



MASTERARBEIT | MASTER'S THESIS

Titel | Title

Abundance and distribution of coral fluorescent proteins in the
genus *Madracis* over a Caribbean reef slope

verfasst von | submitted by

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angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of
Master of Science (MSc)

Wien | Vienna, 2024

Studienkennzahl lt. Studienblatt | Degree
programme code as it appears on the
student record sheet:

UA 066 833

Studienrichtung lt. Studienblatt | Degree
programme as it appears on the student
record sheet:

Masterstudium Ecology and Ecosystems

Betreut von | Supervisor:

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Acknowledgement

My deepest gratitude goes to Dr. Pedro Frade, my primary supervisor and mentor throughout this master's thesis. Your unwavering support and dedication to your students made all the difference. I consider myself incredibly fortunate to have had you guiding me. Thank you for your patience and for constantly encouraging me to strive for excellence.

A special thank you to Giulia M. Marchioro, my lab partner, for accompanying me on this entire journey. From navigating me through all the lab work and supervising me with my work in the confocal. Thank you for making this experience so much richer and more meaningful.

I am also grateful to Prof. Igor Adameyko from the Center of Brain Research at the Medical University of Vienna. Your provision of the lab and microscope was invaluable, and your inspiration has been a driving force behind this work.

I would like to express my sincere thanks to Dr. Daniel Abed-Navandi for sharing his extensive knowledge on corals. Your expertise greatly enriched this thesis.

A huge thank you to Dr. Thibault Boudier, from whom I've learned so much. Your guidance in the lab, as well as your instruction on confocal microscopy, was instrumental in my research. I deeply appreciate the time and effort you invested in teaching me both lab techniques and the nuances of working in a research environment.

Lastly, I would like to thank Univ.-Prof. Dr. Gerhard Herndl, my official supervisor at the University of Vienna. Your support and guidance throughout this process have been greatly appreciated.

Abstract German

Diese Studie untersucht die komplexe Dynamik des Holobionten, bestehend aus dem Korallenwirt und seinen Zooxanthellen-Symbionten, mit einem Fokus auf fluoreszierenden Proteinen (FPs) und deren ökologischer Rolle. Ziel ist es, die räumliche Verteilung und die funktionellen Rollen verschiedener FPs in drei *Madracis*-Korallenarten (*M. senaria*, *M. pharensis* und *M. decactis*; insgesamt n=82) aus unterschiedlichen Tiefen (5m und 20m) sowie an verschiedenen Positionen innerhalb der Kolonien (obere Seite der Kolonie und seitliche Kolonie) zu untersuchen. Die Studie kombiniert histologische Techniken, in-situ-Photobiologie Messungen und konfokale Mikroskopie. Die Forschung zielt darauf ab, die FP-Intensität und ihre Vorkommens Häufigkeit zu quantifizieren sowie FP-Symbionten-Interaktionen zu analysieren, um neue Einblicke in die dynamische Beziehung zwischen Korallen und ihren Symbionten zu gewinnen. Die Ergebnisse zeigen ein erhöhtes Vorkommen und eine höhere Intensität von grün fluoreszierendem Protein (GFP) und cyan fluoreszierendem Protein (CFP) in Kolonien aus flachem Wasser sowie in den oberen Teilen der Kolonien. Eine vergleichende Analyse des Verhältnisses von GFP zur Korallengewebe-Fläche zeigt ein Verhältnis von $1,25 \pm 0,04$ in Korallen aus flachem Gewässer und $1,13 \pm 0,21$ in Korallen aus tieferen Gewässern. Diese Ergebnisse deuten weiter auf eine Zunahme von GFP in lichtreicheren Umgebungen hin. Diese Ergebnisse unterstützen die Hypothese, dass FPs eine Schutzfunktion erfüllen, indem sie das Korallengewebe und die Symbionten vor erhöhter Strahlung schützen. Die erhöhte Fluoreszenzintensität der FPs in Korallen nahe der Wasseroberfläche unterstreicht das Potenzial der FPs, ihre Symbionten vor intensiver Sonneneinstrahlung zu schützen, was zur Widerstandsfähigkeit der Korallen beiträgt.

Schlüsselwörter: fluoreszierende Proteine, GFP, Laser-Scanning-Konfokal Mikroskopie, Lichtgradient

Abstract

This study explores the complex dynamics of the holobiont, consisting of the coral host and its zooxanthellae symbionts, with a focus on fluorescent proteins (FPs) and their ecological roles. The aim is to elucidate the spatial distribution and functional roles of different FPs in three *Madracis* coral species (*M. senaria*, *M. pharensis* and *M. decactis*; total n=82) across varying depths (5m and 20m) and colony positions (top of the colony and side of the colony). The study combines histological techniques, *in situ* photobiology measurements, and confocal microscopy. The research seeks to quantify FP intensity, FP occurrence and analyze FP-symbiont interactions, offering new insights into the dynamic relationship between corals and their symbionts. The results reveal an increased occurrence and intensity of green fluorescent protein (GFP) and cyan fluorescent protein (CFP) in shallow colonies and on the top parts of the colony. Comparative analysis of GFP to coral tissue area reveals a ratio of 1.25 ± 0.04 in shallow corals and 1.13 ± 0.21 in deep corals further suggesting an increase of GFP in higher light environments. These findings support the hypothesis that FPs play a protective role by dissipating excess light energy through fluorescence, thus shielding coral tissues and symbionts from elevated irradiance. The heightened fluorescence intensity of FPs in corals near the water surface highlights the FPs' potential in shading their symbionts from intense solar radiation, thereby contributing to coral resilience and potentially preventing bleaching.

Keywords: fluorescent proteins, GFP, laser scanning confocal microscopy, light gradient

Zusammenfassung

Korallenriffe repräsentieren den marinen Lebensraum mit der höchsten Biodiversität (Schuhmacher, 1976). Sie stellen nicht nur einen essenziellen Lebensraum für diverse marine Gemeinschaften dar, sondern sind auch von erheblicher Bedeutung für den Menschen (Salvat, 1992). Korallenriffe sichern die finanzielle Lebensgrundlage zahlreicher Menschen und fungieren als natürliche Schutzstruktur für Küstenregionen (Sheppard *et al.*, 2005). Riffbildende Korallen gedeihen in einem weiten Tiefenspektrum durch Anpassung an eine Vielzahl von Umweltgradienten, wobei Licht der bedeutendste Faktor ist (Veron 2000). Korallen sind sowohl Gradienten in der Intensität als auch in der spektralen Zusammensetzung des Lichts ausgesetzt, die durch die Abschwächung in der Wassersäule verursacht werden (Kirk *et al.*, 1994).

Der Erfolg der Korallen unter verschiedenen Lichtverhältnissen hängt von der integrierten physiologischen Kapazität ihrer symbiotischen Partner ab. Diese photoautotrophen Dinoflagellaten-Symbiose Algen, bekannt als Zooxanthellen, wandeln Sonnenlicht in chemische Energie und Biomasse um und versorgen die Koralle mit bis zu 95 % ihrer benötigten Energie (Carballo-Bolaños *et al.*, 2019). Obwohl Korallen für ihre vielfältigen Anpassungsstrategien bekannt sind, hat sich der Gesundheitszustand der Korallenriffe in den letzten Jahrzehnten dramatisch verschlechtert. Dies ist auf Krankheiten (Aronson und Precht, 2012; Rosenberg und Loya, 2004), vom Menschen verursachte Stressfaktoren wie Sedimentation, Verschmutzung (Loya *et al.*, 2004; Wiedenmann *et al.*, 2013; D'Angelo und Wiedenmann, 2014) sowie natürlich auf den Klimawandel zurückzuführen. Ein weiteres bekanntes Phänomen ist die sogenannte Korallenbleiche. Als Hauptauslöser gilt die weltweit erhöhte Wassertemperatur. Korallenbleiche zeichnet sich durch den Verlust der Zooxanthellen aus (Good und Bahr, 2021), ohne ihre Symbionten verlieren Korallen ihre Farbe und erscheinen weiß, da das kalkhaltige Skelett durch das nunmehr transparente Gewebe der Koralle sichtbar wird. Während einiger Korallenbleiche-Events wurden Korallen jedoch nicht nur weiß, sondern entwickelten bemerkenswert lebhafte Farben. Dieses Phänomen wird als „Colorful bleaching“ bezeichnet (Voolstra, 2020). Bislang war unklar, was der Auslöser für dies Farbenfrohe Spektakel ist. Eine mögliche Erklärung ist, dass die vorhandenen Pigmente im Korallengewebe sichtbarer werden, sobald die bräunlich-grüne Farbe der Symbionten verschwindet. Als ein weiterer Grund wird die vermehrte Expression von sogenannten Fluoreszenz Proteinen (FPs) vermutet (Roth *et al.*, 2013).

Diese FPs bestimmen die Farbenvielfalt in Korallenriffen und ergänzen die braunen Farbtöne der photosynthetischen Pigmente der symbiotischen Algen. Die häufigsten FP-Farben bei Korallen sind Cyan (CFP), Grün (GFP) und Gelb (YFP), wobei GFP am häufigsten vorkommt

und am auffälligsten ist (Alieva *et al.*, 2008; Field *et al.*, 2006). Neben diesen FPs trägt auch die rote Chlorophyllfluoreszenz (RCF) der Zooxanthellen zur Fluoreszenz bei. Korallen können ein oder mehrere FPs exprimieren, was ihre Färbung je nach Lebensstadium, Stressfaktoren, Umweltbedingungen oder Tiefe verändern kann (Alieva *et al.*, 2008). Unterschiede in der FP-Expression können durch unterschiedliche Anzahl an FP-Genkopien oder durch unterschiedliche Regulationsmechanismen verursacht werden (Gittins *et al.*, 2015; Dizon *et al.*, 2021). Trotz der hohen FP-Vielfalt sind ihre ökologischen Funktionen noch weitgehend ungeklärt und umstritten. Hauptsächlich Hypothesen zur Funktion von FPs beziehen sich auf die Aufrechterhaltung der Symbiose mit Zooxanthellen. Sie könnten potenziell eine wichtige Rolle bei der Anpassung der Korallen an Lichtverhältnisse spielen durch:

1. Lichtschutz: FPs schützen Korallen vor UV-Licht und reduzieren Lichtstress, insbesondere in flachen Gewässern. Unter hohen Lichtbedingungen absorbieren FPs wie GFP und CFP überschüssiges Licht, was zu einer verringerten Lichtbelastung der Symbionten führt. Dieser Schutzmechanismus könnte auch zur Widerstandsfähigkeit gegen Korallenbleiche beitragen (Bollati *et al.*, 2020; Gittings *et al.*, 2015; Salih *et al.*, 2000).
2. Lichtverstärkung: FPs können kürzere Wellenlängen in längere Wellenlängen umwandeln, die für die Photosynthese der Zooxanthellen besser geeignet sind. Dies ist besonders vorteilhaft in lichtarmen Umgebungen, da es den Zooxanthellen ermöglicht, mehr Licht für die Photosynthese zu nutzen (Bollati *et al.*, 2017; Smith *et al.*, 2017).

