33  Ridge  4.326926
> shapiro.test(subset(Herb_fish$Biomass, Herb_fish$Site=="Sampela"))

Shapiro-Wilk normality test

data:  subset(Herb_fish$Biomass, Herb_fish$Site == "Sampela")
W = 0.9636, p-value = 0.8351
```

```
> shapiro.test(subset(Herb_fish$Biomass, Herb_fish$Site=="PK"))
```

Shapiro-Wilk normality test

```
data: subset(Herb_fish$Biomass, Herb_fish$Site == "PK")
```

```
W = 0.77508, p-value = 0.01057
```

```
> Herb_fish$Biomass_log = log(Herb_fish$Biomass + 1)
```

```
> Herb_fish
```

	Site	Biomass	Biomass_log
1	Sampela	7.818898	2.1768969
2	Sampela	84.131330	4.4441951
3	Sampela	40.371604	3.7225948
4	Sampela	58.905418	4.0927670
5	Sampela	143.665918	4.9744271
6	Sampela	88.902593	4.4987268
7	Sampela	52.022278	3.9707122
8	Sampela	93.730825	4.5510394
9	Sampela	46.848892	3.8680480
10	PK	159.146875	5.0760914
11	PK	80.161602	4.3964423
12	PK	23.516809	3.1993590
13	PK	52.422861	3.9782388
14	PK	33.184760	3.5317799
15	PK	24.558812	3.2409822
16	PK	43.022286	3.7846960
17	PK	13.750251	2.6912601
18	PK	11.942260	2.5604979
19	Buoy	60.914362	4.1257522
20	Buoy	55.403929	4.0325388
21	Buoy	62.609837	4.1527681
22	Buoy	2.814021	1.3386841
23	Buoy	1.433281	0.8892404
24	Buoy	20.566547	3.0711433
25	Buoy	4.748136	1.7488757
26	Buoy	0.000000	0.0000000
27	Buoy	2.550014	1.2669514
28	Ridge	31.979830	3.4958962
29	Ridge	37.330064	3.6462346
30	Ridge	59.179640	4.0973341
31	Ridge	4.694060	1.7394236
32	Ridge	33.117487	3.5298101
33	Ridge	4.326926	1.6727744


```

> shapiro.test(subset(Herb_fish$Biomass_log, Herb_fish$Site=="Sampela"))

      Shapiro-Wilk normality test

data:  subset(Herb_fish$Biomass_log, Herb_fish$Site == "Sampela")
W = 0.84999, p-value = 0.07449

> shapiro.test(subset(Herb_fish$Biomass_log, Herb_fish$Site=="PK"))

      Shapiro-Wilk normality test

data:  subset(Herb_fish$Biomass_log, Herb_fish$Site == "PK")
W = 0.96659, p-value = 0.8641

> shapiro.test(subset(Herb_fish$Biomass_log, Herb_fish$Site=="Buoy"))

      Shapiro-Wilk normality test

data:  subset(Herb_fish$Biomass_log, Herb_fish$Site == "Buoy")
W = 0.88771, p-value = 0.189

> shapiro.test(subset(Herb_fish$Biomass_log, Herb_fish$Site=="Ridge"))

      Shapiro-Wilk normality test

data:  subset(Herb_fish$Biomass_log, Herb_fish$Site == "Ridge")
W = 0.80062, p-value = 0.05953

```

Assumption 3: all samples must have equal variance. Met. As data from biomass of total herbivorous fish among sites had equal variance using Bartlett test ($P=0.1705>0.05$)

```

> bartlett.test(Biomass_log ~ Site, data=Herb_fish)

      Bartlett test of homogeneity of variances

data:  Biomass_log by Site
Bartlett's K-squared = 5.0178, df = 3, p-value = 0.1705

```

Calculation of one way ANOVA on herbivorous fish

```
> attach(Herb_fish)
> attach(Herb_fish)
The following objects are masked from Herb_fish (pos = 3):

    Biomass, Biomass_log, Site

> Biomass_log
 [1] 2.1768969 4.4441951 3.7225948 4.0927670 4.9744271 4.4987268 3.9707122
 [8] 4.5510394 3.8680480 5.0760914 4.3964423 3.1993590 3.9782388 3.5317799
[15] 3.2409822 3.7846960 2.6912601 2.5604979 4.1257522 4.0325388 4.1527681
[22] 1.3386841 0.8892404 3.0711433 1.7488757 0.0000000 1.2669514 3.4958962
[29] 3.6462346 4.0973341 1.7394236 3.5298101 1.6727744

> Site
 [1] Sampela Sampela Sampela Sampela Sampela Sampela Sampela Sampela Sampela
[10] PK      PK      PK      PK      PK      PK      PK      PK      PK
[19] Buoy    Buoy    Buoy    Buoy    Buoy    Buoy    Buoy    Buoy    Buoy
[28] Ridge   Ridge   Ridge   Ridge   Ridge   Ridge

Levels: Buoy PK Ridge Sampela
> fit <- aov(Biomass_log~Site)
> summary(fit)
              Df Sum Sq Mean Sq F value Pr(>F)
Site           3  15.22    5.072   4.116 0.0151 *
Residuals     29  35.74    1.232
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> TukeyHSD(fit)
    Tukey multiple comparisons of means
      95% family-wise confidence level

Fit: aov(formula = Biomass_log ~ Site)

$Site
              diff            lwr            upr           p adj
PK-Buoy         1.3148215 -0.1109396  2.740583 0.0789483
Ridge-Buoy       0.7384728 -0.8555766  2.332522 0.5935586
Sampela-Buoy     1.7414948  0.3157337  3.167256 0.0120832
Ridge-PK        -0.5763487 -2.1703980  1.017701 0.7589646
Sampela-PK       0.4266733 -0.9990878  1.852434 0.8467241
Sampela-Ridge   1.0030220 -0.5910274  2.597071 0.3348017
```

Appendix 8. One Way ANOVA's Grazing fish among sites

Assumptions

To carry out one-way ANOVA, three assumptions must be met:

Assumption 1: all samples must be independent to each other. Met. Site was independent.

Assumption 2: Data must be normally distributed. Met. Data are normally distributed after $\log(x+1)$ transformation.

