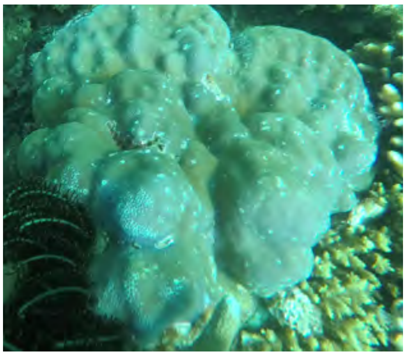
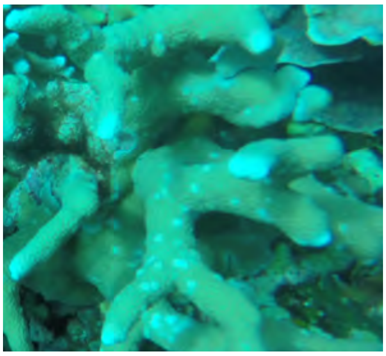
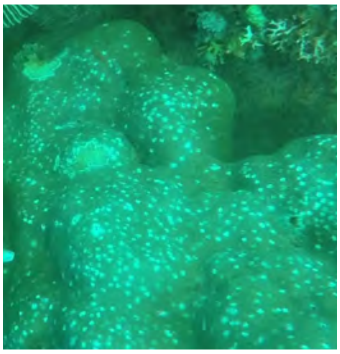
New records of *Porites* Ulcerative White Spot Disease in the Philippines; Implications for the Future of MPA Management

Niamh Meyler

2022

Supervised by Michael Tedengren

Submitted as part of M.Sc Marine Biology Degree at Stockholm University

Stockholm University

Contents

1. Abstract:

Disease in an underestimated and understudied threat to coral reefs. The diverse range of Poritids makes them an excellent candidate to observe the change in coral disease prevalence over several years. The contribution of these data to the knowledge from prior studies brings us closer to a full and educated description of *Porites* ulcerative white spot (PUWS) disease and its growth. This study determined that PUWS has been consistently present on 100% of the sampled shallow reef systems in Dauin, Philippines since long-term monitoring began in 2019. New records of PUWS have been observed in Apo Island Marine Sanctuary where previous studies found it to be free of PUWS as of 2005. Future analysis using the genetic samples collected during this sampling event may further elucidate the question of the infectious agent responsible for PUWS and the species within the genus Porites which may be most susceptible.

2. Introduction:

2.1 Coral Ecology and Contributions:

The biodiversity of our oceans is considered an important bioindicator of the planet's health. Endless ecosystem services provided to us by nature are the result of the complicated community structures present today in our marine systems (Roitman et al., 2018). In 2011, it was estimated that there are over 8.7 million species of extant eukaryotic organisms on our planet (Sweetlove, 2011), 2.2 million of which, are marine (Mora et al., 2011). Coral reefs are often called the rainforest of the sea to illustrate their incredible diversity of life (Mulhall 2008). At least 605 species of reef-building coral were described in the Indo-Pacific, making these reefs the most biodiverse on record. Compare this to the 61 species described in the Western Atlantic Ocean (Veron 2000; Allen 2007). As diversity is an indicator of reef health, it is clearly worth investigating the Indo-Pacific reefs due to their extensive coral diversity.

In recent years, we have seen an increase in coral diseases, most of which are attributed to the warmer, tropical areas of our oceans. (Kaczmarsky, 2009; Weil et al., 2012). Some of the many challenges that coral reef scientists are increasingly facing, are the issues of global climate change, pollution, ocean acidification, overfishing and eutrophication. More recently, it has been realised that we must also consider the consequences following outbreaks of new and persistent diseases (Kaczmarsky, 2009).

With climate change comes higher water temperatures, longer bleaching events, range shift of marine organisms and changing water levels. Those who are most dependant on coral reefs for their livelihood, protection from storms and a source of food, are the least contributing to carbon emissions (Donner & Potere, 2007).

Dauin's Coastal Resource Management program has kept the resources of local dive tourism in the hands of the municipality rather than the larger corporate entities. The Fisheries Code in the Philippines gives preference to local fishers (Oracion, 2007). Over the years, there has been an increase in the number of resorts in Dauin, which is attributed to an increase in dive tourism (Tubog & Tayco, 2017). The increase in revenue from tourism can be a benefit to the local communities and contribute to funding marine protected areas (MPAs) both directly, through entry fees for diving the reefs, and indirectly by bringing revenue to the municipality (Oracion, 2007). Diving and snorkelling tourism can also be a threat to coral reefs in MPAs with less effective management. Coral reefs with low tourist traffic have been shown to harbour up to twice as many healthy corals as the more tourism-intensive sites (Lamb et al., 2014). Destructive fishing practices and poor water quality are some of the most prevalent issues in Philippines waters, with over 95% of reefs at risk from anthropogenic activities (Burke et al. 2002).

One of the more devastating and highly publicised outbreaks of this nature is the continuing issue of white band diseases (WBD) in the Caribbean shallow reef systems. Over 80% of *Acropora* colonies in Florida were lost to this disease (Kline & Vollmer, 2011). The Caribbean is often the epicentre of disease research but there is much to be learned about the Pacific region also. WBD distribution has become almost world-wide and have very different hosts across its range (Antonius 1985, 1988). There is an upward trend in the number of diseases that have been recorded in marine organisms (Ward & Lafferty 2004). It is suspected that climate change is an important driver in the future potential increase of susceptibility of host species and the ease with which diseases will be transmitted (Harvell et al. 2002).

There is still much work to be done in quantifying and describing coral diseases in the Philippines.

2.3 *Porites spp.* and *Porites* ulcerative white spot disease (PUWS):

Porites is a genus within the Scleractinian corals with 68 accepted species and a total of 296 accepted varieties (WoRMS, 2022). *Porites spp.* displays different morphotypes depending on species and the environment in which the colony has settled (reference here). Threats to Porites spp. are listed on the IUCN Red List and include disease as well as urban wastewater and runoff ((Edwards et al., *The IUCN Red List of Threatened Species* 2008). This genus is an important habitat builder within the Coral triangle and is thought to be a tough coral, resistant to disease and bleaching (Kaczmarsky, 2009; Raymundo et al., 2005), living long enough that several studies have used cores to investigate the historical climate of our planet (Sayani et al., 2019).

PUWS was first identified and described in 1996 of a shallow reef in the Philippines (Reynolds et al., 2002). Raymundo et al., 2003, gave this definition of PUWS; "(PUWS) is characterized by discrete, bleached, round foci, 3 to 5 mm in diameter, that may either regress or progress to full tissue-thickness ulcerations that coalesce, occasionally resulting in colony mortality." From this description, it is possible to identify PUWS in the Philippine reefs.

Though the visually similar "white-spot syndrome" has been reported in *Porites spp.* from the Caribbean, no PUWS has yet been recorded there. This is interesting as *Porites* is a very cosmopolitan coral found globally (Woodley et al., 2016). Raymundo et al., 2005 found that *Porites* was the only genus regularly diseased in the reefs near Negros Oriental, despite there being extensive coral diversity. They found that the Poritids were the most appropriate genus to study as it was more consistently observed to be diseased than other corals. With this recommendation, our study will also focus on the diseases present in Porites species.

The pathogen associated with PUWS is yet to be confirmed but is suspected to be *Vibrio sp.*, more specifically, a variety closely related to *V. parahaemolyticus* and *V. natriegens* (Arboleda & Reichardt, 2010) or *V. carchiariae* and *V. nereis* (Reynolds et al., 2002). Another study shows bacteria associated with PUWS to be *Pseudoalteromonas sp.*, and *Bacillus sp.* (Sa'adah et al., 2018).

There is work to be done to discover how human activities can impact coral disease transmission but the Raymundo et al. 2003 study showed that it takes about 5 weeks for a healthy coral colony to develop visual PUWS after being repeatedly exposed to the disease. No information could be found regarding the recovery rate or survival rate of *Porites spp.* once a colony has contracted PUWS. The factors that effect a coral's susceptibility to disease may not be the same factors that affect the spread of the disease once infection occurs, or the same factors that affect the coral's ability to recover (Putchim et al., 2012; Wulff, 2006).

Different growth forms in corals affect their thermal stress tolerance (Liang et al., 2017). Porites attenuata, P. cylindrica, P. nigrescens, P. horizontallata, P. rus and P. annae can have a branching morphology and were present in previous reef surveys of the Dumaguete and Apo Island region (Raymundo et al., 2003). Massive species P. australiensis, P. lobata, P. lutea and P. solida are also common on Philippines shores (Raymundo et al., 2003). Coral colonies of different morphology represent different species though it can be difficult to identify them in the field. Disease often affects different species of coral differently, so it was important for our study that this be recorded (Lawrence et al., 2014). PUWS, so far has been attributed only to *Porites spp.* though the disease ulcerative white spot disease (UWS) is being investigated as being a larger category of disease that affects *Porites* as well as other coral genera such as *Echinopora sp.* (Séré et al. 2015a, AllenWaller 2015)

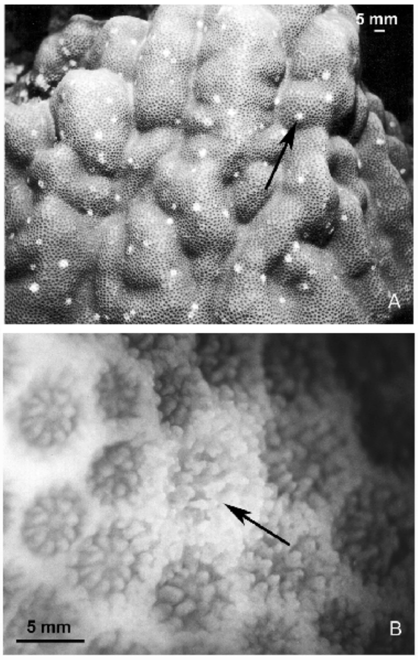


Fig 1. (A) PUWS on a massive colony, (B) close-up of PUWS, taken from (Raymundo, 2003)

2.4 Aims of this study:

This study aims to provide insight into the status of PUWS in Dauin, Negros Oriental, Philippines and produce data that can be useful to the management of the relevant MPAs by local government authorities. This report will outline two phases of research activities to achieve this goal. Phase 1 will be a field portion, establishing the history and current trends of PUWS in Dauin reefs. Phase 2 will not be fully outlined in this report but will be part of a continuation where mucus samples obtained from the Dauin shores may be investigated in a laboratory setting.