Zusätzlich werden andere mögliche Funktionen von FPs diskutiert, darunter antioxidative Eigenschaften, Immunantwort der Korallen und Anziehung von Zooxanthellen und Zooplankton durch grüne Fluoreszenz. Es wird vermutet, dass verschiedene FPs unterschiedliche und möglicherweise ergänzende Funktionen für Korallen und ihre Symbionten haben könnten (Roth *et al.*, 2013).

Diese Studie untersucht die räumliche Verteilung und funktionale Rolle von Fluoreszenzproteinen (FPs) in drei verschiedenen *Madracis* Arten (*M. pharensis*, *M. decactis*; *M. senaria*) aus zwei unterschiedlichen Tiefen (5m und 20m) und Koloniepositionen (vom oberen Teil der Kolonie und vom seitlichen Teil der Kolonie). Dazu werden verschiedene histologische Techniken, *in situ* photobiologische Messungen und konfokale Mikroskopie kombiniert. Diese innovative Methodik bietet neue Einblicke in die Dynamik von FPs und der Symbionten Photobiologie in Korallen. Das zentrale Ziel ist es, herauszufinden, ob die Tiefe und Lichtverfügbarkeit die Verteilung und Menge der FPs beeinflussen. Es soll

die Fluoreszenzintensität der FPs quantifiziert und ihre relative Position zu den Symbionten analysiert werden. Es wird angenommen, dass FPs die Lichtmodulation innerhalb des Korallengewebes bewirken und den Korallen helfen, sich an Lichtveränderungen in unterschiedlichen Tiefen anzupassen (Ben-Zvi *et al.*, 2019). Daher werden auch die Verteilung und Zelldichte der Symbionten untersucht. Die drei Hauptziele dieses Projekts sind: (1.) Identifikation und Quantifizierung verschiedener FPs in *Madracis*-Korallen durch konfokale Bildanalyse. (2) Kartierung der FP-Verteilung im Korallengewebe und Aufdeckung potenzieller funktionaler Unterschiede mittels histologischer Techniken und konfokaler Mikroskopie. (3.) Charakterisierung photosynthetischen Leistung der Symbionten durch Quantifizierung von Chlorophyll *a* und *c2* sowie der Zelldichte.

Die Analyse zeigt, dass grünes Fluoreszenzprotein (GFP) in allen drei Arten vorhanden ist und am häufigsten in flachen und oberen Positionen der Kolonien vorkommt. Dies deutet darauf hin, dass GFP eine Schutzfunktion gegen hohe Lichtintensität bietet (Bollati *et al.*, 2020). Auch das Cyan Fluoreszenzprotein (CFP) zeigte höhere Präsenz in hellen Bedingungen und das gelbe Fluoreszenzprotein (YFP) war ebenfalls in flachen Proben häufiger und deutet auf eine ähnliche Schutzfunktion hin. Die Analyse der Fluoreszenz Intensität von GFP und YFP, liefert ähnliche Ergebnisse und war entsprechend in flachen Kolonien höher, was die Annahme der Schutzfunktion weiter stützt (Gittings *et al.*, 2015). Bei *M. pharensis* wurde eine positive Korrelation zwischen GFP-Intensität und Lichtintensität festgestellt. Auch die Analyse des Verhältnisses von GFP zur Gewebeoberfläche bestätigte, dass flache Korallen eine höhere GFP-Intensität im Verhältnis zur Gewebeoberfläche aufweisen, was auf einen UV-Schutz hinweist.

Die Ergebnisse deuten darauf hin, dass Korallen ihre FPs nutzen, um sich an verschiedene Lichtbedingungen anzupassen und sich zu schützen. Flache Proben und solche, die zur Wasseroberfläche hin ausgerichtet waren, zeigten eine höhere Vorkommen und mittlere fluoreszierende Intensität der FPs. Diese Ergebnisse unterstützen die Hypothese, dass die Expression von FPs im Korallengewebe als Schutz vor UV-Strahlung dient und überschüssige Lichtenergie durch Fluoreszenz und Lichtstreuung ableiten kann (Mazel *et al.*, 2002; Roth *et al.*, 2008).

Auf der nächsten Ebene, den Symbionten, stimmen die Ergebnisse dieser Studie mit der Literatur überein. Anpassungsmechanismen innerhalb der Kolonie umfassen die Erhöhung der Zooxanthellen Dichte pro cm² in den zur Oberfläche hin ausgerichteten Teilen der Kolonie und im Gegensatz dazu die Verringerung der Zooxanthellen Zahlen in den mehr beschatteten Teilen, um den Effekt der Selbstbeschattung bei minimaler Bestrahlung zu minimieren (Mass *et al.*, 2007). Zudem scheint eine erhöhte Chlorophyllkonzentration in den Symbionten Zellen eine universelle Reaktion auf niedrige Lichtniveaus und die Notwendigkeit

einer verstärkten Lichtaufnahme zu sein (McCloskey und Muscatine, 1984; Mass *et al.*, 2007).

Die Studie liefert neue Einblicke in die Beziehung zwischen dem Korallenwirt und dem Zooxanthellen-Symbionten sowie die komplexen Mechanismen, die eine optimale Lichtaufnahme und UV-Schutz gleichzeitig gewährleisten. Insgesamt wird die Fluoreszenz in Korallen durch Licht beeinflusst, was zu Akklimatisierungsreaktionen führt, die sowohl auf interspezifischer als auch intraspezifischer Ebene erkennbar sind. Die Ergebnisse stimmen mit ähnlichen Studien überein, die eine Korrelation zwischen Bleich-Resistenz und der Konzentration von FPs im Gewebe berichten (Roth *et al.*, 2008; Salih *et al.*, 2000). Diese Ergebnisse wurden den lichtstreuenden Eigenschaften der FPs zugeschrieben, die ein wichtiger Faktor für ihre Sonnenfilterfunktion sind (Salih *et al.*, 2000). Die multivariate statistische Analyse hebt die zentrale Rolle von hoher Lichteinwirkung hervor, zeigt aber auch, dass die Expression von FPs nicht nur von einem Faktor, sondern von einer Reihe unterschiedlicher Variablen abhängt. Diese Studie betont die Komplexität des Holobionten, der aus dem Korallenwirt und dem Zooxanthellen-Symbionten besteht. Beide symbiotischen Partner tragen zu den physiologischen Reaktionen sowie den Anpassungs- und Akklimatisierungsmechanismen bei (Frade *et al.*, 2008; Roth *et al.*, 2008; Einbinder *et al.*, 2016).

INDEX

1	General Introduction	10
1.1	Coral reefs - a hotspot of biodiversity	10
1.1.1	Reef Ecology	10
1.1.2	Light – The primary source of energy for reef life	10
1.1.3	Threats and Climate Change	10
1.1.4	Conservation and restoration	12
1.2	Coral Physiology	14
1.3	Fluorescent Proteins (FPs)	15
1.3.1	Fluorescent Proteins – in General	15
1.3.2	Fluorescent Proteins - in Corals	15
1.3.3	Fluorescent proteins - their role in “Colorful Bleaching”	16
1.4	The coral genus „Madracis“	17
1.5	Objectives	17
2	Glowing Reefs: Understanding the distribution and function of fluorescent proteins in <i>Madracis</i> species across a caribbean reef slope	19
2.1	Introduction - Fluorescent proteins (FPs) in corals	19
2.2	Materials and Methods	21
2.2.1	Sample Collection & Target Species	21
2.2.2	Symbiont photobiology and irradiance	21
2.3	Data Analyses	24
2.4	Results	26
2.4.1	Irradiance	26
2.4.2	Identification of fluorescent proteins	27
2.4.3	Occurrence of distinct FP signatures	28
2.4.4	Ratios of different fluorescence signals across species	38
2.4.5	FP mean intensities and ratios (of fluorescence signals) within species	40
2.4.6	Symbiont and photobiology analyses	47
2.5	Discussion	51
2.5.1	Irradiance	52
2.5.2	Fluorescent proteins in corals	52
2.6	Symbionts and photobiology	56
2.7	Conclusion	59
3	Limitations and recommendations for future studies	61
4	General Discussion and Personal Thoughts	63
5	Supplementaries:	66
5.1	Materials and Methods	66

5.1.1	Sample collection & Target species	66
5.1.2	Symbiont photobiology	66
5.1.3	Fluorescent Proteins	68
6	Gallery	72
6.1	Photos taken during fieldwork	72
6.2	Confocal Images	Fehler! Textmarke nicht definiert.
7	Literature	72

1 General Introduction

1.1 Coral reefs- a hotspot of biodiversity

1.1.1 Reef Ecology

Coral reefs cover an area of 123 million km² of tropical oceans and are the world's most biodiverse marine ecosystem (Caspers and Schuhmacher, 1976). Reefs also provide fundamental support for human populations, as they are providing a source of income (Salvat, 1992) as well as acting as protective structures near shorelines (Sheppard *et al.*, 2005). Coral reefs are also one of the most important habitats for many marine organisms (Nagelkerken *et al.*, 2000). Reef-building corals are known to thrive across large depth ranges. They can do so through adaptation to a broad set of environmental gradients, the most important of which is light (Vernon, 2000).

1.1.2 Light – The primary source of energy for reef life

Marine organisms are subject to gradients in the intensity and spectral composition of light, due to attenuation by the water column (Osborne *et al.*, 1995; Mass *et al.*, 2010).

Communities found in deeper water must be able to cope with a light environment that is characterized by reduced spectral composition (towards the blue) and low irradiance intensity.

In the mesophotic zone (30-150m), light intensities are significantly lower compared to those encountered by shallow corals (0–30m), with reductions of up to 99% of available light. Most reduction of light happens in the first meters of water. For example, in the clear waters of the Caribbean, at subsurface level (0m) one would find an irradiance of 1400-2000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 500-1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at 7m and 50-200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at 30m (Frade *et al.*, 2008). These values might vary, depending on the location, but also on the time of the year, rainfall, terrestrial run-off, or anything affecting turbidity.

1.1.3 Threats and Climate Change

Although corals are known for their large range of adaptation strategies, the last few decades have led to a dramatic decline in coral-reef health. This decline is due to disease (Aronson and Precht, 2012; Rosenberg and Loya, 2004), human derived stressors such as sedimentation (Goatley and Bellwood, 2013), pollution and eutrophication (Wiedenmann *et al.*, 2013; Zaho *et al.*, 2021), overfishing (Shantz *et al.*, 2020; Zaneveld *et al.*, 2016), and of course climate change (Hoegh-Guldberg *et al.*, 2017).