```
> Grazers <- read.csv(file='Grazers_v2.csv',header=T)
> Grazers
  Site    Biomass
1  Sampela  7.818898
2  Sampela 81.991673
3  Sampela 35.262076
4  Sampela 20.618735
5  Sampela 140.528317
6  Sampela 88.902593
7  Sampela 30.899038
8  Sampela 91.278518
9  Sampela 42.888437
10 PK 144.370009
11 PK 77.446947
12 PK 12.219781
13 PK 44.071531
14 PK 33.184760
15 PK 24.558812
16 PK 30.865034
17 PK 13.750251
18 PK 11.942260
19 Buoy 60.914362
20 Buoy 48.364889
21 Buoy 62.609837
22 Buoy 2.814021
23 Buoy 1.433281
24 Buoy 20.566547
25 Buoy 4.748136
26 Buoy 0.000000
27 Buoy 2.550014
28 Ridge 2.678249
29 Ridge 8.285877
30 Ridge 26.962413
31 Ridge 4.694060
32 Ridge 21.664146
33 Ridge 4.326926
> shapiro.test(subset(Grazers$Biomass, Grazers$Site=="Sampela"))

      Shapiro-Wilk normality test

data:  subset(Grazers$Biomass, Grazers$Site == "Sampela")
W = 0.92405, p-value = 0.4268
```

```
> shapiro.test(subset(Grazers$Biomass, Grazers$Site=="PK"))
```

Shapiro-Wilk normality test

```
data: subset(Grazers$Biomass, Grazers$Site == "PK")
W = 0.75769, p-value = 0.006656
```

```
> Grazers$Biomass_log = log(Grazers$Biomass + 1)
```

```
> Grazers
```

	Site	Biomass	Biomass_log
1	Sampela	7.818898	2.1768969
2	Sampela	81.991673	4.4187403
3	Sampela	35.262076	3.5907724
4	Sampela	20.618735	3.0735603
5	Sampela	140.528317	4.9524998
6	Sampela	88.902593	4.4987268
7	Sampela	30.899038	3.4625759
8	Sampela	91.278518	4.5248114
9	Sampela	42.888437	3.7816509
10	PK	144.370009	4.9792823
11	PK	77.446947	4.3624226
12	PK	12.219781	2.5817143
13	PK	44.071531	3.8082508
14	PK	33.184760	3.5317799
15	PK	24.558812	3.2409822
16	PK	30.865034	3.4615093
17	PK	13.750251	2.6912601
18	PK	11.942260	2.5604979
19	Buoy	60.914362	4.1257522
20	Buoy	48.364889	3.8992394
21	Buoy	62.609837	4.1527681
22	Buoy	2.814021	1.3386841
23	Buoy	1.433281	0.8892404
24	Buoy	20.566547	3.0711433
25	Buoy	4.748136	1.7488757
26	Buoy	0.000000	0.0000000
27	Buoy	2.550014	1.2669514
28	Ridge	2.678249	1.3024368
29	Ridge	8.285877	2.2284947
30	Ridge	26.962413	3.3308612
31	Ridge	4.694060	1.7394236
32	Ridge	21.664146	3.1207842
33	Ridge	4.326926	1.6727744

```
> shapiro.test(subset(Grazers$Biomass_log, Grazers$Site=="Sampela"))
```

```
Shapiro-Wilk normality test
```

```
data: subset(Grazers$Biomass_log, Grazers$Site == "Sampela")  
W = 0.94521, p-value = 0.6377
```

```
> shapiro.test(subset(Grazers$Biomass_log, Grazers$Site=="PK"))
```

```
Shapiro-Wilk normality test
```

```
data: subset(Grazers$Biomass_log, Grazers$Site == "PK")  
W = 0.92614, p-value = 0.4454
```

```
> shapiro.test(subset(Grazers$Biomass_log, Grazers$Site=="Buoy"))
```

```
Shapiro-Wilk normality test
```

```
data: subset(Grazers$Biomass_log, Grazers$Site == "Buoy")  
W = 0.89676, p-value = 0.2338
```

```
> shapiro.test(subset(Grazers$Biomass_log, Grazers$Site=="Ridge"))
```

```
Shapiro-Wilk normality test
```

```
data: subset(Grazers$Biomass_log, Grazers$Site == "Ridge")  
W = 0.90023, p-value = 0.3753
```

Assumption 3: all samples must have equal variance. Met. As data from biomass of grazing fish among sites had equal variance using Bartlett test ($P=0.1908>0.05$).

```
> bartlett.test(Grazers$Biomass_log ~ Grazers$Site, data=Grazers)
```

```
Bartlett test of homogeneity of variances
```

```
data: Grazers$Biomass_log by Grazers$Site  
Bartlett's K-squared = 4.7531, df = 3, p-value = 0.1908
```

Calculation of one way ANOVA on grazing fish

```
> fit <- aov(Grazers$Biomass_log~Grazers$Site)
> summary(fit)
              Df Sum Sq Mean Sq F value Pr(>F)
Grazers$Site   3  16.42    5.474   4.614 0.0093 **
Residuals     29  34.41    1.187
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> TukeyHSD(fit)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Grazers$Biomass_log ~ Grazers$Site)