The objective of Phase 1 of this study was to conduct a reef survey of three shore sites along Dauin, and one site at the nearby Apo Island, and build up a description of Porites ulcerative white spot (PUWS) disease in terms of coral depth, season and location over several years. Apo Island Marine Sanctuary is a community-led MPA where destructive fishing practices are prohibited. We hope to be able to compare the area of Apo Island which is known for its healthy reefs, to the Dauin Municipailty areas along the coast of Negro Oriental. These sites are also in close proximity to MPAs that have had varying success in improving reefs and crating spill over effects outside the MPA (Hind et al., 2010). An analysis of disease prevalence was also performed to investigate the relationship between *Porites* growth forms and PUWS prevalence. This study aspires to further investigate and evaluate the extent and nature of PUWS prevalence in the Dauin area. Furthermore, Phase 2 of this study will work closely with the 4 tenants of Koch's postulates for ascertaining a causal relationship between a microorganism and a disease (as found in the appendix of Richardson, 1998).

- The pathogen must always be found associated with a particular disease.
- It must be possible to isolate the pathogen from the disease and grow the culture of the same pathogen.
- The cultured pathogen must produce the disease when inoculated into or onto a healthy animal.
- You must be able to re-isolate the same pathogen from this inoculated animal now presenting disease.

This study aims to collect mucus samples from both infected and healthy *Porites spp.* colonies from the reef transect. Using Koch's guidelines, the samples may be used to corroborate previous attempts to identify the pathogen associated with PUWS and to investigate the patterns in infection, recovery, and transmission.

In order to form a conclusion, the following null hypotheses were tested within this report:

H0: There is no significant difference between the prevalence of PUWS across the years 2019, 2020, 2021, 2022

H0: There is no significant difference between the prevalence of PUWS across all three growth forms

H0: There is no significant difference between the prevalence of PUWS across the Dauin and Apo Island reefs

3. Methodology:

3.1 Survey area:

This study set out to analyse PUWS in existing long-term monitoring data to gain more knowledge about its transmission and prevalence in Philippine waters. The Institute for Marine Research (IMR) of Dauin, Philippines, has been monitoring the Dauin reefs for benthic cover and diversity since 2019 (Waters et al., 2020). IMR first recorded PUWS on Dauin shores in 2020 (Brand et al., 2020).

There was limited time and resources for this project and not all 19 sites monitored by IMR could be reviewed. The sites most likely to be affected by PUWS were identified as the 3 reef sites at Poblacion I, Dauin South, and Masaplod Norte (Brand et al., 2020). At each site, two transects were carefully surveyed. For additional comparison, two transects at Apo Island were chosen (fig, 2) to serve as a reference site that is expected to have less or no PUWS. The Apo Island site is part of a well-managed MPA and has historically better water quality than the sites on the Dauin shores. Apo island was determined to have the least anthropogenic influences of all the sites, also noted by Raymundo et al. (2005).

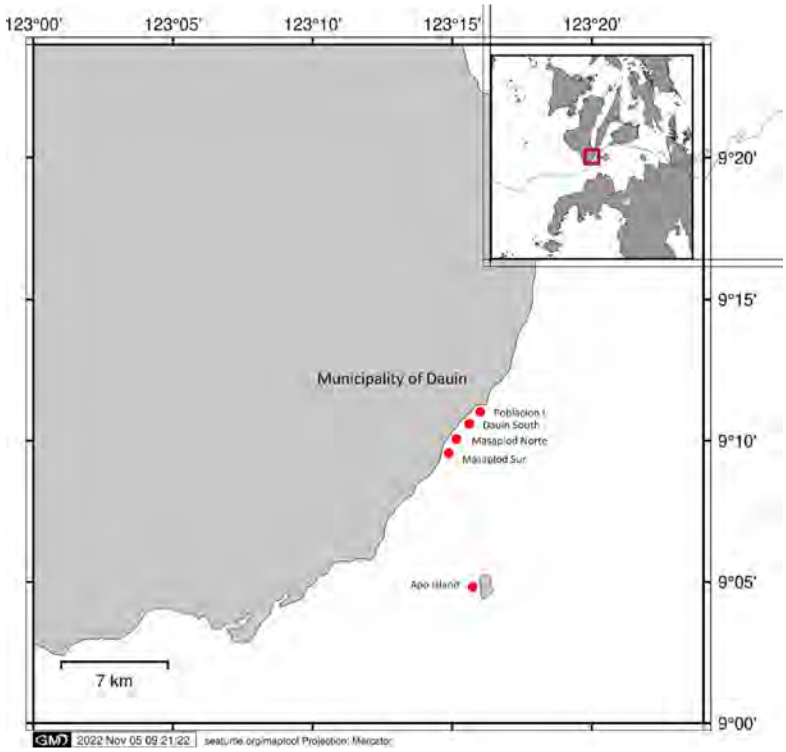
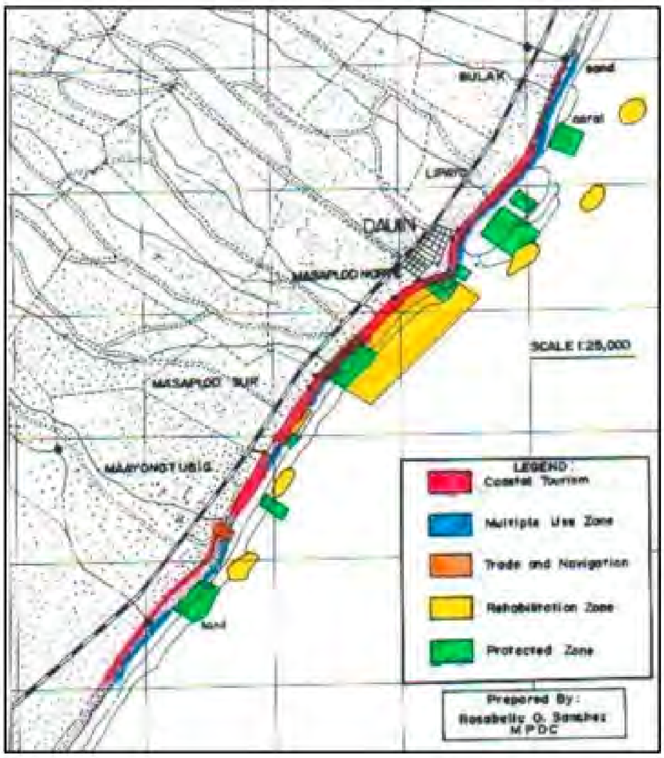


Fig 2. Survey area with 5 sites Poblacion I, Dauin South, Masaplod Norte, Masaplod Sur and Apo Island. Maps created using Maptool program for analysis and graphics in this paper. Maptool is a product of SEATURTLE.ORG. (Information is available at www.seaturtle.org)



f

Fig 3. Dauin coastal zoning map with MPAs shown in green. Taken from (Bianchessi, 2012).

3.2 Reef survey:

Over the years 2019, 2020, 2021 and 2022, IMR has had a consistent photographic method of recording coral cover and diversity following the Australian Institute of Marine Science (AIMS) LTMP methodology. IMR revisits 19 sites, each with a 50m transects. Each transect is one of two depth ranges: 1-6m or 7-12m. All sites are monitored both in the dry season and the wet season, totalling two monitoring events per year. To ensure the photographs were collected from the same area as the previous year, IMR had metal stakes to mark the start and end of each transect.

To calculate the coral diversity and cover, an IMR SCUBA diver will lay out a 50m transect tape and use a GoPro camera to photograph at each cumulative 1m mark from 0.5 above the substrate, totalling fifty photographs per 50m transect. These files are then white balanced for clarity and uploaded to the program CPCe.

Abiotic data was also collected by IMR. At each site during a monitoring event, abiotic data was recorded. This includes date, high tide, number of fishing vessels present, number of recreational vessels present. Water temperature was not recorded by IMR.

3.3 Mucus collection

In order to confirm that the pathogen thought to be associated with PUWS or another potential infectious agent was present within the selected transects, mucus secretions were collected from Porites colonies within the surveyed transects. Due to the variety of species and morphotypes present on the reef, one sample each was collected from an infected and an apparently healthy branching, submassive and massive *Porites* colony for each site and depth. These samples were collected in the 2022 dry season within 14 days of the transect survey. The donor colonies were photographed before collection and secretions were encouraged with metal tweezers. All collection tubes and tweezers were sanitised before use with boiling water.

Collected samples were placed in a freezer until they could be transported to Stockholm University for future analysis. rRNA sequencing of the 16S will be run on each sample regardless of their original visual appraisal of healthy or diseased.

3.4 Data processing and statistical analysis

Once the reef survey was complete and generated 50 photographs per transect, they were uploaded to a computer and analysed. The program CPCe (Coral Point Count with Excel Extensions) was chosen as it is currently in use by IMR to calculate the coral and substrate coverage for their reefs. CPCe is convenient for use as it can export to Excel and will calculate the sum, mean, standard deviation, standard error (Kohler & Gill, 2006).