Sedimentation - Sedimentation, a well-studied phenomenon, impacts corals in three primary ways: through photosynthetic interference, physical disruption, and chemical changes. Reef-building corals rely heavily on nutrients provided by their photosynthetic partners, the so-called zooxanthellae (Muscatine *et al.*, 1981). Therefore, any decrease in the quality or quantity of available light negatively affects coral nutrition, growth, reproduction, and depth distribution (Richmond, 1993).

Pollution & Eutrophication – Eutrophication and pollution (through e.g. wastewater, agricultural run-off, fertilizers) in coastal waters have been shown to severely affect coral-reef ecosystems (Wiedenmann *et al.*, 2013; Prouty *et al.*, 2017). One of these negative impacts is the increased input of inorganic nutrients. Dissolved inorganic nitrogen (DIN) in particular, has also been linked to reef decline and to a reduction of the temperature threshold for coral bleaching (Wooldridge, 2009). Nitrogen enrichment can not only directly affect coral physiology, it can also indirectly affect the resilience of coral reefs through promoting the growth of other organisms such as algae. It has also been shown that the settling of juvenile corals decreases with increasing eutrophication (Zaho *et al.*, 2021). The exposure to wastewater, which is also associated with higher phosphate levels, has also been shown to inhibit both the growth rate and the calcification rate of coralline algae. Considering the crucial role of coralline algae as reef builders, these effects are extremely concerning for corals as well (Björk *et al.*, 1995).

Destructive Fishing Practice and over-exploitation – The practice of dynamite fishing and the use of poisons such as cyanide has been responsible for the destruction of coral reefs throughout the world. Especially in the Indo-Pacific, dynamite ("blast") fishing is a significant contributor to reef degradation. The resulting fragmented coral cannot survive, leading to unstable rubble fields that are unsuitable for new coral recruitment (Fox *et al.*, 2004; Raymundo *et al.*, 2007). Another impact that is damaging to coral reefs are several different types of net fishing. They have also been responsible for over-exploitation of reef resources (Richmond, 1993).

Climate change and coral bleaching – Bleaching is defined as the loss of color caused by the partial up to total loss of the coral endosymbiotic Symbiodiniaceae population, or degradation of its pigments. Symbiodiniaceae is a family of dinoflagellate microalgae commonly known as zooxanthellae.

Bleaching can be displayed by all organisms living in symbiosis with these dinoflagellate algae (e.g. zoanthids, sea anemones, scleractinian and octocorals). Zooxanthellae symbionts not only possess chlorophyll *a* and *c2*, but also various carotenoids (e.g. peridinin which confer the brown color)(Douglas, 2003). The color of healthy living coral tissue is therefore partially influenced by the coral's symbiont community. If the coral host expels its symbionts, the then bleached coral appears to be white, the color of the underlying skeleton

of calcium carbonate (Douglas, 2003). Despite considerable research on this topic, there is still no simple explanation for the causes of bleaching. Bleaching can be triggered not only by one single factor, but by multiple factors: extremes of temperature (heat or cold stress), high irradiance, prolonged darkness, and pathogenic microorganisms (Hoegh-Guldberg, 1999; Brown, 2000). Recent, largescale bleaching events, their frequency and intensity, have been irrefutably attributed to increasing sea surface temperatures (SST) which are consequence to climate change (Sully *et al.*, 2019; Good and Bahr, 2021). About half of coral reefs worldwide have already been lost due to climate change (Good & Bahr, 2021). Bleached corals need to recover their symbiont populations quickly, or else they will they become subject to starvation and die within a few weeks (Hughes *et al.*, 2017).

Typically, coral mass bleaching precedes the mass mortality of corals, and this phenomenon has been registered not only in shallow-water but also into the deeper sections of the reef (Frade *et al.*, 2018). On April 15, 2024, the National Oceanic and Atmospheric Administration (NOAA) and the International Coral Reef Initiative (ICRI) officially confirmed the Fourth Global Coral Bleaching Event. They issued press releases and a coordinated call to action to address the impacts of climate change (<https://icriforum.org/4gbe/>). Extensive heat stress, monitored via satellite since 1985, has impacted coral reefs across the Atlantic, Pacific, and Indian Oceans from February 2023 to April 2024 (Reimer *et al.*, 2024). In the last year, significant bleaching has been observed in more than half of the world's coral reefs, including regions such as Florida (U.S.A.), the Caribbean, Brazil, the eastern Tropical Pacific, Australia's Great Barrier Reef, the South Pacific, the Red Sea, the Persian Gulf, the Gulf of Aden, and parts of the Indian Ocean, including Tanzania, Kenya, Mauritius, and Indonesia.

1.1.4 Conservation and restoration

A growing number of coral reefs are facing threats. Because of that, there is strong motivation for scientists and coral reef managers to create non-invasive tools for directly monitoring coral growth and the health status of our coral reefs. For a significant global impact, existing protocols and technologies for marine ecosystem restoration need to be effective on a larger spatial scale (Aronson *et al.*, 2021). Typically, conservation practices employ a gradual, "passive" approach, focusing on removing stressors and allowing the ecosystem to recover naturally (e.g., in Marine Protected Areas) (Aguzzi *et al.*, 2024). However, many studies have highlighted the need for more "active" approaches, such as reintroducing key species (e.g., ecosystem engineers like seagrasses, corals, and sponges) or providing substrates for colonization (Da Ros *et al.*, 2019; Jones *et al.*, 2018). The future of coral reefs relies on swift and effective action at local, national, and international levels, which necessitates improved science for better management. This entails generating and analyzing more data, faster, and across larger scales than ever before. Emerging technologies such as drones, autonomous underwater vehicles (AUVs), 3D mapping tools,

high-resolution satellite imagery, and monitoring tool are already implemented and can greatly contribute to addressing key issues in coral reef conservation (Obura *et al.*, 2019; Apprill *et al.*, 2023; Franceschini *et al.*, 2023; Madin *et al.*, 2019).

As for many other conservation targets, approaches based on genetic information, also gained more importance recently. Teixeira and Huber (2021) emphasize the need to better understand genetic variation that is important for adapting to future environmental and climatic changes. In the study they argue that to study these genetic variations the following three conditions must be met: 1) the identification of the environmental factors negatively impacting a species and predicting their future geographical and/or temporal spread, 2) to ensure that tolerance or resilience to these changes varies within the species and has a significant genetic basis and 3) to be able to map the relevant genetic variants and predict which individuals will be most tolerant or resilient to the adverse conditions (Teixeira and Huber, 2021).

Other methods which are used in conservation include sampling of corals to measure cellular and molecular markers of stress (Downs *et al.*, 2005) or the measurement of endosymbiotic chlorophyll fluorescence as a representative variable of overall photosynthetic functionality (Warner *et al.*, 2012; Manzello *et al.*, 2009).

A variable that has been attributed to coral recovery from bleaching is the increased expression of photoprotective green fluorescent protein (GFP)-like pigments in bleached tissue (Bollati *et al.*, 2020)(see chapter 1.3.3). Fluorescent proteins are known to be amongst the most dynamic proteins which respond to environmental change (DeSalvo *et al.*, 2008). Because of the ability to respond quick, they might have the potential to help understand the adaptive physiology of corals to environmental factors and possibly be used to non-invasively monitor coral health (Roth *et al.*, 2010; Mazel *et al.*, 1995).

Another conservation effort lies in on-site reef restoration. Several different restoration methods have been developed over the last few decades. The most used one is coral transplantation, in which coral colonies and fragments are directly planted onto a reef substrate (Ferse *et al.*, 2021). Usually, small fragments are kept in so called „nurseries“ and are later transplanted to the natural reef. Projects like „Coral gardeners“, claim to have transplanted 30,980 corals by 2024 (<https://coralgardeners.org>) and are actively working together with scientists also raising awareness for the public. This restoration is done to return the reef to a previous state, rescue or promote a specific species, increase live cover, biomass and diversity (Polak *et al.*, 2012). Fast-growing, branching corals are used in most of these transplantation projects (Berger *et al.*, 2020).

Besides the commonly used coral fragmentation (asexual reproduction) technique, a rather new approach in coral reef restoration is to use in vitro fertilization (sexual reproduction), sometimes also coupled to cryopreservation (Hagedorn *et al.*, 2017; Dela Cruz and Harrison,

2022). The loss of adult breeding populations prevents the reproductive success of the corals and the natural supply and settlement of larvae, both of which is crucial for reef recovery. One way to approach this is the collection of gametes (eggs and sperm) during natural spawning events in the wild. These gametes are then combined and cultured in specially designed floating pools to produce millions of coral larvae (Dela Cruz and Harrison, 2022). Once the larvae are ready, they can be settled onto damaged reefs to aid in their recovery and restoration. Coral restoration using the sexual production of millions of genetically diverse coral larvae can aid the restoration of damaged coral reefs and their communities (Dela Cruz and Harrison, 2022).

1.2 Coral Physiology

Stony (reef-building) corals, belonging to the phylum Cnidaria (class: Hexacorallia; order: Scleractinia), utilize their photoautotrophic dinoflagellate symbiotic algae (known as zooxanthellae, belonging to the family Symbiodiniaceae) to convert sunlight into chemical energy and biomass, forming the foundation for reef ecosystems. Under different light regimes the success of symbiotic corals depends on the integrated physiological capacity of both symbiotic partners: the animal host on the one hand and the phototrophic dinoflagellate endosymbiont on the other hand. Together they are referred to as the coral holobiont (Trench, 1993). A lot of the productivity of coral reefs relies on this symbiotic relationship between the coral animal and the photosynthetic dinoflagellates living inside its tissue. Under optimal light conditions, photosynthesis provides up to 95% of energy requirements in form of organic carbon for the coral host (Carballo-Bolaños *et al.*, 2019). To support the zooxanthellae photosynthesis the coral in return provides them metabolic waste products and protection (Muscatine *et al.*, 1981). Light quantity and quality are among the most important factors determining the physiology and stress response of zooxanthellate corals (Wangpraseurt *et al.*, 2012). Corals use different strategies to photoacclimatize to the present light conditions and assist in maintaining a successful symbiosis with their photoautotrophic dinoflagellate endosymbionts.

Some known strategies are certain skeletal features that can modify the internal light environment (Kahng *et al.*, 2012) and the modification of the composition of photosynthetic pigments (Einbinder *et al.*, 2016). Another important strategy is to host genetically and functionally different types or lineages of symbionts. The family Symbiodiniaceae is a taxonomically diverse group (Coffroth and Santos, 2005). The coral host can modify the genetic composition of its Symbiodiniaceae community as well as the cell density (Frade *et al.*, 2008). This can potentially confer different advantages or disadvantages according to the light (and temperatures) corals are acclimated to.

Communities found in deeper water must be able to cope with a light environment that is characterized by reduced spectral composition and low irradiance intensity. In the mesophotic zone (30-150m), light intensities are significantly lower compared to those encountered by shallow corals (0–30m), with reductions of up to 99%. Typically, corals found at greater depths are more effective at utilizing minimal light levels (Einbinder *et al.*, 2016; Ben-Zvi *et al.*, 2021). They also tend to have a lower density of Symbiodiniaceae cells while exhibiting higher chlorophyll concentration within the symbiont cells (Mass *et al.*, 2007). Additionally, they show a diminished ability to regulate excessive light exposure (Einbinder *et al.*, 2016; Ben-Zvi *et al.*, 2021).