$`Grazers$Site`
              diff          lwr          upr          p adj
PK-Buoy         1.19167162 -0.2073452  2.5906885  0.1165846
Ridge-Buoy      -0.04449914 -1.6086475  1.5196493  0.9998289
Sampela-Buoy     1.55417555  0.1551587  2.9531924  0.0250397
Ridge-PK        -1.23617076 -2.8003192  0.3279776  0.1605639
Sampela-PK       0.36250393 -1.0365129  1.7615208  0.8939073
Sampela-Ridge    1.59867470  0.0345263  3.1628231  0.0437264
```


Appendix 9. Two Way ANOVA's Algae of benthic composition among sites

Justification of using one way ANOVA

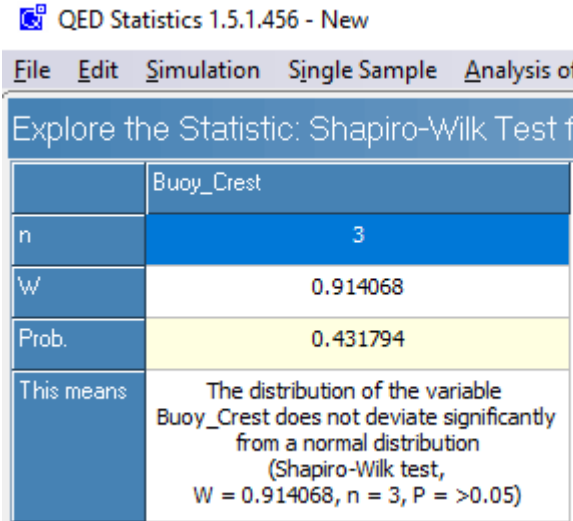
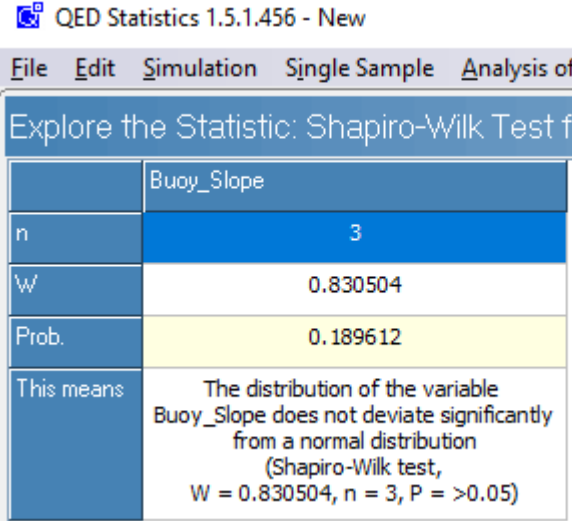
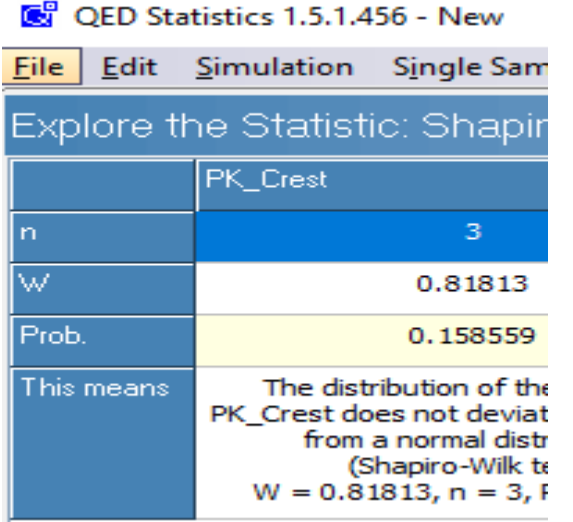
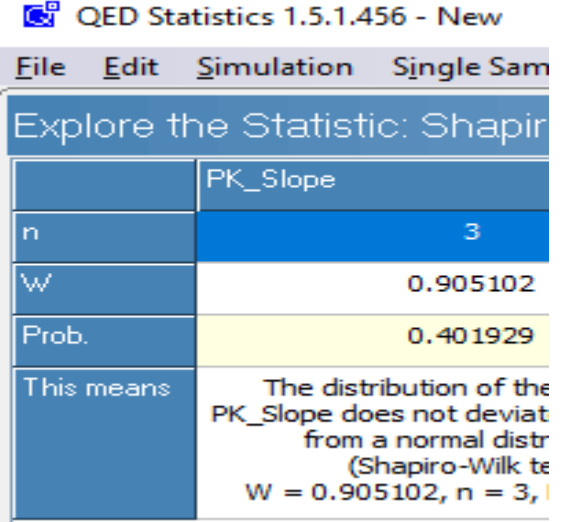
As there are two sets of factors (Site and Habitat) per sample and not only findings on two sets of comparisons, but also their interaction effect, so two-way ANOVA is best fit to use (Stevens 1998).

Assumptions

To carry out two-way ANOVA, three assumptions must be met:

Assumption 1: all samples must be independent to each other. Met. Site and Habitat were independent.

Assumption 2: Data must be normally distributed. Met. Data are normally distributed.

(a)  QED Statistics 1.5.1.456 - New File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test f <table border="1"> <tr> <td></td> <td>Buoy_Crest</td> </tr> <tr> <td>n</td> <td>3</td> </tr> <tr> <td>W</td> <td>0.914068</td> </tr> <tr> <td>Prob.</td> <td>0.431794</td> </tr> <tr> <td>This means</td> <td>The distribution of the variable Buoy_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.914068, n = 3, P = >0.05)</td> </tr> </table>		Buoy_Crest	n	3	W	0.914068	Prob.	0.431794	This means	The distribution of the variable Buoy_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.914068, n = 3, P = >0.05)	(b)  QED Statistics 1.5.1.456 - New File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test f <table border="1"> <tr> <td></td> <td>Buoy_Slope</td> </tr> <tr> <td>n</td> <td>3</td> </tr> <tr> <td>W</td> <td>0.830504</td> </tr> <tr> <td>Prob.</td> <td>0.189612</td> </tr> <tr> <td>This means</td> <td>The distribution of the variable Buoy_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.830504, n = 3, P = >0.05)</td> </tr> </table>		Buoy_Slope	n	3	W	0.830504	Prob.	0.189612	This means	The distribution of the variable Buoy_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.830504, n = 3, P = >0.05)
	Buoy_Crest																				
n	3																				
W	0.914068																				
Prob.	0.431794																				
This means	The distribution of the variable Buoy_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.914068, n = 3, P = >0.05)																				
	Buoy_Slope																				
n	3																				
W	0.830504																				
Prob.	0.189612																				
This means	The distribution of the variable Buoy_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.830504, n = 3, P = >0.05)																				
(c)  QED Statistics 1.5.1.456 - New File Edit Simulation Single Sam Explore the Statistic: Shapir <table border="1"> <tr> <td></td> <td>PK_Crest</td> </tr> <tr> <td>n</td> <td>3</td> </tr> <tr> <td>W</td> <td>0.81813</td> </tr> <tr> <td>Prob.</td> <td>0.158559</td> </tr> <tr> <td>This means</td> <td>The distribution of the PK_Crest does not deviat from a normal distr (Shapiro-Wilk te W = 0.81813, n = 3, f</td> </tr> </table>		PK_Crest	n	3	W	0.81813	Prob.	0.158559	This means	The distribution of the PK_Crest does not deviat from a normal distr (Shapiro-Wilk te W = 0.81813, n = 3, f	(d)  QED Statistics 1.5.1.456 - New File Edit Simulation Single Sam Explore the Statistic: Shapir <table border="1"> <tr> <td></td> <td>PK_Slope</td> </tr> <tr> <td>n</td> <td>3</td> </tr> <tr> <td>W</td> <td>0.905102</td> </tr> <tr> <td>Prob.</td> <td>0.401929</td> </tr> <tr> <td>This means</td> <td>The distribution of the PK_Slope does not deviat from a normal distr (Shapiro-Wilk te W = 0.905102, n = 3, l</td> </tr> </table>		PK_Slope	n	3	W	0.905102	Prob.	0.401929	This means	The distribution of the PK_Slope does not deviat from a normal distr (Shapiro-Wilk te W = 0.905102, n = 3, l
	PK_Crest																				
n	3																				
W	0.81813																				
Prob.	0.158559																				
This means	The distribution of the PK_Crest does not deviat from a normal distr (Shapiro-Wilk te W = 0.81813, n = 3, f																				
	PK_Slope																				
n	3																				