The species code data for CPCe was edited for this specific study. 39 points were overlayed on top of each photograph in stratified rows and each point was identified as "*Porites spp.*", another "hard coral", or "other". Those classified as *Porites spp.*, could be subclassified as branching, sub-massive or massive form colonies, as well as being either healthy, or in early, mid-, or advanced stage of PUWS depending on the number of lesions visible. Colonies with 5 or less lesions were in the early stage (unless the colony was particularly small), between 5 and 30 lesions relatively spread out on a colony was classified as mid-stage, advanced stage was generally over 30 lesions and when the coral looked particularly unhealthy. This is different from the original CPCe code file method but keeps a comparable format to IMR. Photographs from the same transect (50 files at a time) were grouped together. For each transect. the mean percentage cover of each of these categories is autogenerated in the Excel extension for CPCe.

Since IMR did not actively record PUWS until they suspected an outbreak in 2020, this study chose a different method to analyse their long-term monitoring data for PUWS.

Kruskal-Wallis tests were used to investigate whether there was a difference in PUWS in relation to (i) year and (ii) site location (iii) depth and (iv) season.

4. Results:

4.1 PUWS Prevalence

Due to our small sample size, it was necessary to first determine the distribution of our variables for the total PUWS cover, total coral cover, cover over the years, locations, seasons, and depths. For this, we performed a Shapiro-Wilks test which showed evidence of non-normality ($p<0.05$). Based on these results, non-parametric tests were used for all variables.

Several trends can be seen in the data plotted for the Dauin area;

(i) A higher percent cover of PUWS was seen in the wet season when plotted against the dry season. Kruskal-Wallis chi-squared = 2.6677, df = 1, p-value = 0.1024.

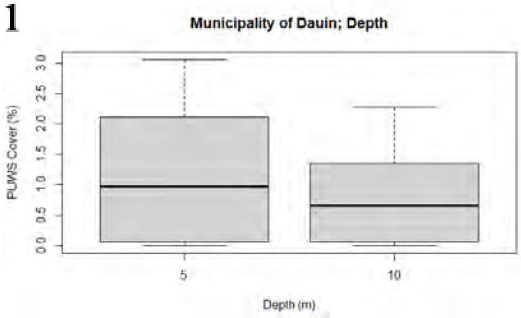
(ii) A higher percent cover of PUWS was seen in the 5m transect when plotted against the 10m transect. Kruskal-Wallis chi-squared = 0.49378, df = 1, p-value = 0.4822.

(iii) Dauin South was the location with the highest percent cover of PUWS. Kruskal-Wallis chi-squared = 10.759, df = 3, p-value = 0.0131.

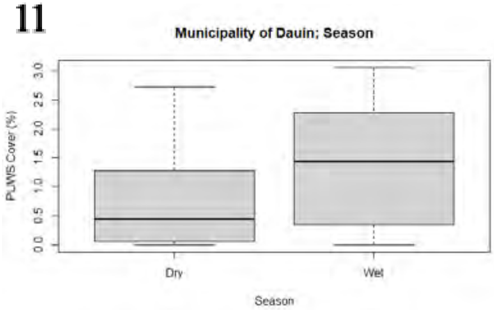
(iv) There was an increase in percent cover of PUWS from 2019 to 2020, a decrease in PUWS from 2020 to 2021 and a further decrease from 2021 to 2022. Kruskal-Wallis chi-squared = 0.89103, df = 3, p-value = 0.8276.

(v) The overall upward trend in PUWS over the years becomes more obvious when the Apo Island data is not included as the dataset is incomplete. Kruskal-Wallis chi-squared = 1.8147, df = 3, p-value = 0.6117.

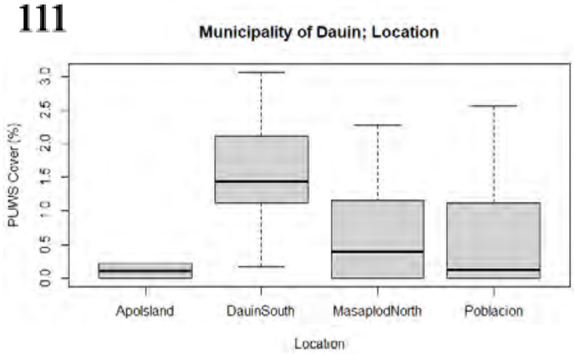
i


Municipality of Dauin; Depth
PUWS Cover (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
5
10
Depth (m)

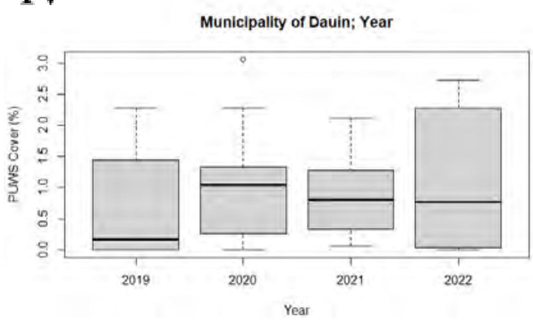
ii


Municipality of Dauin; Season
PUWS Cover (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Dry
Wet
Season

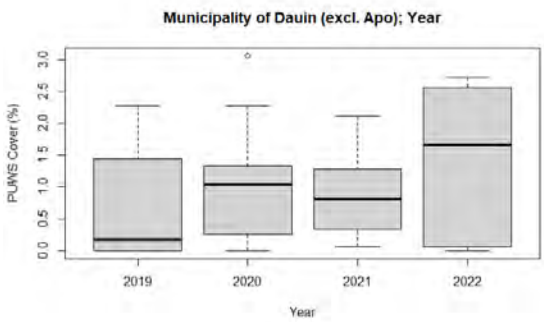
iii


Municipality of Dauin; Location
PUWS Cover (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
ApoIsland
DauinSouth
MasaplodNorth
Poblacion
Location

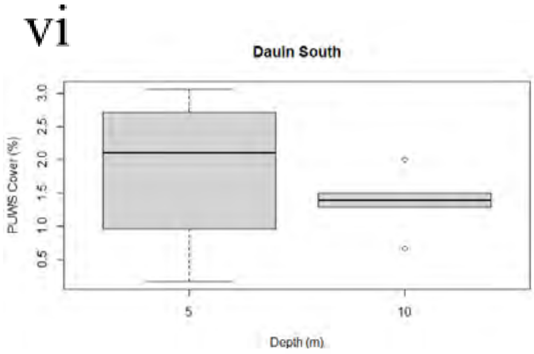
iv

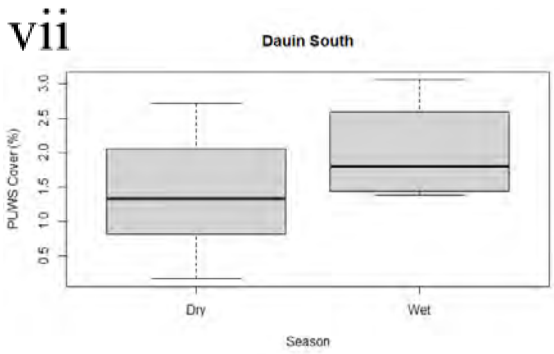

Municipality of Dauin; Year
PUWS Cover (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
2019
2020
2021
2022
Year

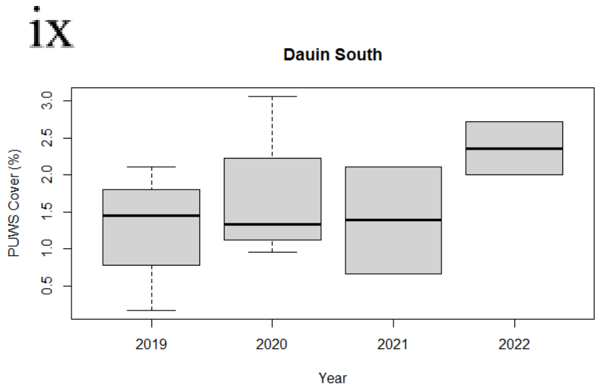
v


Municipality of Dauin (excl. Apo); Year
PUWS Cover (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
2019
2020
2021
2022
Year

Dauin South;







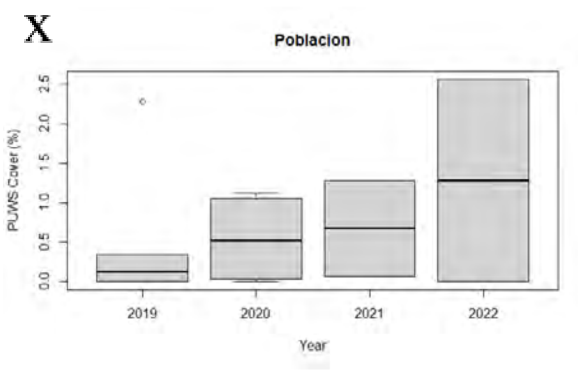
Analysis of the Dauin South data

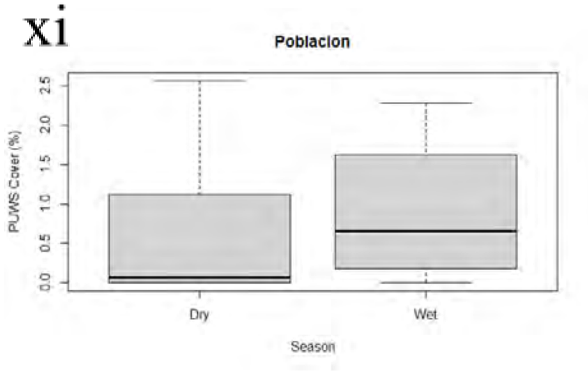
(vi) shows a higher percent PUWS cover on the 5m depth transect than the 10m depth transect. Kruskal-Wallis chi-squared = 1.2608, df = 1, p-value = 0.2615

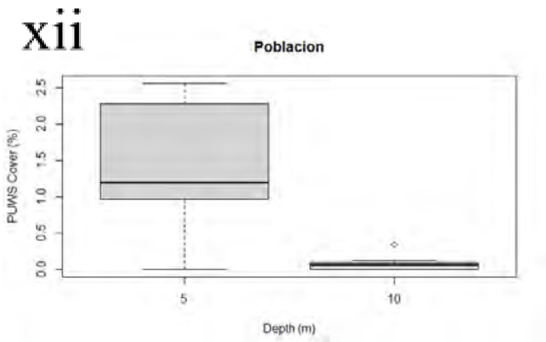
(vii) There is a higher percent PUWS cover during the wet season sampling than the dry season. Kruskal-Wallis chi-squared = 1.6283, df = 1, p-value = 0.2019