Excessive light exposure in shallow-water, on the other hand, can lead to photoinhibition and the generation of harmful reactive oxygen species, and this happens synergistically with increasing sea surface temperatures (SST) (Coles and Brown, 2003; Lesser, 2006).

Another known strategy that may help corals adapt to different light conditions is the regulation of fluorescent proteins.

1.3 Fluorescent Proteins (FPs)

1.3.1 Fluorescent Proteins – in General

“Fluorescence is a physical phenomenon, comprising the emission of light by a substance that has absorbed light with a different wavelength” (Ben-Zvi *et al.*, 2015). This phenomenon that has been reported in many marine organisms, including invertebrates and fishes. Corals are among the most fluorescent marine organisms (Mazel *et al.*, 2004; Sparks *et al.*, 2014). Green fluorescent protein (GFP) was originally discovered in the *Aequorea victoria* jellyfish (class Hydrozoa) in 1961 (Shimomura *et al.*, 1962). Since then, the study of fluorescent proteins (FPs) has introduced a new era in the cell biology field.

1.3.2 Fluorescent Proteins - in Corals

FPs are natural pigments whose color is dictated by the sequence of a single gene. Since they are the only natural pigment known to do this, FPs present a unique opportunity to directly investigate the molecular evolution of coral reef color (Alieva *et al.*, 2008; Field *et al.*, 2006). Under short-wavelength lighting conditions corals are extremely colorful. This is attributed to the phenomenon of fluorescence (Ben-Zvi *et al.*, 2021). FPs are an abundant and diverse class of proteins in reef-building corals. They strongly determine the color diversity of corals and the reef environment, complementing the brownish color given by the photosynthetic pigments of the coral’s algal symbionts (Matz *et al.*, 2006). Fluorescent proteins (FPs) are widespread in shallow reef-building corals (Alieva *et al.*, 2008) and can constitute a substantial portion of the total soluble protein in a coral (Leutenegger *et al.*,

2007). FPs absorb potentially harmful high-energy photons and re-emit them as lower-energy light. Corals exhibit a wide variety of FPs (Alieva *et al.*, 2008) and this also includes chromoproteins, GFP-like homologues that absorb light but do not re-emit it as fluorescence (Dove *et al.*, 2001).

There are three basic and common colour types of coral FPs: cyan (CFP), green (GFP), and red (RFP), and other occasional FP colours, such as yellow FPs (YFP) and chromo-red FPs (Alieva *et al.*, 2008). GFP is the most common FP in corals (Matz *et al.*, 2006). Despite the notoriously high diversity of FPs, their ecological functions in reef environments remain largely undetermined and controversial among researchers (Nakaema and Hidaka, 2015). There are two main hypotheses for the ecological function of FPs in corals; one of them being, that FPs can modulate symbiont light use, by re-emitting light at photosynthetically active wavelengths in deep-water environments and thus increasing light available to the symbionts (Smith *et al.*, 2017); and the other that FPs protect the symbionts from light stress in shallow-water environments, by shading them from extreme irradiance and UV (Bollati *et al.*, 2017; Smith *et al.*, 2017).

1.3.3 Fluorescent proteins - their role in “Colorful Bleaching”

Elevated seawater temperatures are considered the primary cause of coral bleaching, which results from the loss of symbionts (Good and Bahr, 2021). During some bleaching events, corals do not just turn white; they develop remarkably vivid fluorescent colors often described as 'glowing' (Voolstra, 2020). Up until now it was unclear why corals on some occasions became colorful rather than just white. One possible explanation is that the existing pigments in the coral tissue become more visible once the brownish-greenish color of the symbionts is depleted. Additionally, the fluorescence of these pigments might be more effectively excited in the absence of the symbionts' 'shading' effect (Roth *et al.*, 2013). In the severe 1998 coral mass bleaching event on the Great Barrier Reef, a correlation was found between bleaching resistance and concentration of FPs in coral tissue (Salih *et al.*, 2000). Green FPs were more abundant in temperature tolerant species of corals, compared to temperature sensitive species (Dizon *et al.*, 2021). A recent study (Bollati *et al.*, 2020) revealed that the vibrant bleaching observed is due to elevated expression of photoprotective GFP-like protein pigments, prompted by intensified light fluxes within the coral tissue depleted of symbionts. In healthy corals, significant amounts of incident sunlight are absorbed by the photosynthetic pigments of symbionts, leading to the downregulation of colourful host pigment production. In the bleached tissue, this light is no longer absorbed by the symbiont pigments. Instead, the white skeleton facilitates the light to reflect within the tissue. This heightened internal light fluxes trigger the expression of light-responsive FPs, which then leads to the colourful appearance. The induced stronger expression of FPs (GFP/CFP) on the ectodermal layer of the coral tissue absorbs the excessive light contributing to the decrease of the levels of

internal light stress for the symbionts, facilitating their recovery. As the abundance in symbionts goes up again, they progressively absorb more light, further diminishing internal light levels. Consequently, host pigment production is subsequently downregulated, restoring the system to its normal state (Bollati *et al.*, 2020; Voolstra, 2020). Therefore, the authors suggest that vibrant bleaching may serve as an indicator of bleaching events with favourable recovery prospects for corals. This phenomenon only happens after the elevated temperatures that triggered the initial bleaching were returning to normal. Prolonged exposure to mild heat stress also seems to trigger the colour response (Bollati *et al.*, 2020).

1.4 The coral genus „*Madracis*“

As elucidated by our research team's documented findings (Frade *et al.*, 2010; Bongaerts *et al.*, 2015), the genus *Madracis* reveals a remarkable depth distribution spanning from 2 to over 100 meters in Caribbean reefs. Because of its unique vertical distribution patterns and the diverse zooxanthellae types found at different depths, the genus *Madracis* offers a suitable framework for investigating how coral hosts and their symbionts adapt and acclimatize in response to varying light conditions across extensive depth ranges (Frade *et al.*, 2008). This study used three different species of the genus *Madracis*. The encrusting or submassive *Madracis pharensis* (Heller 1868) and *Madracis senaria* (Wells 1973a). The two of them can be considered depth generalists with distribution from 5 to >60 m (Vermeij and Bak, 2003; Frade *et al.*, 2008) and the nodular *Madracis decactis* (Lyman 1859) which occurs from 5 to 40 m.

1.5 Objectives

My study aims to elucidate the spatial distribution and functional role of FPs in *Madracis* corals across varying depths and colony positions, using an integrative approach that combines histological techniques, *in situ* photobiology measurements of the symbionts in hospite and confocal microscopy. The application of various techniques and the integration of multiple variables for simultaneous analysis, including potential interactions, represents a relatively novel approach. This methodology holds significant promise for elucidating the complex dynamics of fluorescent proteins (FPs) and symbiont photobiology in corals.

One of the key questions of my research is, if depth, and therefore also light availability, play a role in the distribution of FPs and the amount that is being expressed. My goal was to quantify the FP regarding their fluorescence intensity as well as to analyze the relative position of FPs and symbionts. Depth-light gradients in this field of FPs have remained largely unexplored until now. More innovative work using advanced microscopy techniques is

needed to study the distribution of fluorescence within the tissue of this depth generalist coral genus.

Leveraging the mentioned extensive vertical range, my study seeks to delve into the spatial distribution of FPs within the tissues of *Madracis* corals across varying depths and between different colony positions (top and side) leading up to the next question – Are there species-specific differences and is it possible to characterize certain species by potentially unique FP patterns?

As mentioned, FPs are hypothesized to contribute to the modulation of light inside the coral tissue and thus help corals and their photosymbionts to adjust to acute depth-light gradients, particularly for such coral genera exhibiting broad depth distributions (Bollati *et al.*, 2017; Smith *et al.*, 2017). Therefore, the coral photosymbionts, their distribution and cell density, will also be targets of my study. One dimension of my research that I would like to highlight is the innovative cellular-level analyses of fluorescent protein distribution—a rather new approach in this field.

The project comprises three key objectives:

- I. **To differentiate and quantify FPs:** By employing quantitative confocal image analysis, I aim to identify, quantify and distinguish different FPs in *Madracis* corals.
- II. **To localize FPs within coral tissue:** By using advanced histological techniques and confocal microscopy, I aim to map FPs distribution within *Madracis* coral tissues revealing potential functional distinctions.
- III. **Symbiont specific analysis:** By quantifying chlorophyll *a* and *c2* as well as symbiont cell density I aim to characterize the symbionts photobiological response and photosynthetic performance.

2 Glowing Reefs: Understanding the distribution and function of fluorescent proteins in *Madracis* species across a Caribbean reef slope

2.1 Introduction- Fluorescent proteins (FPs) in corals

Corals are well known for their vivid coloration, for which fluorescent proteins (FPs) are largely responsible (Matz *et al.*, 1999). FPs are an abundant and diverse class of proteins in reef-building corals. They strongly determine the color diversity of reef environments, and the corals themselves by complementing the brownish color given by the photosynthetic pigments of the coral's algal symbionts (Matz *et al.*, 1999). There are three basic and common color types of coral FPs: cyan (CFP), green (GFP), and red (RFP), and other occasional FP colors, such as yellow FPs (YFP) and chromo-red FPs (Alieva *et al.*, 2008). GFP is the most common in corals (Matz *et al.*, 2006). In addition, another contributor to coral fluorescence is the red chlorophyll fluorescence (RCF) from the symbiotic zooxanthellae (Warner *et al.*, 2010). Corals can either express one or multiple FPs. This can alter their coloring during different life stages, under changing stressors, environmental conditions, or along a depth gradient (Ben-Zvi *et al.*, 2019). Variation in FP expression among individuals within the same species may be due to differences in the number of FP gene copies, as well as to differences in the regulation of FP expression (Gittins *et al.*, 2015; Dizon *et al.*, 2021; Ferreira *et al.*, 2023).

Despite the notoriously high diversity of FPs, their ecological functions in reef environments remain largely undetermined and controversial among researchers (Nakaema and Hidaka, 2015). The most prominent hypotheses of FP function within corals are related to the maintenance of the obligate symbiosis with their photosynthetic algae. This means FPs can play an important role in coral adaptation to low light conditions (Bollati *et al.*, 2017; Smith *et al.*, 2017). FPs can convert shorter wavelengths of light into longer wavelengths. This has led to the suggestion of two main functions being photo protection and light enhancement.