W	0.905102																				
Prob.	0.401929																				
This means	The distribution of the PK_Slope does not deviat from a normal distr (Shapiro-Wilk te W = 0.905102, n = 3, l																				
(e)	(f)																				

QED Statistics 1.5.1.456 - New File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr><td></td><td>Ridge_Crest</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.992483</td></tr> <tr><td>Prob.</td><td>0.834203</td></tr> <tr><td>This means</td><td>The distribution of the variable Ridge_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.992483, n = 3, P = >0.05)</td></tr> </table>		Ridge_Crest	n	3	W	0.992483	Prob.	0.834203	This means	The distribution of the variable Ridge_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.992483, n = 3, P = >0.05)	QED Statistics 1.5.1.456 - New File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr><td></td><td>Ridge_Slope</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.847674</td></tr> <tr><td>Prob.</td><td>0.23425</td></tr> <tr><td>This means</td><td>The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.847674, n = 3, P = >0.05)</td></tr> </table>		Ridge_Slope	n	3	W	0.847674	Prob.	0.23425	This means	The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.847674, n = 3, P = >0.05)
	Ridge_Crest																				
n	3																				
W	0.992483																				
Prob.	0.834203																				
This means	The distribution of the variable Ridge_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.992483, n = 3, P = >0.05)																				
	Ridge_Slope																				
n	3																				
W	0.847674																				
Prob.	0.23425																				
This means	The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.847674, n = 3, P = >0.05)																				
(g) QED Statistics 1.5.1.456 - New File Edit Simulation Single Sample Analysis of Fr Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr><td></td><td>Sampela_Crest</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.991908</td></tr> <tr><td>Prob.</td><td>0.82796</td></tr> <tr><td>This means</td><td>The distribution of the variable Sampela_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.991908, n = 3, P = >0.05)</td></tr> </table>		Sampela_Crest	n	3	W	0.991908	Prob.	0.82796	This means	The distribution of the variable Sampela_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.991908, n = 3, P = >0.05)	(h) QED Statistics 1.5.1.456 - New File Edit Simulation Single Sample Analysis of Fr Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr><td></td><td>Sampela_Slope</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.921576</td></tr> <tr><td>Prob.</td><td>0.457907</td></tr> <tr><td>This means</td><td>The distribution of the variable Sampela_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.921576, n = 3, P = >0.05)</td></tr> </table>		Sampela_Slope	n	3	W	0.921576	Prob.	0.457907	This means	The distribution of the variable Sampela_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.921576, n = 3, P = >0.05)
	Sampela_Crest																				
n	3																				
W	0.991908																				
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Appendix 9.1. Shapiro-Wilk test proved all data in normality with all P values>0.05 (a) Buoy and crest, P=0.432. (b) Buoy and slope, P=0.190. (c) Pak Ksim and crest, P=0.159. (d) Pak Ksim and slope, P=0.402. (e) Ridge and crest, P=0.834. (f) Ridge and slope, P=0.234. (g) Sampela and crest, P=0.828. (h) Sampela and slope, P=0.458																					

Assumption 3: all samples must have equal variance. Met. Site and Habitat had equal variance by Bartlett's test, $P=0.456681 > 0.05$

Homogeneity of Variance Tests			
Test	Statistic	DF1	DF2
Hartley Fmax	9.16245	8	2
Cochran C	0.264283	8	2
Bartlett Chi-square	6.7378	7	
Bartlett Probability > Chi-Square	0.456681		

Calculations

Three null hypotheses, H_0 :

01. The percentage cover of algae by site (Buoy, Pak Ksim, Ridge and Sampela) factor were the same.
02. The percentage cover of algae by habitat (Crest and Slope) factor were the same.
03. There was no interaction between the two factors (Site and Habitat)

Two way ANOVA

Column Effect Type
☒ Fixed ☐ Random

2
 Rows Effect Type
☒ Fixed ☐ Random

	Column: 1	Column: 2	Column: 3	Column: 4
Row: 1	Buoy_Crest	PK_Crest	Ridge_Crest	Sampela_Crest
Row: 2	Buoy_Slope	PK_Slope	Ridge_Slope	Sampela_Slope

Multiple Comparisons Test level
 0.050

Multiple Comparisons