(ix), it can be observed that there is a relatively steady percent PUWS cover overall in the years 2019, 2020, 2021 and an upward trend in 2022. Kruskal-Wallis chi-squared = 1.6741, df = 3, p-value = 0.6427

Poblacion District I;







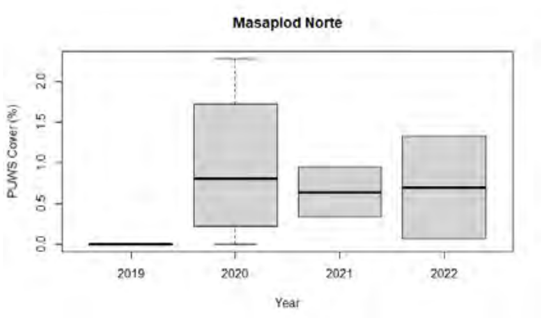
(x) for the Poblacion I area of Dauin shows an upward trend in percent PUWS cover each year over 2019, 2020, 2021 and 2022. Kruskal-Wallis chi-squared = 0.42833, df = 3, p-value = 0.9343

(xi) There is a higher percent PUWS cover in the wet season. Kruskal-Wallis chi-squared = 0.30076, df = 1, p-value = 0.5834

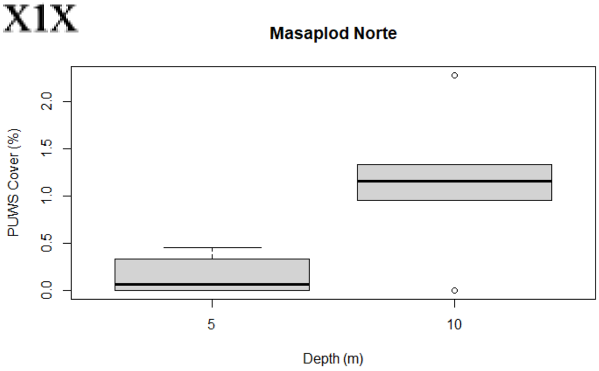
(xii)There is a higher percent cover PUWS at the 5m depth then the 10m depth. Kruskal-Wallis chi-squared = 5.0558, df = 1, p-value = 0.02454

Masaplod Norte;

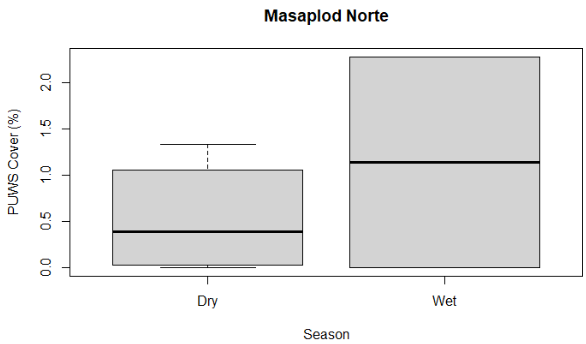
xiii



xix



xx

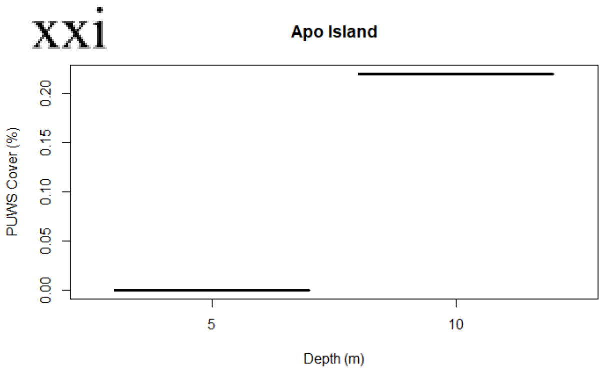


(xiii) No PUWS was observed at Masaplod Norte in 2019, there is PUWS recorded at a similar percent cover through 2020, 2021, 2022. Kruskal-Wallis chi-squared = 3.4658, df = 3, p-value = 0.3252

(xix) There was a higher rate of PUWS cover at the 10m depth. Kruskal-Wallis chi-squared = 3.2311, df = 1, p-value = 0.07225

(xx) during the wet season. Kruskal-Wallis chi-squared = 0.069876, df = 1, p-value = 0.7915

Apo Island;



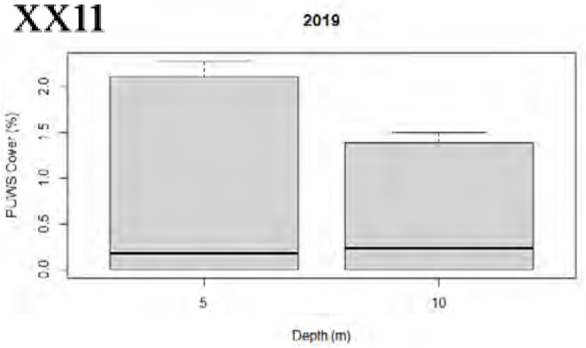
Apo Island was only sampled in the dry season 2022, thus there is no data available to compare the site over previous years or wet and dry seasons.

(xxi)No PUWS was found within the 5m transect, there was a 0.22 percent PUWS cover in the 10m transect. Kruskal-Wallis chi-squared = 1, df = 1, p-value = 0.3173

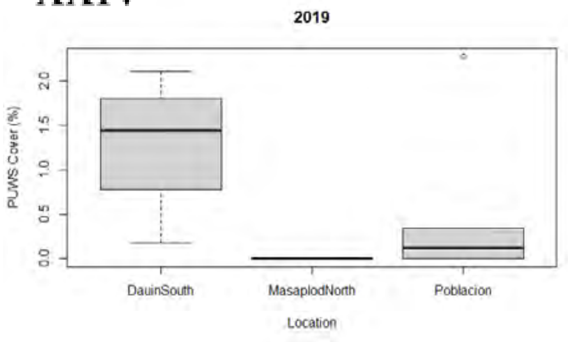
Year 2019;

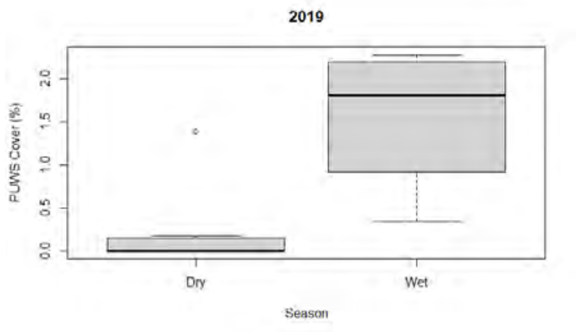
xxii

xxiii



xxiv





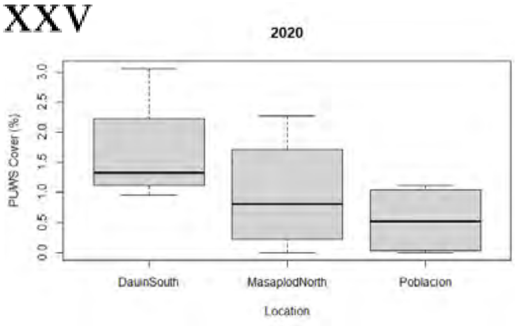
In 2019, (xxii) there is a relatively equal percent cover of PUWS between the 5m and 10m transects across all locations (excluding Apo Island as no data is available). Kruskal-Wallis chi-squared = 0.13968, df = 1, p-value = 0.7086

(xxiii) Dauin South had the highest percent cover of PUWS among the three sites, Masaplod Norte had no PUWS recorded. Kruskal-Wallis chi-squared = 4.3381, df = 2, p-value = 0.1143

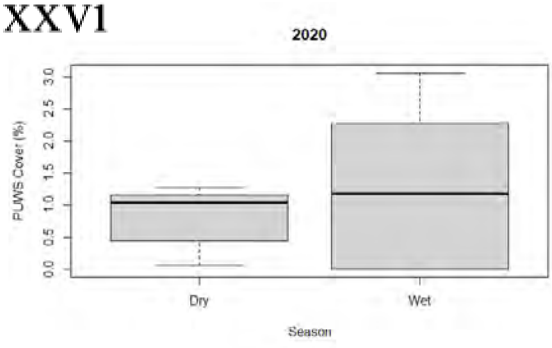
(xxiv) More PUWS was recorded during the wet season than in the dry season in 2019. Kruskal-Wallis chi-squared = 6.3231, df = 1, p-value = 0.01192

Year 2020;

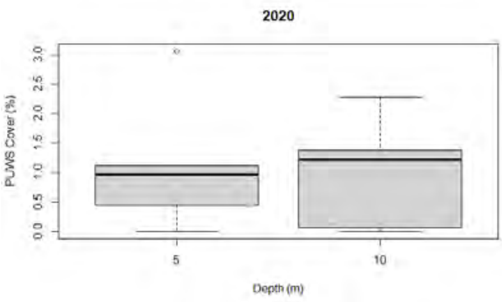
xxv



xxvi



xxvii



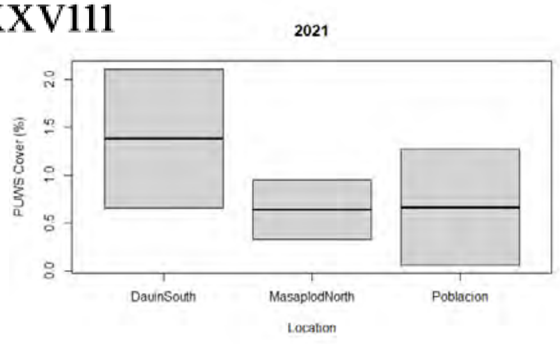
(xxv)In 2020, the data continues to show Dauin South as the location with the highest percent cover of PUWS. Kruskal-Wallis chi-squared = 3.3675, df = 2, p-value = 0.1857