Photoprotection. FPs play a critical role in providing UV protection for corals and mitigating light stress. Under high-light conditions, corals express photoprotective pigments that absorb light, thereby reducing the amount of light available to the photo symbionts within their tissues (Gittins *et al.*, 2015; Salih *et al.*, 2000). This photoprotection mechanism is particularly pronounced in shallow environments, where high irradiance induces the expression of FPs, such as GFP and CFP, which absorb excessive light and help decrease internal light stress (Bollati *et al.*, 2020). Many of these FPs are localized in the ectoderm of coral tissues and are regulated by light intensity, especially within the blue spectral range (Gittins *et al.*, 2015). In

the severe 1998 coral mass bleaching event on the Great Barrier Reef, a correlation was found between bleaching resistance and concentration of FPs in coral tissue (Salih *et al.*, 2000). The increased expression of photoprotective GFP-like pigments in bleached tissue (i.e., “colourful bleaching”) has been implicated in coral recovery from bleaching (Bollati *et al.*, 2020) and is providing some hope for the future of coral reefs.

Light Enhancement. FPs also have been demonstrated to play an important role in coral adaptation to low light conditions (Bollati *et al.*, 2017; Smith *et al.*, 2017). FPs can serve the purpose of photoconversion of certain wavelengths into ones that are more favorable for symbiont photosynthesis. They provide longer wavelengths to shaded zooxanthellae as an adaptation of corals to life in light-limited environments (Bollati *et al.*, 2017; Ben-Zvi *et al.*, 2019). Blue light, which is increasingly abundant in relative proportion at greater depths (eg. >20 m), is being converted into red-orange light. This red-orange light (which is of a longer wavelength) can penetrate deeper into the coral tissues, and thus leads to an increase in the utilized light (Bollati *et al.*, 2017; Ben-Zvi *et al.*, 2019). This could constitute an adaptation of corals to less light by optimizing photosynthesis (Bollati *et al.*, 2017). FPs known to serve this function are known as photoconvertible red FPs (pcRFP). Additional hypotheses on the functions of FPs include antioxidant capacity (Bou-Abdallah *et al.*, 2006), regulation of dinoflagellate population (Dove *et al.*, 2008) and coral innate immune response (Palmer *et al.*, 2009; D’angelo *et al.*, 2012). Another potential function of coral FP is to attract both free-living zooxanthellae and zooplankton since both exhibit phototactic attraction towards green fluorescence (Aihara *et al.*, 2019, Ben-Zvi *et al.*, 2022). All these functions may not be mutually exclusive. It is likely that different fluorescent proteins serve distinct functions, thereby offering corals a diverse array of functional advantages (Roth *et al.*, 2013). The evolution of GFP-like homologues suggests that the absorption properties of FPs might be crucial to their ecological roles. Different types of fluorescent proteins may serve distinct and potentially complementary functions for the coral and the symbionts (Roth *et al.*, 2013 Ferreira *et al.*, 2023).

2.2 Materials and Methods

2.2.1 Sample Collection & Target Species

The genus *Madracis* ranges from 2 to over 100 meters deep in Caribbean reefs (Frade *et al.*, 2010; Bongaerts *et al.*, 2015). Its wide depth distribution and diverse zooxanthellae make it ideal for studying how corals and their symbionts adapt to different light conditions (Frade *et al.*, 2008). This study used three different *Madracis* species. The encrusting or submassive *Madracis pharensis* (Heller 1868) and *Madracis senaria* (Wells 1973a), and the nodular *Madracis decactis* (Lyman 1859). Samples of *Madracis pharensis*, *Madracis decactis* and *Madracis senaria* were collected in Snake Bay, Curaçao, southern Caribbean, in September 2022, to analyze host and symbiont physiological parameters described in the following sections. The samples were collected from two different depths; deeper samples were collected between 17 to 22 m depth, while shallower samples were collected between 4.5 to 11 m. In addition, of each colony two different orientations (top and side of the colony) have been sampled. Over all only light-exposed colonies were included in the study. *M. decactis* shallowest sample was collected at 5m and the deepest at 18m. *M. senaria* shallowest sample was collected at 8m and the deepest at 21m. *M. pharensis* shallowest sample was collected at 6m and the deepest at 20m. In total 10 samples of *M. decactis*, 14 samples of *M. pharensis* and 12 samples of *M. senaria* have been collected.

Before sampling, all samples were photographed under blue light (for colony fluorescence) and ambient light. In addition, before collection, sampled colonies were measured for photosynthetic activity (photochemical efficiency or yield), reflectance, and fluorescence using a pulse amplitude modulated (PAM) fluorometer (Diving-PAM; Walz GmbH, Germany) in the field. Samples were divided into fragments: one for tissue visualization under confocal microscopy, and one for analysis of symbiont physiological parameters.

Coral fragments for confocal microscopy were brought to the laboratory in seawater after sampling in the field and fixed in paraformaldehyde (PFA) solution 4% for 6 h at 4°C, transferred to phosphate-buffered saline (PBS) 1x and stored at 4°C. Coral fragments for symbiont physiology were snap-frozen in liquid nitrogen immediately after leaving the water in the field. All samples were returned to Vienna in liquid nitrogen or dry ice.

2.2.2 Symbiont photobiology and irradiance

The tissue was removed from the skeleton using an air-jet with a pipette tip attached. The tissue was brushed off while inside a ziplock bag filled with 10 ml 1x PBS and then further processed for further analysis. To characterize the photobiology, zooxanthellae were counted using a hemocytometer. A minimum 8 independent counts (each for a 0.1 µL per well) of 200

cells per sample were established to ensure a robust and precise counting of zooxanthellae, aiming for a coefficient of variation below 10%. The cell density per coral surface area was then calculated accordingly. Surface area was determined using the aluminum foil method (Marsh 1970). The chlorophyll quantification was done using a spectrophotometer. Absorbance was measured at 630, 663, and 750 nm using a BioTek™ microplate reader. The Jeffrey & Humphrey (1975) formulas were used to calculate chlorophyll *a* and *c*₂. The surface area and zooxanthellae density per area, were then used to calculate chlorophyll per area and per cell. The incident irradiance was calculated using a published formula for the field location, which converts depth to irradiance (Frade *et al.*, 2008).

$$E(z) = E(0) * e^{-k*z}$$

(*E* = PAR irradiance (in $\mu\text{mol}/\text{m}^2/\text{s}$), *k* = attenuation coefficient; *z*=depth (in m)).

2.2.3 Fluorescent Proteins

2.2.3.1 Sample preparation and Laser scanning confocal microscopy (LSCM)

Fragments collected for LSCM were transferred to Ethylenediaminetetraacetic acid (EDTA 10%) for decalcification. After decalcification, the remaining tissue samples were extracted and transferred to a 30% sucrose medium for overnight rehydration. Before sectioning, the rehydrated tissue was embedded in OCT in small plastic molds. Tissue samples were sectioned using a cryostat. A slice thickness of 20 μm was defined. To further prepare the samples for confocal microscopy tissue samples were mounted in the mounting medium Mowiol, followed by covering them with a coverslip. Samples were then allowed to dry for an hour, and then ready for confocal microscopy.

2.2.3.2 LSCM – Laser scanning confocal microscopy

A Zeiss LSCM 780 microscope and Zen Black processing software (2011 v14.0.16.201) were used to image the coral tissue and its fluorescence. The samples were analyzed at a magnification of 20x with different excitation lasers at specific wavelengths ($\lambda=405\text{nm}$, $\lambda=488\text{nm}$, $\lambda=546\text{nm}$ and $\lambda=633\text{nm}$).

From each sample, 9 tissue slices have been analysed in the confocal. Four regions of interest were chosen in each image to be quantified for their fluorescence intensity: green (GFP), blue (blue tissue fluorescence; BTF), yellow (YFP) and red (red chlorophyll autofluorescence; RCF). For each species, different settings had to be used (settings within species and treatments remained the same).

Lambda scans were performed to acquire the emission spectrum of the fluorescent proteins (FPs). This procedure was executed for a chosen optical field (one that had all regions of interest present if possible) using each of the six available laser lines ($\lambda=405\text{nm}$, $\lambda=458\text{nm}$,

$\lambda=488\text{nm}$, $\lambda=514\text{nm}$, $\lambda=458\text{nm}$, $\lambda=561\text{nm}$, and $\lambda=633\text{nm}$). The identification of FPs was primarily based on their emission peaks as previously described (Alieva *et al.* 2008). In all images, the blue color was identified autofluorescence from the host tissue (BTF), rather than an actual fluorescent protein. This was based on its distribution characteristics and the fact, that the fluorescence intensity was low and almost not possible to detect. Consequently, it was therefore not included in the FP analyses but used as a proxy for tissue area.

2.2.3.3 Image analyses from confocal microscopy - Occurrence of distinct FP signatures

To further characterize FP patterns, images taken in the confocal were scored as follows. The following categories were quantified by using a binary variable, with values of "1" indicating the presence of the category and "0" indicating its absence: Granular green fluorescent protein (granular GFP), diffused green fluorescent protein (diffused GFP), granular blue fluorescent protein (granular BFP), diffused blue fluorescent protein (diffused BTF), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP) and also endolithic filamentous structures, which are most likely not part of the host tissue (yellow not from tissue) (Fig.1). These structures exhibited the same emission peak as the yellow originating from the coral tissue and were consequently quantified as yellow fluorescence by the LSCM. Given that our study focuses solely on analyzing host fluorescence, images containing non-identified structures were excluded from the analyses. In total, 403 images have been scored for presence/absence of these categories.

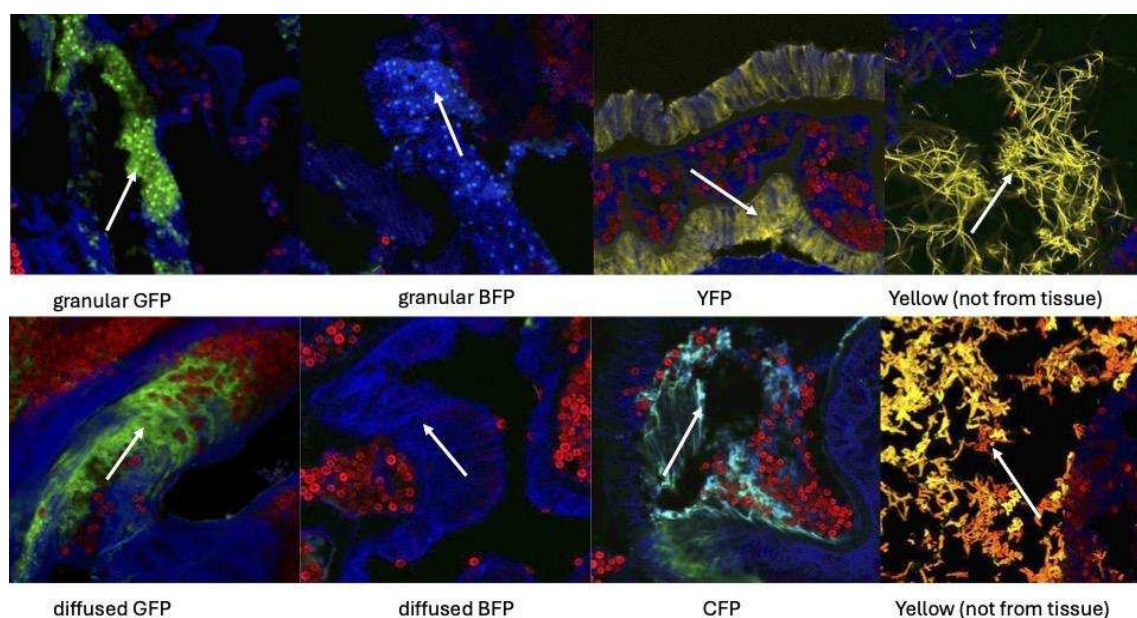


Figure 1. Representative examples for each category displaying the characteristics of the quantified FP – granular GFP/BFP: small, bright green/blue dots embedded in a less bright green/blue background; YFP: dispersed yellow; Yellow not from tissue: filamentous yellow to orange; diffused GFP/BFP: dispersed green/blue; CFP: bright light blue (cyan) usually dispersed.