☒ None ☐ Scheffe ☐ Tukey-Kramer
☐ Tukey ☐ Newman-Keuls ☐ Bonferroni

Available data

	Buoy_Crest	PK_Crest	Ridge_Crest	Sampela_Crest	Buoy_Slope	PK_Slope	Ridge_Slope	Sampela_Slope
Row 1	0.099253731	0.029030266	0.028699369	0.246252677	0.070895522	0.023843489	0.0550585	0.1838
Row 2	0.080348259	0.139320185	0.090909091	0.049749164	0.061442786	0.157338308	0.046434494	0.239433551
Row 3	0.174875622	0.02247191	0.15920398	0.114427861	0.009452736	0.04309996	0.129353234	0.059701493

OK

File	Edit	Simulation	Single Sample	Analysis of Frequency	Two Samples	Regression	ANOVAs	GLM	Planning
Explore the Statistic: Two way ANOVA									
Two Way ANOVA									
	DF	SS	MS	F	Prob. >F	Omega ²			
Between Columns	3	0.0228899	0.00762995	1.60849	0.226731	0.0769597			
Between Rows	1	0.000997016	0.000997016	0.210183	0.65279	0			
Interaction	3	0.00798847	0.00266282	0.561356	0.648205	0			
Within Groups/Error	16	0.0758969	0.00474356						
Total	23	0.107772	0.00468575						
Omega ²	0								
	Means and Variability								
Group	ID	Mean	Variance	StdD	N				
Cell	Buoy_Crest	0.118159	0.00250192	0.0500192	3				
Cell	Buoy_Slope	0.0472637	0.00109459	0.0330846	3				
Cell	PK_Crest	0.0636075	0.00431007	0.0656511	3				
Cell	PK_Slope	0.0747606	0.00520701	0.0721596	3				
Cell	Ridge_Crest	0.0929375	0.00426095	0.0652759	3				
Cell	Ridge_Slope	0.0769487	0.00207827	0.045588	3				
Cell	Sampela_Crest	0.13681	0.0100291	0.100146	3				
Cell	Sampela_Slope	0.160978	0.00846652	0.0920137	3				
Column	1	0.0827114	0.00294646	0.0542813	6				
Column	2	0.069184	0.00384415	0.0620012	6				
Column	3	0.0849431	0.00261238	0.0511114	6				
Column	4	0.148894	0.00757349	0.0870258	6				
Row	1	0.102879	0.00466198	0.0682787	12				
Row	2	0.0899878	0.00504486	0.0710272	12				
	Total	0.0964332	0.00468575	0.0684525	24				

Appendix 9.2: The result screen performed on algae data by the two-way ANOVA.

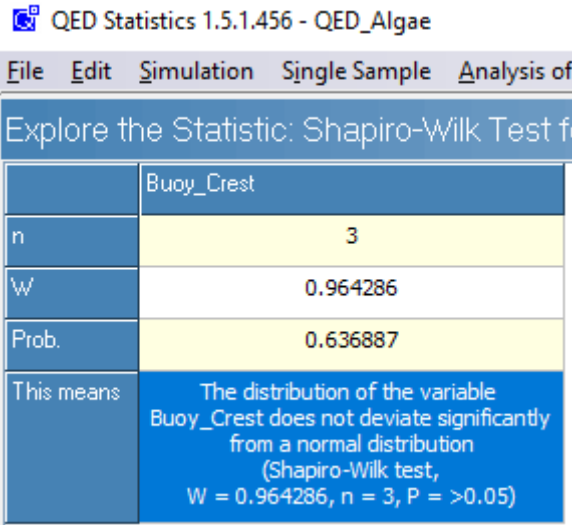
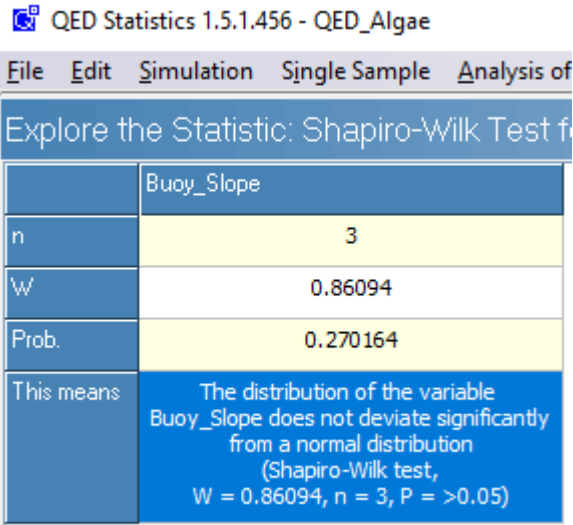
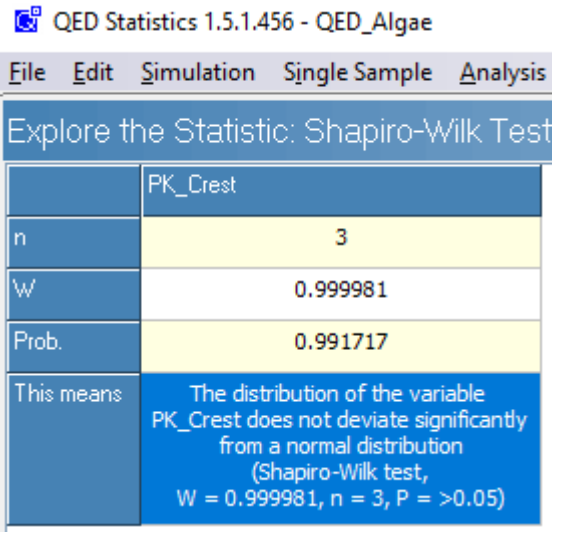
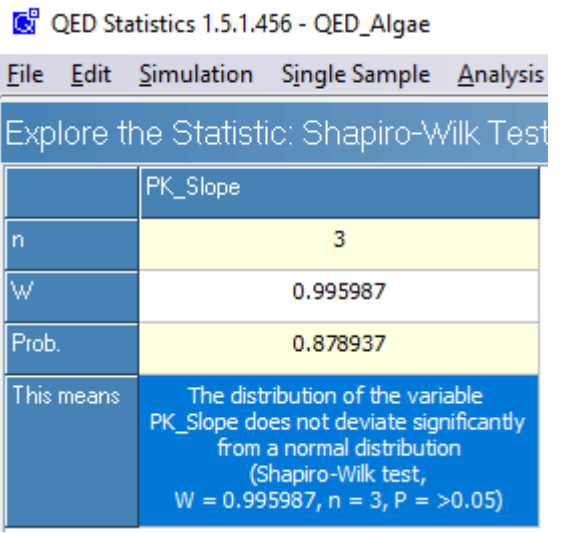
Appendix 10. Two Way ANOVA's Hard Coral of benthic composition among sites

Assumptions

To carry out two-way ANOVA, three assumptions must be met:

Assumption 1: all samples must be independent to each other. Met. Site and Habitat were independent.

Assumption 2: Data must be normally distributed. Met. Data are normally distributed.

(a)  QED Statistics 1.5.1.456 - QED_Algaee File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr> <td></td> <td>Buoy_Crest</td> </tr> <tr> <td>n</td> <td>3</td> </tr> <tr> <td>W</td> <td>0.964286</td> </tr> <tr> <td>Prob.</td> <td>0.636887</td> </tr> <tr> <td>This means</td> <td>The distribution of the variable Buoy_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.964286$, $n = 3$, $P = >0.05$)</td> </tr> </table>		Buoy_Crest	n	3	W	0.964286	Prob.	0.636887	This means	The distribution of the variable Buoy_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.964286$, $n = 3$, $P = >0.05$)	(b)  QED Statistics 1.5.1.456 - QED_Algaee File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr> <td></td> <td>Buoy_Slope</td> </tr> <tr> <td>n</td> <td>3</td> </tr> <tr> <td>W</td> <td>0.86094</td> </tr> <tr> <td>Prob.</td> <td>0.270164</td> </tr> <tr> <td>This means</td> <td>The distribution of the variable Buoy_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.86094$, $n = 3$, $P = >0.05$)</td> </tr> </table>		Buoy_Slope	n	3	W	0.86094	Prob.	0.270164	This means	The distribution of the variable Buoy_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.86094$, $n = 3$, $P = >0.05$)
	Buoy_Crest																				
n	3																				
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(c)  QED Statistics 1.5.1.456 - QED_Algaee File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr> <td></td> <td>PK_Crest</td> </tr> <tr> <td>n</td> <td>3</td> </tr> <tr> <td>W</td> <td>0.999981</td> </tr> <tr> <td>Prob.</td> <td>0.991717</td> </tr> <tr> <td>This means</td> <td>The distribution of the variable PK_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.999981$, $n = 3$, $P = >0.05$)</td> </tr> </table>		PK_Crest	n	3	W	0.999981	Prob.	0.991717	This means	The distribution of the variable PK_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.999981$, $n = 3$, $P = >0.05$)	(d)  QED Statistics 1.5.1.456 - QED_Algaee File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr> <td></td> <td>PK_Slope</td> </tr> <tr> <td>n</td> <td>3</td> </tr> <tr> <td>W</td> <td>0.995987</td> </tr> <tr> <td>Prob.</td> <td>0.878937</td> </tr> <tr> <td>This means</td> <td>The distribution of the variable PK_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.995987$, $n = 3$, $P = >0.05$)</td> </tr> </table>		PK_Slope	n	3	W	0.995987	Prob.	0.878937	This means	The distribution of the variable PK_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.995987$, $n = 3$, $P = >0.05$)