(xxvi)Similar PUWS percent cover was present during the dry and wet seasons. Kruskal-Wallis chi-squared = 0.23158, df = 1, p-value = 0.6304

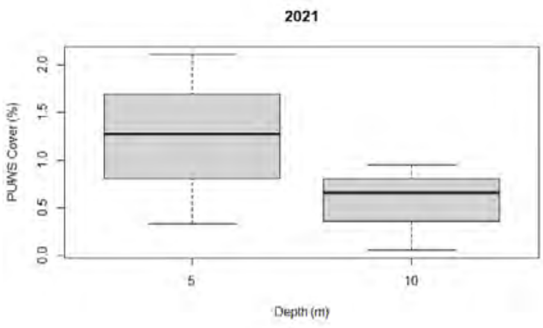
(xxvii) and at the 5m and 10m depths in 2020. Kruskal-Wallis chi-squared = 0.3152, df = 1, p-value = 0.5745

Year 2021;

xxviii



xxix



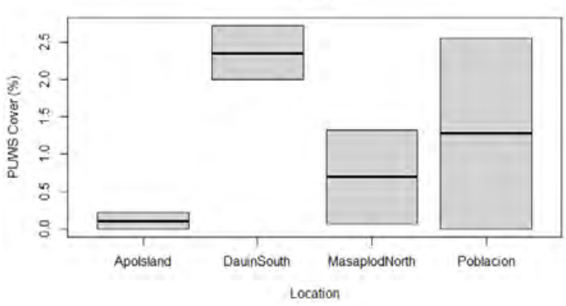
(xxviii)In 2021, Dauin South had the highest PUWS cover of the three locations, Masaplod Norte and Poblacion I had a similar percent cover PUWS. Kruskal-Wallis chi-squared = 0.85714, df = 2, p-value = 0.6514

(xxix) There was a higher percent cover of PUWS at 5m depth in 2021. Kruskal-Wallis chi-squared = 1.1905, df = 1, p-value = 0.2752

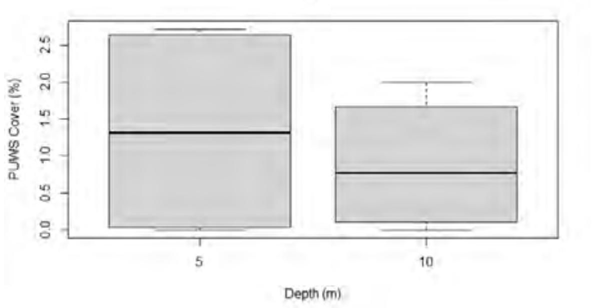
No data was available for the 2021 wet season.

Year 2022;

XXX



xxxi



(xxx)Dauin South continues to have the highest percent cover PUWS in 2022 and Apo Island had the lowest coverage. Kruskal-Wallis chi-squared = 3.247, df = 3, p-value = 0.3551

(xxxi)The figure shows a slightly higher percent cover PUWS at 5m than 10m. Kruskal-Wallis chi-squared = 0.18976, df = 1, p-value = 0.6631

IMR provided a table with their observations of PUWS on all of their shore sites over the years;

Site Name	2019 Dry	2019 Wet	2020 Dry	2020 Wet	2021 Dry	2022 Dry	Total (Site)
Bulak II				6		2	8
Lipayo I						3	3
Lipayo I Norte					1		1
Lipayo I Sur						7	7
Maayong Tubig		1				10	11
Masaplod Norte					2	26	28
Masaplod Sur					1	17	18
Masaplod Sur MPA				3	1	10	14
Poblacion District I		3		4	1	6	14
Dauin South			3	27	11	53	94
Total (Year)	0	4	3	40	17	134	

Table 1. Data provided by IMR showing their counts (no. of Poritid colonies) infected with PUWS between 2019 and 2022 for all of their sampling sites, including the sites relevant to our study, Dauin South, Poblacion District I and Masaplod Norte.

4.2 PUWS and *Porites* morphotypes

Porites spp. total cover was recorded for all transects well as the percent cover of the 3 categories of morphotypes; branching, massive and submassive.

A

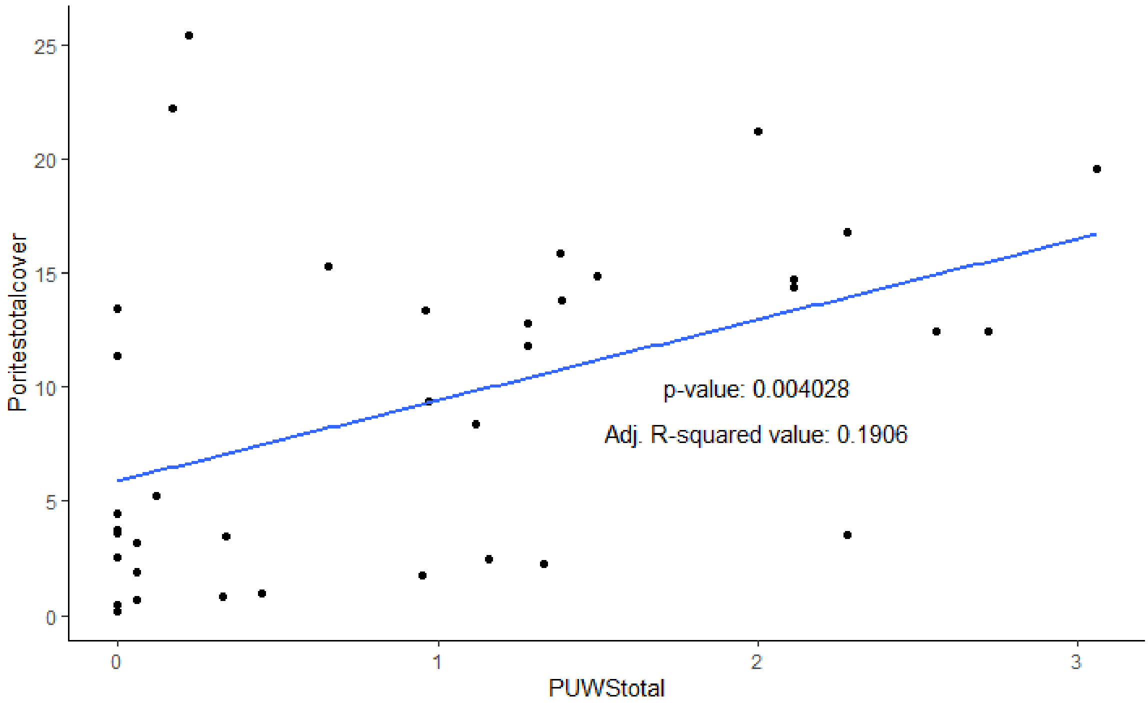


Figure (A) graphs the regression line for PUWS total cover against the total percent cover of *Porites spp.* in all transects. The adj. R-squared value = 0.1906 and the p-value of confidence in the graph is 0.004028.

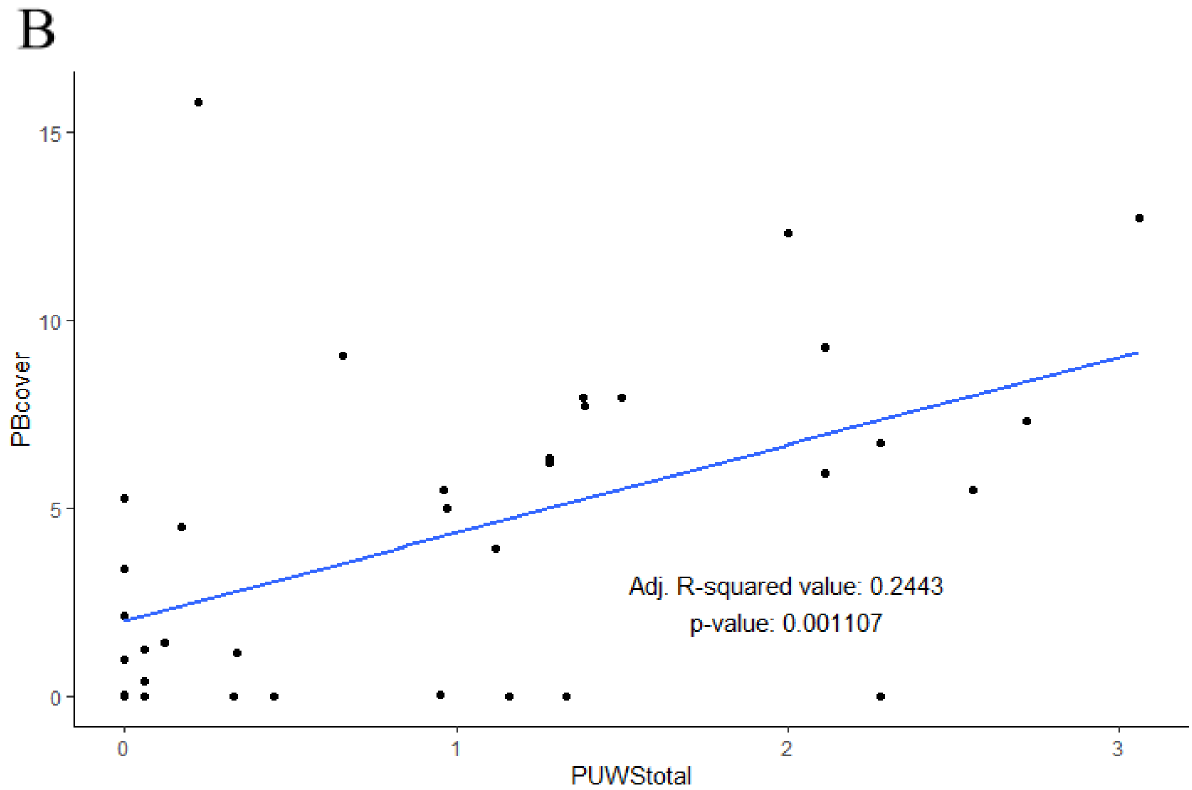


Figure (B) shows a regression between the total PUWS for all transects and the *Porites spp.* of the branching morphotype. The adj. R-squared value = 0.2443 and p-value of confidence in the graph is 0.001107.