2.3 Data Analyses

Statistics were used to assess significant differences between sampling treatments using RStudio version 2022.07.0 Build 548 (<http://www.rstudio.com>). The treatments were compared regarding their differences in FPs occurrence and FP intensity. Comparisons were done among species with the occurrence (presence vs. absence) data, as well as using ratios of different FP signals.

Occurrence of distinct FP signatures. First step was a Principal coordinate analysis (PCoA with Jaccard-Index) for the binominal presence vs. absence data from the image analyses (granular and diffused GFP, granular and diffused BTF, RCF, YFP) to check the data for a possible distribution pattern regarding depth, orientation and species. The presence vs. absence data was then further analyzed with χ^2 -tests to check for differences between depth, orientation and species

As a final step for the analyses of the presence vs. absence data, a logistic regression with a model selection process was conducted to calculate the importance and influence of each predictor variable (depth, orientation, species, irradiance) and their potential interactions and the impact on the likelihood of expressing different FPs. The significance of each predictor on the response variable (such as orientation, depth, species) was assessed using the likelihood ratio χ^2 -tests. The logistic regression model's goodness-of-fit was assessed using Hosmer-Lemeshow test.

FP fluorescent intensities. In the confocal, the fluorescent intensity (in relative fluorescent units) from the GFP, BTF, RCF and YFP was measured. For the statistical analyses, the first step was the calculation of the mean fluorescent intensity of each FP for each sample. (meaning the mean fluorescent intensity for each FPs, of the 9 analyzed tissue slices per sample). This data was only suitable for analyses within species, since for each of the three species, a different setting of the confocal was used to measure the FPs intensities, hence the fluorescent intensities cannot be compared between species.

To compare the mean intensities of each FP between depth and orientation, multiple ANOVAs followed by Tukey tests to test pairwise significances have been conducted within each sample set of the three species.

To compare the three species with each other, three different ratios of fluorescent mean intensities have been calculated.

GFP:RCF. Is the ratio of the GFP fluorescent intensity to RCF fluorescent intensity. This ratio has been calculated based on the mean fluorescent intensity of GFP and RCF for each sample. The RCF can be seen as a proxy for the symbionts since the chlorophyll of the symbionts will appear as red chlorophyll fluorescence.

GFP:BTF. Is the ratio of the GFP fluorescent intensity to BTF fluorescent intensity. This ratio

has been calculated based on the mean fluorescent intensity of GFP and BTF for each sample. The BTF can be seen as a proxy for the area of tissue visualized in the confocal. *RCF:BTF*. Is the ratio of the RCF fluorescent intensity to BTF fluorescent intensity. This ratio has been calculated based on the mean fluorescent intensity of RCF and BTF for each sample.

The ratios have been compared within and between species, and between depths and orientations by conducting multiple ANOVAs followed by Tukey tests to test pairwise significances.

Symbiont and photobiology analyses. Besides analyzing FPs this study aimed to identify any differences in host symbiont photobiology. As a first step the data was explored by performing a principal component analysis (PCA) with the symbiont data (zoox density, chlorophyll *a* and *c₂* per area and cell and yield).

The PCA on the symbiont data was followed by conducting multiple ANOVAs followed by Tukey tests to test pairwise significances, to check for differences between depth, orientation and species regarding their chlorophyll (*a* and *c₂*) per area and cell, zooxanthellae density per area and the measured yield. Symbiont data was analyzed only across species, since the sample size for each treatment was too small for within species comparison.

Effect of light on symbiont photobiology was analyzed with a Pearson correlation between total PAR irradiance and the quantum yield of photosynthesis and between cellular chlorophyll *a* and total PAR irradiance.

Before any testing was done, outliers have been removed from the dataset using the interquartile range (IQR) method. Quartiles were first calculated to determine the first quartile (Q1) and the third quartile (Q3). The IQR was then computed as the difference between Q3 and Q1. Data points falling below $Q1 - 1.5 * IQR$ or above $Q3 + 1.5 * IQR$ were identified as outliers and removed from the dataset. This approach helps ensure that extreme values do not unduly influence the analysis, resulting in a cleaner and more reliable dataset.

All other requirements for parametric testing have been checked as well; Histograms, QQ-plots and Shapiro-Wilk tests have been used to check for normality and as an assessment for homogeneity of variance the Levene's test was conducted.

If the requirements for parametric testing were not met, non-parametric Kruskal-Wallis tests was used followed by the Dunn test.

2.4 Results

2.4.1 Irradiance

Total PAR irradiance decreases exponentially with depth for both the top and side of colonies (Fig.2(A)). For the shallowest collected sample from 5 m, the top part of the colony was exposed to a total PAR irradiance of $1047 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, while the side of the colony was exposed to only $186 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (as calculated using the formula in section 2.2.2). In contrast, for the deepest sampled colony, which was located at a depth of 22.3 m, the top part of the colony was exposed to a total PAR irradiance of $299 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, while the side part was exposed only $73 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ from the same colony. The greatest discrepancy is observed between deep colonies side samples and shallow colonies top samples. The shallow/top samples are exposed to an irradiance that is over 10x higher than what the deep/side samples are exposed to. (Fig.2(B))

Colonies situated closer to the surface, experience a greater variation of light over their colony surfaces. With depth, this variation of light over the colony surface decreases. In shallow waters, the light intensity is approximately 7 times higher in the top compared to side positions, while in deep waters, the intensity at the top position is about 6 times higher than at the side position. Both differences are relatively close in value, but they represent different contexts. Shallow waters generally have more variation in light intensity between positions compared to deep waters, where light is more attenuated, and the difference is more constant. The smaller difference in deep waters suggests that the change in light intensity is more consistent, but both scenarios show significant variations in light depending on depth and position.

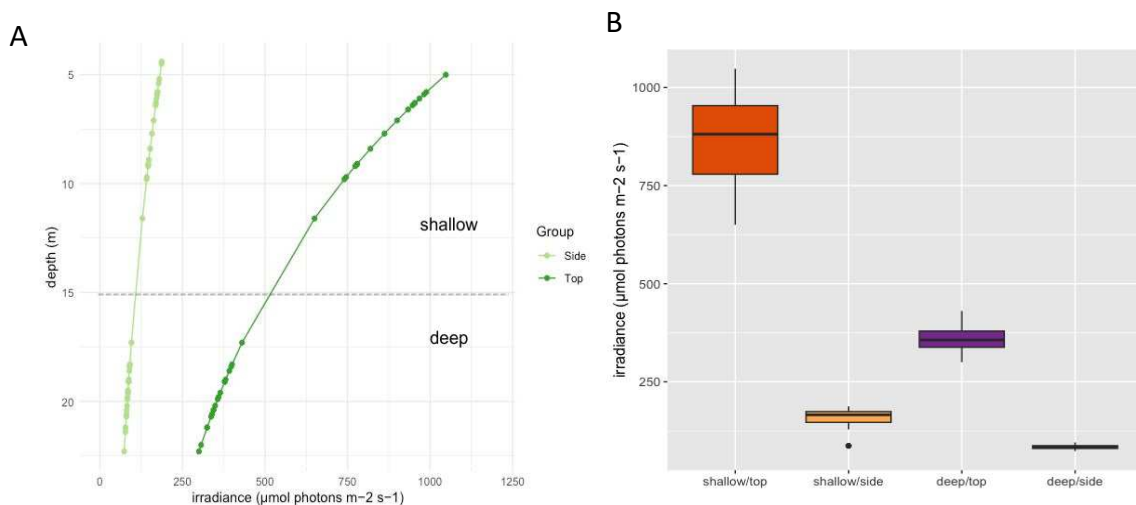


Figure 2. (A) Total PAR irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) measured for two orientations (top & side) within the coral colony (B) Boxplot representing two different depth and two different orientations and the total PAR irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)

2.4.2 Identification of fluorescent proteins

Utilizing LSCM, I was able to detect three types of FPs (CFP, GFP, YFP) in the coral tissue and in addition the chlorophyll *a* in the zooxanthellae. Figure 3 illustrates the five different regions of interest that have been quantified by their mean intensity. The first region, which focused on the symbionts containing the chlorophyll (RCF), emits fluorescence predominantly in the far-red spectral region (~ 679nm). The second region was associated with GFPs, with emission peaks varying between species: *M. decactis* exhibited an emission peak at 521nm, while *M. pharensis* and *M. senaria* exhibited an emission peak at 516 nm. CFP was identified with an emission peak at 501-504nm. The YFP was identified with an emission peak at 572nm and the hosts tissue (BTF) with 453 nm. Table 1 gives an overview of the used lasers and the emission peaks.

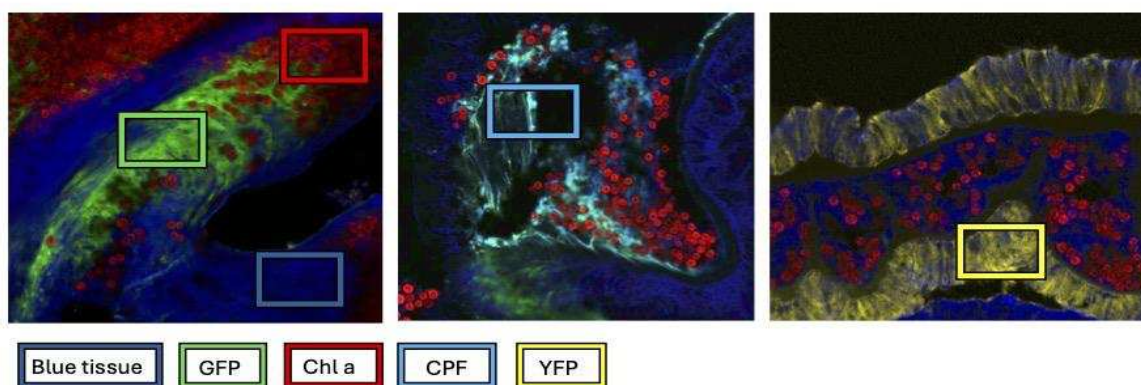


Figure 3. Five regions of interest that have been targeted in the confocal and quantified by their mean intensity in each image that was taken for all three species.