	PK_Crest																				
n	3																				
W	0.999981																				
Prob.	0.991717																				
This means	The distribution of the variable PK_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, $W = 0.999981$, $n = 3$, $P = >0.05$)																				
	PK_Slope																				
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(e)	(f)																				

QED Statistics 1.5.1.456 - QED_Algae File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test for <table> <tr><td></td><td>Ridge_Crest</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.968626</td></tr> <tr><td>Prob.</td><td>0.659916</td></tr> <tr><td>This means</td><td>The distribution of the variable Ridge_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.968626, n = 3, P = >0.05)</td></tr> </table>		Ridge_Crest	n	3	W	0.968626	Prob.	0.659916	This means	The distribution of the variable Ridge_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.968626, n = 3, P = >0.05)	QED Statistics 1.5.1.456 - QED_Algae File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test for <table> <tr><td></td><td>Ridge_Slope</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.976591</td></tr> <tr><td>Prob.</td><td>0.706638</td></tr> <tr><td>This means</td><td>The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.976591, n = 3, P = >0.05)</td></tr> </table>		Ridge_Slope	n	3	W	0.976591	Prob.	0.706638	This means	The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.976591, n = 3, P = >0.05)
	Ridge_Crest																				
n	3																				
W	0.968626																				
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n	3																				
W	0.976591																				
Prob.	0.706638																				
This means	The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.976591, n = 3, P = >0.05)																				
(g) QED Statistics 1.5.1.456 - QED_Algae File Edit Simulation Single Sample Analysis of Fre Explore the Statistic: Shapiro-Wilk Test for <table> <tr><td></td><td>Sampela_Crest</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.846281</td></tr> <tr><td>Prob.</td><td>0.230553</td></tr> <tr><td>This means</td><td>The distribution of the variable Sampela_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.846281, n = 3, P = >0.05)</td></tr> </table>		Sampela_Crest	n	3	W	0.846281	Prob.	0.230553	This means	The distribution of the variable Sampela_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.846281, n = 3, P = >0.05)	(h) QED Statistics 1.5.1.456 - QED_Algae File Edit Simulation Single Sample Analysis of Fre Explore the Statistic: Shapiro-Wilk Test for <table> <tr><td></td><td>Sampela_Slope</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.973808</td></tr> <tr><td>Prob.</td><td>0.689541</td></tr> <tr><td>This means</td><td>The distribution of the variable Sampela_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.973808, n = 3, P = >0.05)</td></tr> </table>		Sampela_Slope	n	3	W	0.973808	Prob.	0.689541	This means	The distribution of the variable Sampela_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.973808, n = 3, P = >0.05)
	Sampela_Crest																				
n	3																				
W	0.846281																				
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Appendix 10.1. Shapiro-Wilk test proved all data in normality with all P values>0.05 (a) Buoy and crest, P=0.637. (b) Buoy and slope, P=0.270. (c) Pak Ksim and crest, P=0.992. (d) Pak Ksim and slope, P=0.879. (e) Ridge and crest, P=0.660. (f) Ridge and slope, P=0.707. (g) Sampela and crest, P=0.231. (h) Sampela and slope, P=0.690																					

Assumption 3: all samples must have equal variance. Met. Site and Habitat had equal variance by Bartlett's test, $P=0.116 > 0.05$

Homogeneity of Variance Tests			
Test	Statistic	DF1	DF2
Hartley Fmax	18.2373	8	2
Cochran C	0.288331	8	2
Bartlett Chi-square	11.547	7	
Bartlett Probability > Chi-Square	0.116473		

Calculations

Three null hypotheses, H_0 :

01. The percentage cover of hard coral by site (Buoy, Pak Ksim, Ridge and Sampela) factor were the same.