C

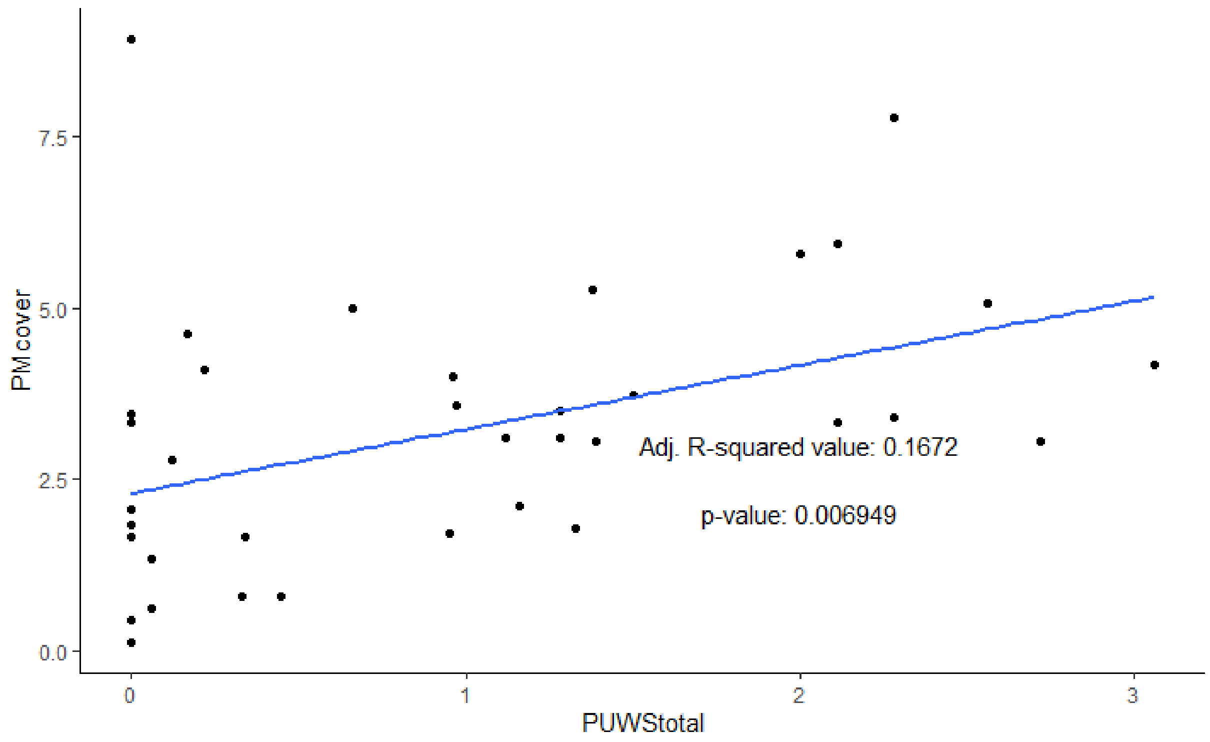


Figure (C) is a regression line between the total PUWS for all transects and the *Porites spp.* of the massive morphotype. The adj. R-squared value = 0.1672 and p-value of confidence in the graph is 0.006949.

D

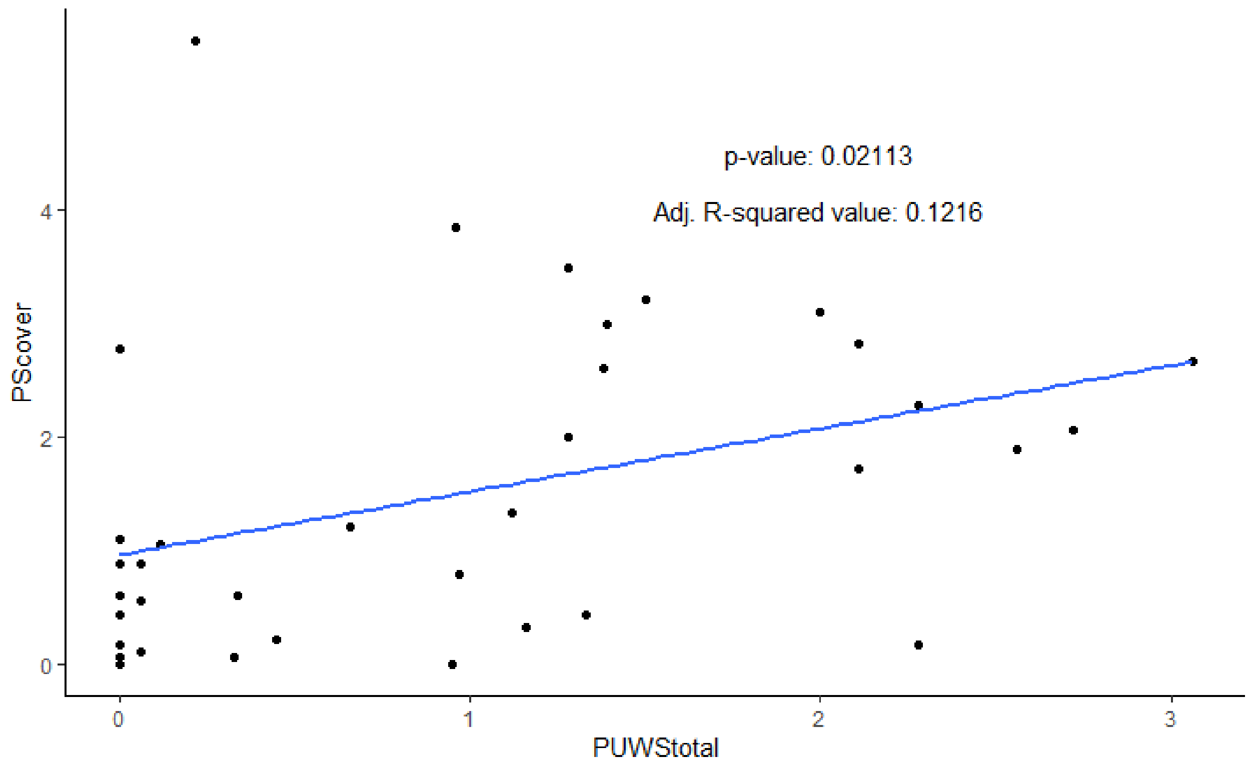


Figure (D) shows a regression between the total PUWS for all transects and the *Porites spp.* of the submassive morphotype. The adj. R-squared value = 0.1216 and p-value of confidence in the graph is 0.02113.

4.3 Corallivore feeding activity

The results of the feeding scar analysis show that overall years and transects feeding scars of either parrotfish or butterflyfish were recorded 18 times on a *Porites* colony. 12 records of scarring coincided with positive signs of PUWS, 6 records of scarring were of apparently healthy *Porites* colonies. The site with the highest rate of feeding activity using feeding scars as the indicator in Poblacion I which had a total of 8 *Porites* colonies with feeding scars recorded; 6 were infected with PUWS, and 2 were apparently healthy.

Site	Depth	Year	Season	Porites with scar and PUWS	Porites with scar healthy	Total scars per site
Dauin South	5	2019	Dry	1		1
Poblacion District I	5	2019	Dry			0
Masaplod Norte	5	2019	Dry			0
Dauin South	5	2019	Wet			0
Poblacion District I	5	2019	Wet	2	1	3
Dauin South	10	2019	Dry	1		1
Poblacion District I	10	2019	Dry			0
Masaplod Norte	10	2019	Dry		1	1
Dauin South	10	2019	Wet	1	1	2
Poblacion District I	10	2019	Wet			0
Dauin South	5	2020	Dry	1		1
Poblacion District I	5	2020	Dry			0
Masaplod Norte	5	2020	Dry			0
Dauin South	5	2020	Wet			0
Poblacion District I	5	2020	Wet	1		1
Masaplod Norte	5	2020	Wet			0
Dauin South	10	2020	Dry			0
Poblacion District I	10	2020	Dry			0
Masaplod Norte	10	2020	Dry			0
Dauin South	10	2020	Wet			0
Poblacion District I	10	2020	Wet			0
Masaplod Norte	10	2020	Wet			0
Dauin South	5	2021	Dry			0
Poblacion District I	5	2021	Dry	2	1	3
Masaplod Norte	5	2021	Dry	2		2
Dauin South	10	2021	Dry			0
Poblacion District I	10	2021	Dry	1		1
Masaplod Norte	10	2021	Dry			0
Dauin South	5	2022	Dry			0
Poblacion District I	5	2022	Dry			0
Masaplod Norte	5	2022	Dry			0
Dauin South	10	2022	Dry		1	1
Poblacion District I	10	2022	Dry			0
Masaplod Norte	10	2022	Dry	1	1	2
Apo Island	5	2022	Dry			0
Apo Island	10	2022	Dry			0
Total (PUWS or healthy)				13	6	

Table 2. Feeding scars in each transect and the health status of the Porites colony; PUWS or healthy.

5. Discussion:

5.1 Analysis of results:

This report describes the earliest occurrences of PUWS on the Dauin reefs. Analysis of IMR's long-term reef monitoring revealed that PUWS was present in the Dauin region since their first sampling event, the 2019 dry season. PUWS was observed in the 2019 Dauin South 5m and 10m transects, as well as the Poblacion I 10m transect. No PUWS was present in the 2019 Masaplod Norte transect, and no data was gathered in 2019 for the Apo Island region. Though many of the Kruskal-Wallis tests came back with no significant difference in ranked means, this could be due to the small sample size and the tendency for non-parametric tests to make type II errors. The first Kruskal-Wallis tests that provided a low p-value ($p<0.05$) were the difference between the dry and wet season in 2019. This means that we can reject the null hypothesis and accept the alternate that the ranked mean for PUWS cover in the wet season was significantly higher than the dry season. The PUWS cover in Poblacion is significantly higher at the 5m depth than the 10m ($p<0.05$).