Table 1. Identified fluorescent proteins and chlorophyll *a* in *M. pharensis*, *M. senaria* and *M. decactis*. The table represents the wavelength at which the emission peak was obtained and the excitation laser that yielded the highest emission of fluorescence.

	Excitation Laser	Emission peak
GFP	488 nm	~ 516/521nm
CFP	488 nm	~ 501-504 nm
YFP	561 nm	~ 572 nm
Chl a (RCF)	561 nm	~ 679 nm
Tissue (BTF)	458 nm	~ 453 nm

2.4.3 Occurrence of distinct FP signatures

PCoA reveals that CFP, diffused GFP and granular GFP are all positively correlated with each other. Biplot A, C and D (Fig. 4) illustrate the overlap of species, depth and orientation regarding their FP pattern. The analyses suggest that the differences may not be strong since the different treatments are mostly overlapping.

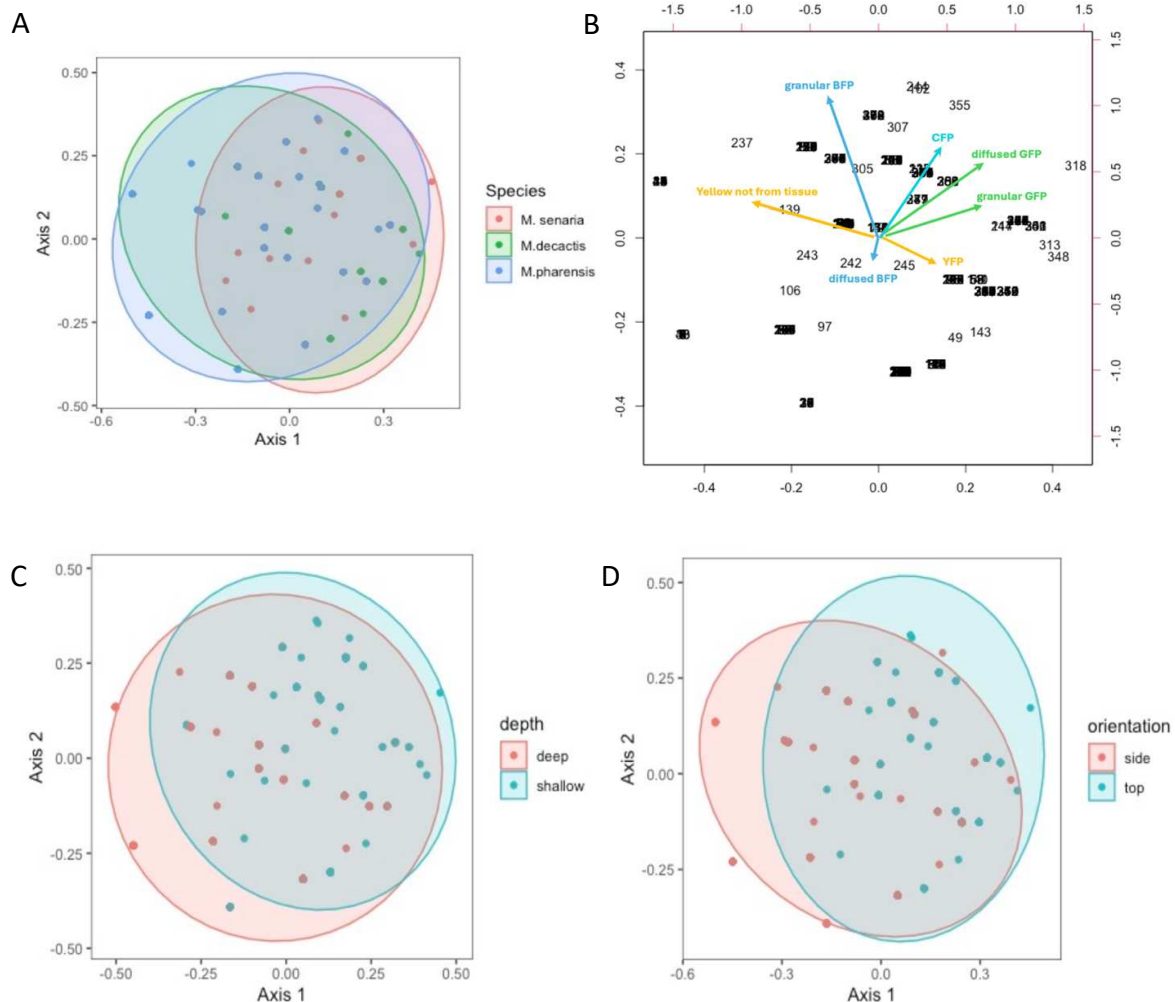


Figure 4. (A-D) PCoA on the occurrence of distinct FP signatures (A) correlation of the different variables in relation to PC1 and PC2 (B-D) relationships between symbiont physiology and the three different treatments, being species, depth, and orientation in the reduced-dimensional space.

Effect of Species. The data from the scoring of presence and absence of different FP categories was analysed using χ^2 -tests. Diffused GFP revealed a significant relationship between species and diffused GFP ($\chi^2 = 23.219$, $df = 2$, $p < 0.001$) (Fig. 5). Post-hoc analysis employing adjusted standardized residuals unveiled that *M. senaria* displayed higher levels of diffused GFP compared to both *M. decatis* and *M. pharensis* (both $p < 0.001$). Species also had a significant effect on the occurrence of granular GFP ($\chi^2 = 42.464$, $df = 2$, $p < 0.001$). Post-hoc analysis, utilizing adjusted standardized residuals, demonstrated that *M. senaria* exhibited higher levels of granular GFP compared to both *M. decatis* and *M. pharensis* (both $p < 0.001$). Moreover, *M. pharensis* displayed significantly higher levels of granular GFP compared to *M. decatis* ($p < 0.0001$). This comprehensive analysis highlights the pronounced differences between species concerning both diffused and granular GFP categories.

The analysis of granular BFP revealed a compelling relationship among species ($\chi^2 = 12.341$, $df = 2$, $p = 0.002$). *M. senaria* exhibited substantially lower levels of granular BFP compared to both *M. decatis* and *M. pharensis* (both $p < 0.001$). The analysis of YFP reveals significant differences among species ($\chi^2 = 18.626$, $df = 2$, $p < 0.001$). *M. pharensis* exhibited lowest levels of YFP occurrence when compared to *M. decatis* and *M. senaria* (both $p < 0.001$).

Occurrence of distinct FP signatures in %

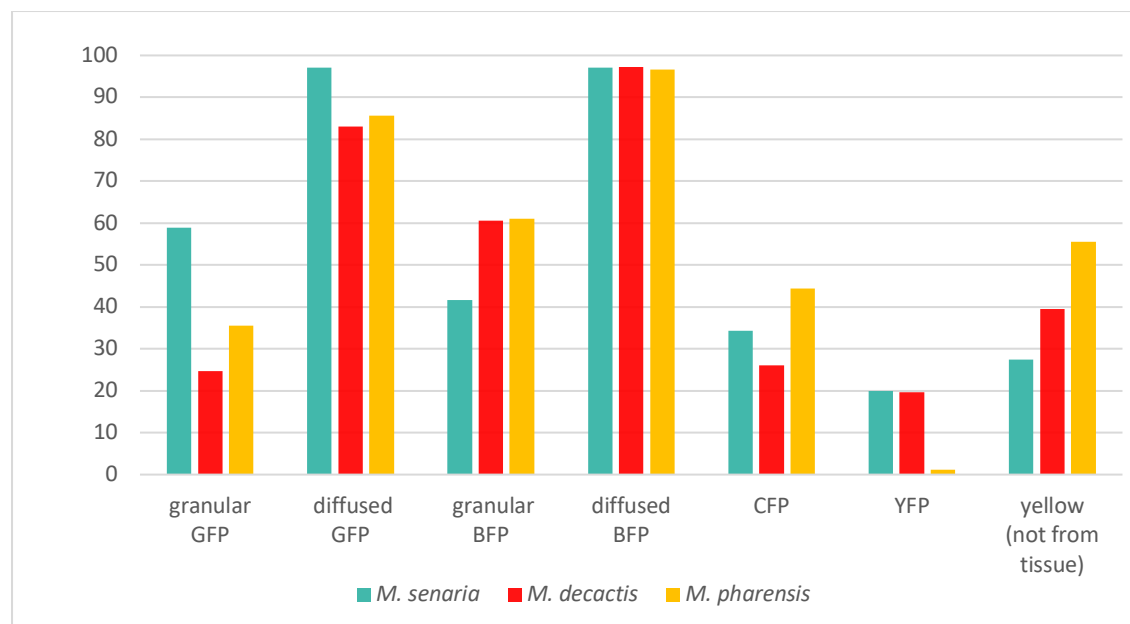


Figure 5. Occurrence of seven different FP categories in the three target species. The assessment involved the calculation of the occurrence of FP categories in percent (%) within the set of images per species revealing *M. senaria* displayed the highest levels of diffused GFP as well as granular GFP and YFP (all $p < 0.001$).

Effect of Depth and Orientation. The results of the χ^2 -tests demonstrated significant differences in FP occurrence between depths , as well as between orientation and FP categories. Regarding orientation, statistically significant differences were observed between tops and sides for granular GFP and CFP, both showing higher abundance in top-oriented samples of the colony ($\chi^2 = 34.652$, $df = 1$, $p < 0.0001$ and $\chi^2 = 22.077$, $df = 1$, $p < 0.001$, respectively). (Fig. 6)

For depth, there was a statistically significant difference for cyan FP (CFP) and granular GFP. Both were significantly more abundant in shallow samples ($\chi^2 = 18.566$, $df = 1$, $p < 0.001$ and $\chi^2 = 5.2466$, $df = 1$, $p = 0.021$, respectively). (Fig. 7)

Differences between top and side in the occurrence of distinct FP signatures in %