02. The percentage cover of hard coral by habitat (Crest and Slope) factor were the same.
03. There was no interaction between the two factors (Site and Habitat)

Two way ANOVA

4
 Rows Effect Type
☒ Fixed
☐ Random

Column Effect Type
☒ Fixed ☐ Random

	Column: 1	Column: 2	Column: 3	Column: 4
Row: 1	Buoy_Crest	PK_Crest	Ridge_Crest	Sampela_Crest
Row: 2	Buoy_Slope	PK_Slope	Ridge_Slope	Sampela_Slope

Multiple Comparisons Test level: 0.050

☒ None
☐ Tukey

☐ Scheffe
☐ Newman-Keuls

☐ Tukey-Kramer
☐ Bonferroni

Available data

	Buoy_Crest	PK_Crest	Ridge_Crest	Sampela_Crest	Buoy_Slope	PK_Slope	Ridge_Slope	Sampela_Slope
Row 1	0.345074627	0.143710109	0.144718095	0.078372591	0.111641791	0.12125535	0.278733655	0.0912
Row 2	0.324776119	0.276122535	0.060606061	0.039715719	0.197910448	0.233830846	0.210406302	0.129847495
Row 3	0.355223881	0.209419077	0.089552239	0.044776119	0.213367347	0.183731283	0.253731343	0.069651741

File	Edit	Simulation	Single Sample	Analysis of Frequency	Two Samples	Regression	ANOVAs	GLM	Planning	Help
Explore the Statistic: Two way ANOVA										
Two Way ANOVA										
	DF	SS	MS	F	Prob.>F	Omega ²				
Between Columns	3	0.102969	0.0343229	18.0617	2.17496E-005	0.452751				
Between Rows	1	1.17035E-005	1.17035E-005	0.00615873	0.938422	0				
Interaction	3	0.0795515	0.0265172	13.9541	9.90743E-005	0.343752				
Within Groups/Error	16	0.030405	0.00190031							
Total	23	0.212937	0.00925812							
Omega ²	0.787711									
	Means and Variability									
Group	ID	Mean	Variance	StdD	N					
Cell	Buoy_Crest	0.341692	0.000240351	0.0155033	3					
Cell	Buoy_Slope	0.174307	0.00300488	0.0548168	3					
Cell	PK_Crest	0.209751	0.00438335	0.0662068	3					
Cell	PK_Slope	0.179606	0.00318107	0.056401	3					
Cell	Ridge_Crest	0.0982921	0.001826	0.0427317	3					
Cell	Ridge_Slope	0.247624	0.00119513	0.0345707	3					
Cell	Sampela_Crest	0.0542881	0.000441447	0.0210106	3					
Cell	Sampela_Slope	0.0968997	0.000930248	0.0305	3					
Column	1	0.257999	0.00970342	0.0985059	6					
Column	2	0.194678	0.00329838	0.0574315	6					
Column	3	0.172958	0.00789843	0.0888731	6					
Column	4	0.0755939	0.0010934	0.0330666	6					
Row	1	0.176006	0.014738	0.1214	12					
Row	2	0.174609	0.00461886	0.0679622	12					
	Total	0.175307	0.00925812	0.0962191	24					

Appendix 10.2: The result screen performed on hard coral data by the two-way ANOVA.

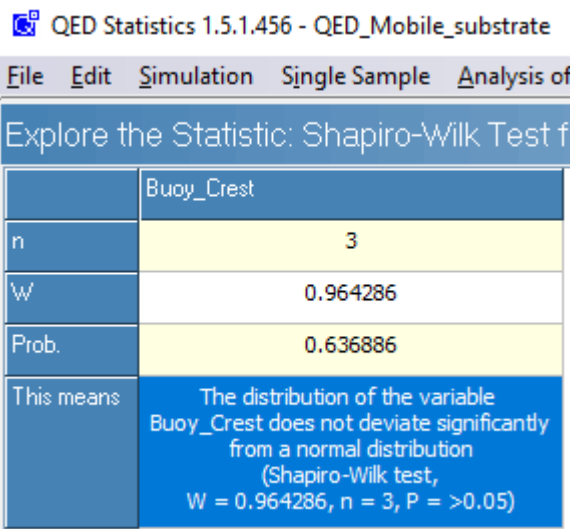
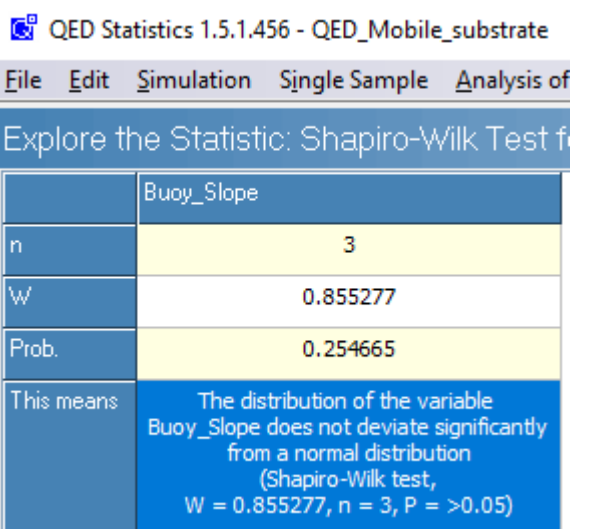
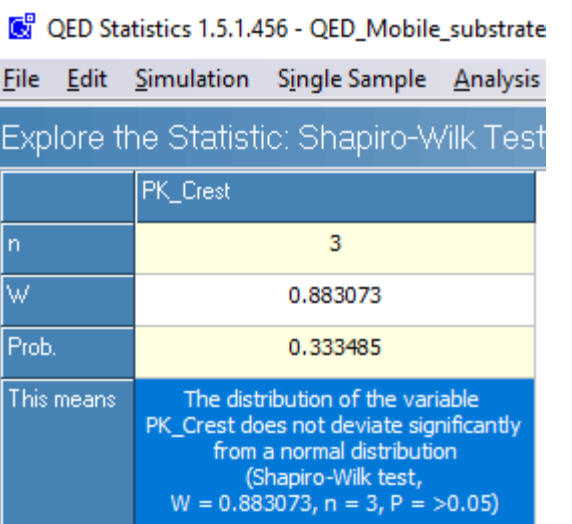
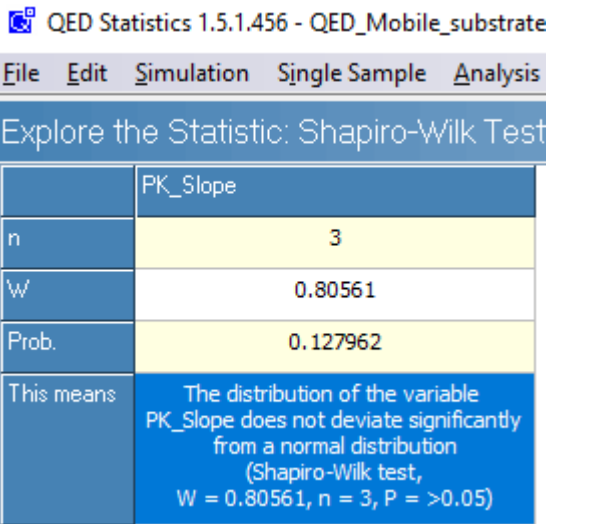
Appendix 11. Two Way ANOVA's Mobile substrate of benthic composition among sites

Assumptions

To carry out two-way ANOVA, three assumptions must be met:

Assumption 1: all samples must be independent to each other. Met. Site and Habitat were independent.

Assumption 2: Data must be normally distributed. Met. Data are normally distributed.

(a) 	(b) 
(c) 	(d) 
(e)	(f)

QED Statistics 1.5.1.456 - QED_Mobile_substrate File Edit Simulation Single Sample Analysis of I Explore the Statistic: Shapiro-Wilk Test fo <table border="1"> <tr><td></td><td>Ridge_Crest</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.974448</td></tr> <tr><td>Prob.</td><td>0.69339</td></tr> <tr><td>This means</td><td>The distribution of the variable Ridge_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.974448, n = 3, P = >0.05)</td></tr> </table>		Ridge_Crest	n	3	W	0.974448	Prob.	0.69339	This means	The distribution of the variable Ridge_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.974448, n = 3, P = >0.05)	QED Statistics 1.5.1.456 - QED_Mobile_substrate File Edit Simulation Single Sample Analysis of Explore the Statistic: Shapiro-Wilk Test fo <table border="1"> <tr><td></td><td>Ridge_Slope</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.934307</td></tr> <tr><td>Prob.</td><td>0.504965</td></tr> <tr><td>This means</td><td>The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.934307, n = 3, P = >0.05)</td></tr> </table>		Ridge_Slope	n	3	W	0.934307	Prob.	0.504965	This means	The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.934307, n = 3, P = >0.05)
	Ridge_Crest																				
n	3																				
W	0.974448																				
Prob.	0.69339																				
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n	3																				
W	0.934307																				
Prob.	0.504965																				
This means	The distribution of the variable Ridge_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.934307, n = 3, P = >0.05)																				