100% of the surveyed reefs had colonies of *Porites spp.* infected with PUWS in 2022. This is not too surprising as the Dauin South, Poblacion I and Masaplod Norte reefs were selected due to their reports of being in poor condition in 2022. However, Apo Island was selected as a site for this survey as a comparison due to its reportedly healthy condition, yet there was still PUWS found. This survey is the first to identify PUWS on the Apo Island reefs. Previous surveys were conducted within the marine reserve in 1997, 1998, 1999, 2003 and 2005 (Raymundo et al., 2003, 2005). It is possible that, were the locations directly outside Dauin to be sampled as well, we may find areas that have not yet been exposed to PUWS. A high diversity of coral species was observed in this area though no correlation was found between the diversity and the amount of PUWS (Raymundo et al., 2005).

Raymundo et al., 2003 studied 30 reefs in the region of Negros Oriental, Philippines and found 80% contained colonies with PUWS and the prevalence of PUWS among the genus was 22% of all colonies. This is somewhat consistent with our current study though we found that mean PUWS cover ranged from 0% to 1.89% (Dauin South 5m, 2020 wet season) as a percent cover of the 50m transect. Dauin South was the only one of the four sites in which PUWS was present for every transect and sampling event. Our survey was not broad enough to compare directly to the Raymundo et al. 2003 study and we are not able to say whether we would have gotten a higher or lower PUWS prevalence if we had expanded the geographic range of our study.

The rates of infection in Dauin reefs lead us to consider an important question; what does this mean for the recovery of Dauin reefs? To address this question, we can look back at the historical data from previous studies. Raymundo et al. 2003, found that only 2 infected *Porites spp.* colonies died over their 17-month-long study. They also found that PUWS has a low rate of tissue loss, and the disease may be present in the colony for quite a long period of time without displaying any more than minor tissue loss. Raymundo's report is consistent with this current study as very little tissue loss was observed in the Dauin reefs despite some observations of highly advanced levels of PUWS lesions on some coral colonies. Unfortunately, the low rates of tissue loss may not be enough to predict any positive trends for the future of the Dauin reefs.

Raymundo et al. 2003, found that within their 17-month study, recovery was extremely rare and only observed in infected colonies which displayed less than 5 lesions, and these recovered only if they were infected less than a month. The lack of any further evidence for the recovery of PUWS-infected colonies makes it difficult to make predictions on future reef health and points towards a focus on preventing the spread of PUWS rather than trying to promote a recovery there is little evidence for. Raymundo's researchers also performed a live inoculation of *Porites spp.* in the field, infecting actual coral reefs with PUWS. experiments showed that 95.5% of colonies exposed to the disease became infected. Their first monitoring event of these colonies showed that PUWS had spread further than expected in the 3-week period and they were unable to definitively trace the source of the lesions to the infected branch grafted to the colony. They also found that grafting healthy coral branches to healthy coral colonies also caused PUWS infections and suggest this may be an indicator that the pathogen is waterborne and can use exposed tissue damage as an entry point. Though Raymundo et al. 2003 suggests further in situ field experiments of this kind, it is the duty of this report to acknowledge the lack of scientific ethics associated with experiments such as this where a disease is exposed to wild corals without knowledge of the mechanisms behind its transmission.

Some coral diseases can cause tissue bleaching and diseases have also been known to be exacerbated by sustained high water temperatures. Tissue loss in white spot disease, also suspected to be caused by Vibrio sp., has been seen to accelerate when water temperatures are above 26˚C (Raymundo et. al., 2003). Since IMR did not directly record water temperature in situ, this study attempted to use NOAA SST data to compare with Dauin PUWS outbreaks. Unfortunately, no data was publicly available to suit the needs of this project. However, a student of Stockholm University has recently placed a continuous temperature recorder at the Dauin shore so we may have data in future.

Raymundo et al. 2005 describes laboratory infection experiments, they found that colonies that had direct contact with the disease contracted PUWS, and colonies that only had waterborne contact were also infected. Suggesting the possibility of water as a vector for spreading PUWS.

5.2 Societal and ethical implications of this research:

Thanks to IMR, we know a lot about the community at Dauin in the context of their dependence on the local reef systems for survival. 53.9% of their study group fished Dauin waters alone and did not fish on nearby shores. They target 23 different taxonomic families of fish, which could become even more unsustainable if the diversity of fish assemblages deteriorates.

It is common in the field of tropical biology that a researcher from a high-income area will travel to a low-income country to conduct their research. This is at times unavoidable as it is necessary for research to be conducted even if there are severe financial or infrastructural limitations on the local community's ability to do so. As a result, we see many researchers extracting data from locations such as the Philippines and failing to provide any benefit or knowledge to the local research efforts. This is an increasingly important topic as the scientific community moves towards ethical research practices and attempts to set new standards of behaviour. Though the need for ethical research planning is expanding, there are little to no resources investigating the effect that travel research has on the Philippines or other low-income populations. In fact, travel research is often lumped together with "ethical tourism" as a sustainable way to travel and support the local people. Not enough evidence has been published for or against this trend and more research is required to make a responsible determination. Though this study failed to locate any published material regarding the necessity for ethical thinking in the marine science when travelling to "developing" countries, the consensus of studies from the medical field is that a strong collaborative effort with local researchers and policy makers is the most ethical way to conduct such research (Benatar, 2002; Morris, 2015; Lund, 2021).

Apo Island has been considered one of the most successful MPAs in the world in terms of local stakeholder satisfaction, this is mostly due to its community-based approach (Hind et al., 2010). Though both the Apo Island and Dauin Municipality shores showed an increase in PUWS of the course of this research project, MPA management is still an important aspect of the future of these reefs. Previous studies have pointed towards MPAs as a way to encourage reef resilience from diseases (Mellin et al., 2016). Many of the reports produced by MPA managers neglect to include meaningful records of the disease prevalence that could help improve the way we protect our reefs (Nguyen, 2021).

5.4 Genetic Evidence

The goal for analysing the genetic samples (mucus secretions), was to determine if the pathogen thought to be associated with PUWS, Vibrio sp., was present in the corals that were infected and absent in the apparently healthy corals. This is in accordance with Koch's postulate that an infectious agent must be found in all diseased samples and no healthy samples. Due to the nature of the disease, in that the visual signs if PUWS may not be present for some time after inoculation, it may be difficult to follow these guidelines as regards the healthy corals not having *Vibrio sp*. present. It is quite possible that an apparently healthy coral may have *Vibrio sp*. and not yet display symptoms.

Since the genetic samples have not yet been analysed within the timeframe of this project, we are unable to discuss any results. Understanding the infectious agent provides an important step towards protecting coral reefs against destructive disease outbreaks. It can help identify a source of the pathogen, the mechanisms of its transmission and can enlighten us about the effect it may have on its host.

5.5 Linking corallivore feeding behaviour to disease transmission:

Intraspecific aggression is a common factor in all animal communities. Coral colonies competing for space often come into contact with each other and act as potential vectors for disease transmission.

Though it is common to find coral disease resources that focus on abiotic drivers, it remains important to investigate the potential roles of corallivores and other biotic factors when tracking disease transmission. Corallivores can expose previously healthy coral tissue and open them up to disease. Several studies have observed an increase in the feeding activities of certain corallivores on already diseased tissue, perhaps carrying this disease to other previously healthy corals through feeding, or alternatively, as some studies have shown, this behaviour can actually slow the progression of diseases when mostly unhealthy tissues are targeted (Allen-Waller 2015; Renzi et al., 2022; Séré et al. 2015a) There is no comprehensive analysis of the relationship between Porites specific corallivores and their role in disease transmission, mitigation and related stressors.

Renzi, et al., 2022 found that *Drupella sp*. and Damselfish activity were correlated with an increase in disease prevalence. Skeletal eroding band disease has also been attributed to *Halofolliculina corallasia* (Antonius & Lipscomb 2000), though there a no records of a corallivore associated with PUWS.

On the Dauin reefs, the observed corallivores were parrotfish, butterflyfish, *Drupella sp*, and crown of thorns starfish (COTS) though none were directly recorded feeding on Poritid species during this study. Feeding scars are a more time-efficient method of recording the presence and rate of feeding activity of corallivores and was chosen over an observational feeding survey which could have been skewed by the presence of the surveyor. The results of this analysis show that there is a higher rate of feeding scars present which coincide with *Porites* infected with PUWS than the rate of feeding scars present on healthy *Porites*. Due to the low count for feeding scars, it is not possible to infer causation between the presence of corallivore activity and the transmission of PUWS. Additionally, the site with the highest prevalence of PUWS does not coincide with the site that had the highest presence of feeding scars, Poblacion I. This count also only includes the feeding scars for parrotfish and butterflyfish as no scars were observed that could be attributed to COTS or *Drupella sp*. though this could be accounted for the difficulty in recording these scars from a still photograph.

6. Conclusion:

The original purpose of this study was to use existing long-term monitoring data to improve our records of PUWS in the Philippines. From our research, we can conclude that there has been PUWS present on Dauin shores longer than IMR's long-term monitoring program had originally discovered. We are also able to make the first official record of PUWS present on Apo Island as it had not been observed in previous surveys done by outside groups. Though we have very little information available regarding the source and transmission of PUWS in Dauin, we have succeeded in the first multi-year observation of PUWS on a single reef. This report also recommends a greater emphasis on coral disease mitigation efforts be incorporated into MPA management plans.

H0: There is no significant difference between the prevalence of PUWS across the years 2019, 2020, 2021, 2022 - Accepted

H1: There is a significant difference between the prevalence of PUWS across the years 2019, 2020, 2021, 2022 -Rejected

H0: There is no significant difference between the prevalence of PUWS across all three growth forms - Accepted

H1: There is a significant difference between the prevalence of PUWS across all three growth forms – Rejected

H0: There is no significant difference between the prevalence of PUWS across the Dauin and Apo Island reefs - Rejected

H1: There is a significant difference between the prevalence of PUWS across the Dauin and Apo Island reefs - Accepted

7. Acknowledgments:

The successful completion of this project could not have been achieved without the participation and guidance of a great many wonderful people amongst the staff and students of Stockholm University. My collaboration with the Institute for Marine Research, Dauin was invaluable and without their team, I would not have the long-term monitoring data I needed to embark on this project.

I would like to express my immense gratitude towards my thesis supervisor Michael Tedengren and examiner Jonas Gunnarsson for their expertise, direction, and continued support throughout the project.

I also want to thank José Ekstedt and Andreas Arleborg for their support and efforts in coral research while we were at IMR together.

8. References:

Allen, G. R. (2007). Conservation hotspots of biodiversity and endemism for indo-pacific coral reef fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*(5), 541–556. https://doi.org/10.1002/aqc.880

Allen-Waller, Luella, "Bleaching, disease, and colonization: The ecology of coral health in southeastern Nosy Be, Madagascar" (2015). Independent Study Project (ISP) Collection. 2149.

Arboleda, M. D. M., & Reichardt, W. T. (2010). Vibrio sp. causing Porites ulcerative white spot disease. *Diseases of Aquatic Organisms*, *90*(2), 93–104. https://doi.org/10.3354/dao02222

Benatar, S. R. (2002). Reflections and recommendations on Research Ethics in developing countries. *Social Science & Medicine*, *54*(7), 1131–1141. https://doi.org/10.1016/s0277-9536(01)00327-6

Bianchessi, A. (2012). A Case Study of the Municipality of Dauin, Negros Oriental, Philippines: A Global Bright Spot for Marine Conservation. *Dauin Municipality*.

Brand, J., Waters, C., Manrique, R., Levy, E. (2020). Annual Outlook Report 2020. *Institute for Marine Research, Dauin.*

Donner, S. D., & Potere, D. (2007). The inequity of the global threat to coral reefs. *BioScience*, *57*(3), 214–215. https://doi.org/10.1641/b570302

Edwards, A., Assessor), D. F. (C. R., Assessor), A. S. (C. R., Assessor), M. A. (C. R., & Assessor), D. O. (C. R. (2008, January 3). *The IUCN Red List of Threatened Species*. IUCN Red List of Threatened Species. Retrieved December 3, 2022, from https://www.iucnredlist.org/species/133082/54191180#threats

Hind, E. J., Hiponia, M. C., & Gray, T. S. (2010). From community-based to centralised National Management—a wrong turning for the governance of the Marine Protected Area in Apo Island, Philippines? *Marine Policy*, *34*(1), 54–62. https://doi.org/10.1016/j.marpol.2009.04.011

Kaczmarsky, L. T. (2009). Characterizations of the major coral diseases of the Philippines: Ulcerative white spot disease and novel growth anomalies of Porites. *FIU Electronic Theses and Dissertations*. https://doi.org/10.25148/etd.fi09120804

Kline, D. I., & Vollmer, S. V. (2011). White Band Disease (type I) of endangered Caribbean acroporid corals is caused by pathogenic bacteria. *Scientific Reports*, *1*(1). https://doi.org/10.1038/srep00007

Kohler, K. E., & Gill, S. M. (2006). Coral Point Count with excel extensions (CPCE): A visual basic program for the determination of coral and substrate coverage using random point count methodology. *Computers & Geosciences*, *32*(9), 1259–1269. https://doi.org/10.1016/j.cageo.2005.11.009

Lamb, J. B., True, J. D., Piromvaragorn, S., & Willis, B. L. (2014). Scuba diving damage and intensity of tourist activities increases coral disease prevalence. *Biological Conservation*, *178*, 88–96. https://doi.org/10.1016/j.biocon.2014.06.027

Lawrence, S. A., Davy, J. E., Wilson, W. H., Hoegh-Guldberg, O., & Davy, S. K. (2014). Porites white patch syndrome: Associated viruses and disease physiology. *Coral Reefs*, *34*(1), 249–257. https://doi.org/10.1007/s00338-014-1218-2

Liang, J., Yu, K., Wang, Y., Huang, X., Huang, W., Qin, Z., Pan, Z., Yao, Q., Wang, W., & Wu, Z. (2017). Distinct bacterial communities associated with massive and branching scleractinian corals and potential linkages to coral susceptibility to thermal or cold stress. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00979

Lund, B. D. (2021). Is academic research and publishing still leaving developing countries behind? *Accountability in Research*, *29*(4), 224–231. https://doi.org/10.1080/08989621.2021.1913124

Mellin, C., Aaron MacNeil, M., Cheal, A. J., Emslie, M. J., & Julian Caley, M. (2016). Marine Protected Areas increase resilience among coral reef communities. *Ecology Letters*, *19*(6), 629–637. https://doi.org/10.1111/ele.12598

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G., & Worm, B. (2011). How many species are there on Earth and in the ocean? *PLoS Biology*, *9*(8). https://doi.org/10.1371/journal.pbio.1001127

Morris, N. (2015). Providing ethical guidance for collaborative research in developing countries. *Research Ethics*, *11*(4), 211–235. https://doi.org/10.1177/1747016115586759

Mulhall M. Saving the rainforest of the sea: an analysis of international efforts to conserve coral reefs. In: Duke environmental law and policy forum, vol. 19; 2008. p. 321.

Nguyen, V. T. (2021). Measuring the Effect of Marine Protected Areas on Coral Disease Prevalence Using a Meta-analysis Approach. *George Mason University*. https://doi.org/http://hdl.handle.net/1920/12130

Oracion, E. G. (2007). Dive tourism, coastal resource management , and local government in Dauin. *Philippine Quarterly of Culture and Society*, *35*, 149–178.

Putchim, L., Yamarunpattana, C., & Phongsuwan, N. (2012). Observations of Coral Disease in Porites lutea in the Andaman Sea Following the 2010 Bleaching. *Phuket Mar. Biol. Cent. Res. Bull.*, *71*, 57–62. https://doi.org/https://www.dmcr.go.th/dmcr/fckupload/upload/44/image/FullpaperPMBC/2012%20Vol.71%20Putchim%2057%2062.pdf

Raymundo, L. J. H. (2003). Porites ulcerative white spot disease: Description, prevalence, and host range of a new coral disease affecting Indo-Pacific reefs. *Diseases of Aquatic Organisms*, *56*, 95–104. https://doi.org/10.3354/dao056095

Raymundo, L. J., Rosell, K. B., Reboton, C. T., & Kaczmarsky, L. (2005). Coral diseases on Philippine reefs: Genus Porites is a dominant host. *Diseases of Aquatic Organisms*, *64*, 181–191. https://doi.org/10.3354/dao064181

Renzi, J. J., Shaver, E. C., Burkepile, D. E., & Silliman, B. R. (2022). The role of predators in coral disease dynamics. *Coral Reefs*, *41*(2), 405–422. https://doi.org/10.1007/s00338-022-02219-w

Reynolds, T. L., Wainwright, N., & Raymundo, L. J. (2002). International Association for Aquatic Animal Medicine. IAAAM Archive.

Richardson, L. (1998). Coral diseases: What is known? *Trends in Ecology & Evolution*, *13*(11), 438–443. https://doi.org/10.1016/s0169-5347(98)01460-8

Roitman, S., Joseph Pollock, F., & Medina, M. (2018). Coral microbiomes as bioindicators of Reef Health. *Population Genomics*, 39–57. https://doi.org/10.1007/13836_2018_29

Sayani, H. R., Cobb, K. M., DeLong, K., Hitt, N. T., & Druffel, E. R. (2019). Intercolony δ 18 O and SR/Ca variability among *porites* spp. corals at Palmyra Atoll: Toward more robust coral‐based estimates of Climate. *Geochemistry, Geophysics, Geosystems*, *20*(11), 5270–5284. https://doi.org/10.1029/2019gc008420

Sa'adah, N., Sabdono, A., & Wijayanti, D. (2018). Identification of antipathogenic bacterial coral symbionts against porites ulcerative white spots disease. *IOP Conference Series: Earth and Environmental Science*, *116*, 012054. https://doi.org/10.1088/1755-1315/116/1/012054

Sweetlove, L. (2011). Number of species on Earth tagged at 8.7 million. *Nature*. https://doi.org/10.1038/news.2011.498

Séré, M. G., Tortosa, P., Chabanet, P., Turquet, J., Quod, J.-P., & Schleyer, M. H. (2013). Bacterial communities associated with Porites White patch syndrome (PWPS) on three

Western Indian Ocean (WIO) coral reefs. *PLoS ONE*, *8*(12). https://doi.org/10.1371/journal.pone.0083746
Tubog, M. V., & Tayco, R. O. (2017). Factors that influence resorts' development in the Municipality of Dauin, Philippines. *UVJOR*, *11*(1).
Waters, C., Brand, J. Manrique, R. (2020). Annual Outlook Report 2019. *Institute for Marine Research, Dauin.*
Weil, E., Irikawa, A., Casareto, B., & Suzuki, Y. (2012). Extended geographic distribution of several Indo-Pacific Coral Reef Diseases. *Diseases of Aquatic Organisms*, *98*(2), 163–170. https://doi.org/10.3354/dao02433
Woodley, C. M., Downs, C., & Bruckner, A. W. (2016). *Diseases of coral*. Wiley Blackwell.
Wulff, J. L. (2006). A simple model of growth form-dependent recovery from disease in coral reef sponges, and implications for monitoring. *Coral Reefs*, *25*(3), 419–426. https://doi.org/10.1007/s00338-006-0110-0
Veron JEN (2000) Corals of the world. Australian Institute of Marine Science, Townsville