(g) QED Statistics 1.5.1.456 - QED_Mobile_substrate File Edit Simulation Single Sample Analysis of Fr Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr><td></td><td>Sampela_Crest</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.990015</td></tr> <tr><td>Prob.</td><td>0.80884</td></tr> <tr><td>This means</td><td>The distribution of the variable Sampela_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.990015, n = 3, P = >0.05)</td></tr> </table>		Sampela_Crest	n	3	W	0.990015	Prob.	0.80884	This means	The distribution of the variable Sampela_Crest does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.990015, n = 3, P = >0.05)	(h) QED Statistics 1.5.1.456 - QED_Mobile_substrate File Edit Simulation Single Sample Analysis of Fre Explore the Statistic: Shapiro-Wilk Test for <table border="1"> <tr><td></td><td>Sampela_Slope</td></tr> <tr><td>n</td><td>3</td></tr> <tr><td>W</td><td>0.951143</td></tr> <tr><td>Prob.</td><td>0.574335</td></tr> <tr><td>This means</td><td>The distribution of the variable Sampela_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.951143, n = 3, P = >0.05)</td></tr> </table>		Sampela_Slope	n	3	W	0.951143	Prob.	0.574335	This means	The distribution of the variable Sampela_Slope does not deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.951143, n = 3, P = >0.05)
	Sampela_Crest																				
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W	0.951143																				
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Appendix 11.1. Shapiro-Wilk test proved all data in normality with all P values>0.05 (a) Buoy and crest, P=0.637. (b) Buoy and slope, P=0.255. (c) Pak Ksim and crest, P=0.333. (d) Pak Ksim and slope, P=0.128. (e) Ridge and crest, P=0.693. (f) Ridge and slope, P=0.505. (g) Sampela and crest, P=0.809. (h) Sampela and slope, P=0.574.																					

Assumption 3: all samples must have equal variance. Not met. Site and Habitat had unequal variance by Bartlett's test, $P=1.08 \times 10^{-6} < 0.05$

Homogeneity of Variance Tests			
Test	Statistic	DF1	DF2
Hartley Fmax	362.848	8	2
Cochran C	0.447448	8	2
Bartlett Chi-square	42.5471	7	
Bartlett Probability > Chi-Square	1.07933E-006		

Appendix 12. Two Way ANOVA's Rock of benthic composition among sites

Assumptions

To carry out two-way ANOVA, three assumptions must be met:

Assumption 1: all samples must be independent to each other. Met. Site and Habitat were independent.

Assumption 2: Data must be normally distributed. Not met. (PK Slope) was not in normality, $P = -8.57 \times 10^{-7} < 0.05$ (Figure xx). So, arcsine squareroot transformation was required (Figure xx). However, transformed data was not in normality.

QED Statistics 1.5.1.456 - QED_Rock	
File Edit Simulation Single Sample Analy	
Explore the Statistic: Shapiro-Wilk Test	
	PK_Slope
n	3
W	0.75
Prob.	-8.57151E-007
This means	The distribution of the variable PK_Slope does deviate significantly from a normal distribution (Shapiro-Wilk test, W = 0.75, n = 3, P = <0.05)

Appendix 12.1. Normality test (Shapiro-Wilk Test) showed one of raw data (PK Slope) was not in normally distributed.

Working Data								
	Buoy_Crest	PK_Crest	Ridge_Crest	Sampela_Crest	Buoy_Slope	PK_Slope	Ridge_Slope	Sampela_Slope
Row 1	0.606374	0.484333	0.491182	0.364673	0.393713	0	0.102909	0
Row 2	0.643812	0.322938	0.314955	0.591169	0.75224	0.0455456	0.205569	0.163763
Row 3	0.492158	0.647759	0.34508	0.533595	0.805812	0	0.20085	0.56158

Appendix 12.2. Arcsine Squareroot transformation on all variables.

Appendix 13. Pearson' / Spearman Correlation table

Test target	Site		Data transformation		Normality		Equal variance	Pearson test if equal variance and normality; Spearman test if unequal variance or not normality	Correlation (Yes/No)
	(i)	(j)	(i)	(j)	(i)	(j)			
Algae vs Grazers	Sampela	Pak Ksim	Arcsine Squareroot	log	Y	Y	Y	$r = -0.636644$, $t = -1.65114$, $df = 3$, $p = 0.1741 > 0.05$	N
	Sampela	Buoy	Arcsine Squareroot	log	Y	Y	N	$r = -0.428571$, $df = 6$, $p > 0.05$	N
	Sampela	Ridge	Arcsine Squareroot	log	Y	Y	N	$r = -0.3$, $df = 5$, $p > 0.05$	N
	Pak Ksim	Buoy	Arcsine Squareroot	log	Y	Y	Y	$r = -0.5057$, $t = -1.17221$, $df = 4$, $p = 0.3062 > 0.05$	N
	Pak Ksim	Ridge	Arcsine Squareroot	log	Y	Y	Y	$r = -0.3279$, $t = -0.6011$, $df = 3$, $p = 0.590 > 0.05$	N
	Ridge	Buoy	Arcsine Squareroot	log	Y	Y	Y	$r = -0.8076$, $t = -2.3715$, $df = 3$, $p = 0.0984 > 0.05$	N