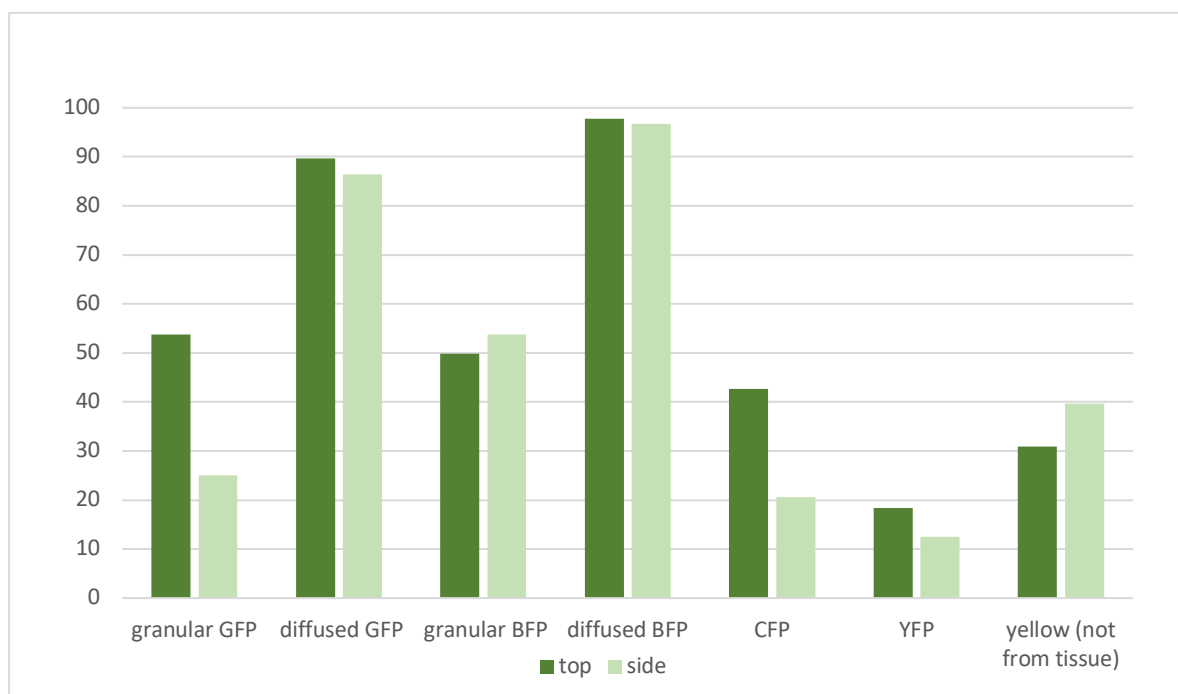


Figure 6. Occurrence of seven different FP categories in to different colony orientations (top& side) The assessment involved the calculation of the occurrence of FP categories in percent (%) within the set of images of the given category; revealing that top oriented samples show a higher occurrence of granular GFP and CFP ($\chi^2 = 34.652$, $df = 1$, $p < 0.001$ and $\chi^2 = 22.077$, $df = 1$, $p < 0.001$, respectively).

Differences between deep and shallow in the occurrence of distinct FP signatures in %

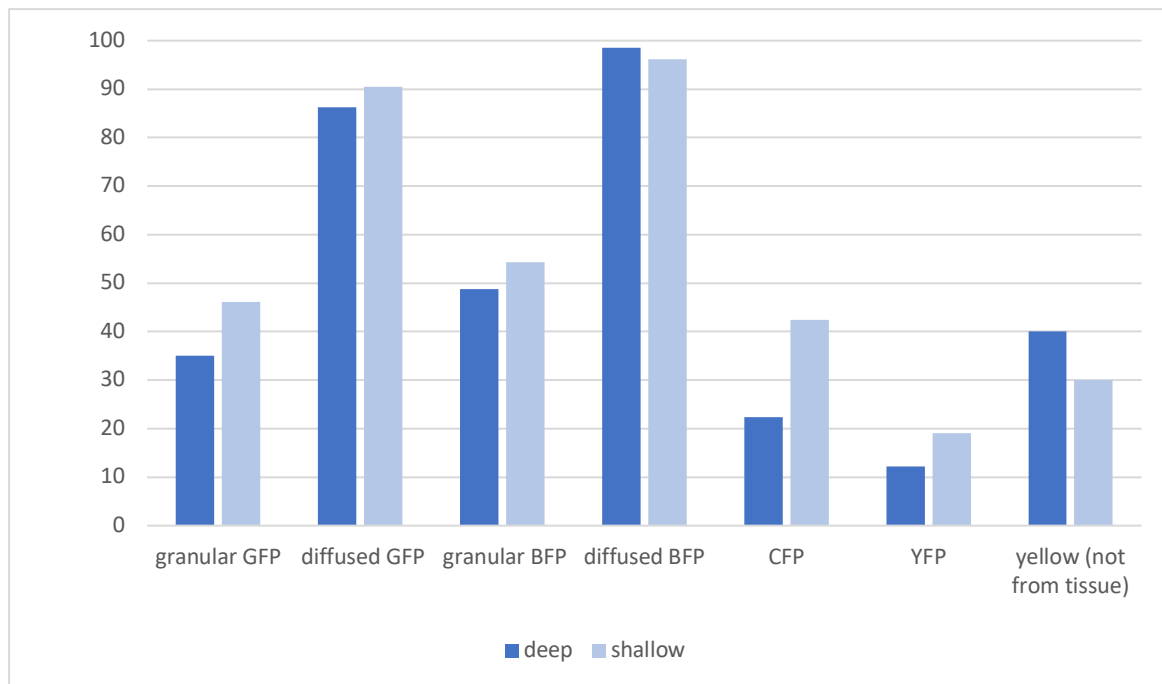


Figure 7. Occurrence of seven different FP categories in to different colony depths (deep & shallow) The assessment involved the calculation of the occurrence of FP categories in percent (%) within the set of images of the given category; revealing that shallow samples showed a higher occurrence of cyan FP (CFP) and granular GFP ($\chi^2 = 18.566$, $df = 1$, $p < 0.0001$ and $\chi^2 = 5.2466$, $df = 1$, $p = 0.021$, respectively).

Occurrence of granular GFP. The significance of each predictor variable was assessed using the likelihood ratio chi-square test. Species was found to be a significant predictor for the occurrence of granular GFP ($\chi^2 = 43.048$, $df = 2$, $p < 0.001$). The inclusion of orientation ($\chi^2 = 54.186$, $df = 1$, $p < 0.001$) and depth ($\chi^2 = 7.688$, $df = 1$, $p = 0.006$) as a predictor variable demonstrated a significant improvement in the model fit as well as including interactions between species and orientation ($\chi^2 = 12.395$, $df = 2$, $p = 0.002$). (Fig. 8) The model with species, depth, orientation and interactions between species and orientation as a predictor provides a significantly better fit to the data than other tested models. (Hosmer-Lemeshow $p > 0.05$; no evidence of lack of fit in the model) The model's pseudo-R² value of 0.7782 suggests that approximately 77.82% of the variability in the granular GFP outcome accounted for by the model.

Differences in the occurrence of granular GFP signatures in %

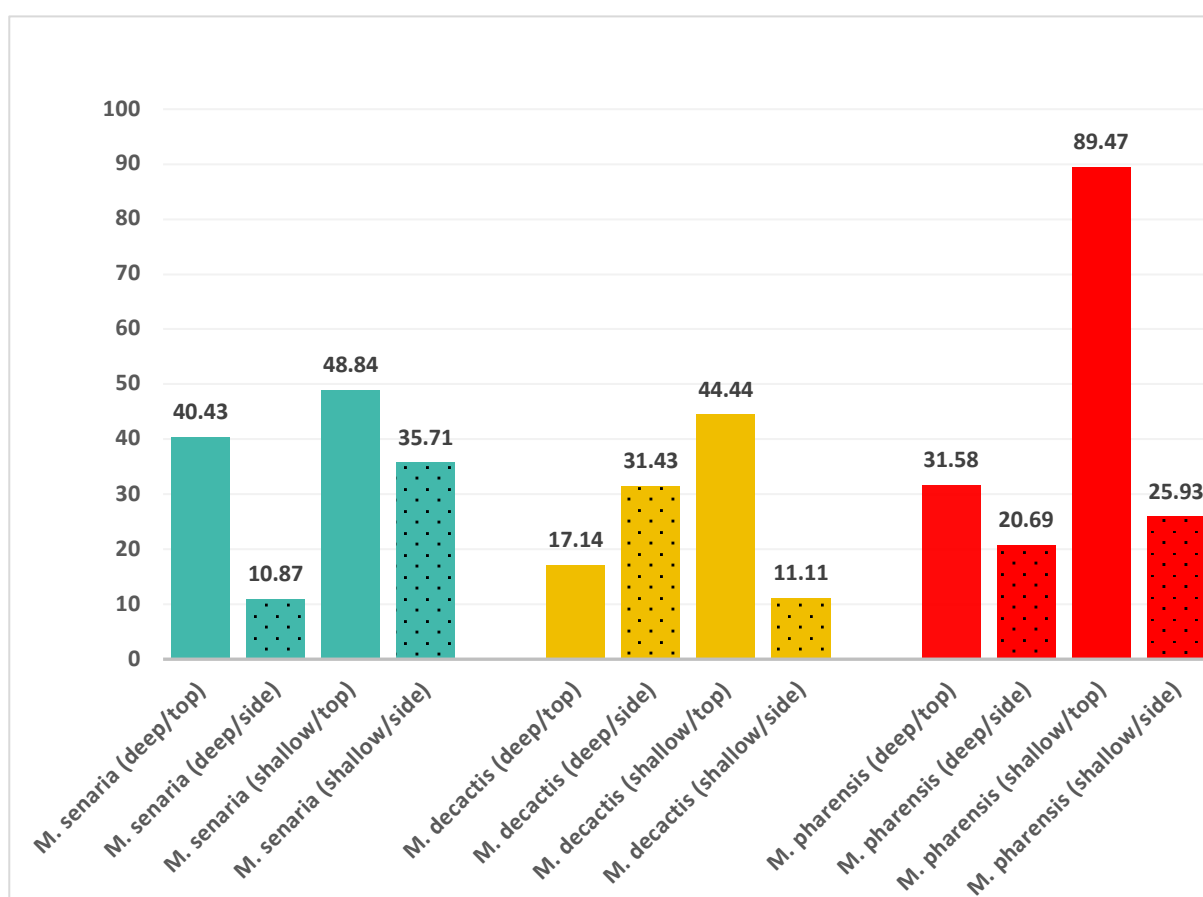


Figure 8. Occurrence of granular GFP in the three target species and two different treatments (orientation & depth). The assessment involved the calculation of the occurrence of granular GFP in percent (%) within the set of images per species and treatment. The Logistic regression analyses revealed species ($\chi^2 = 43.048$, $df = 2$, $p < 0.001$), orientation ($\chi^2 = 54.186$, $df = 1$, $p < 0.001$), depth ($\chi^2 = 7.688$, $df = 1$, $p = 0.006$) and the interactions between species and orientation ($\chi^2 = 12.395$, $df = 2$, $p = 0.002$) as significant predictors for the occurrence of granular GFP.

For top samples, there is a significant increase in the odds of expressing granular GFP with an odds ratio (OR) of 5.34 (95% CI: [2.72-10.47], $p < 0.05$). Also, shallow corals, have a significant increase in the odds of expressing granular GFP with an OR of 2.11 (95% CI: [1.08, 4.12], $p < 0.05$). For species, the analysis yielded an OR of 0.21 for *M. decactis* (95% CI: [0.06, 0.67], $p < 0.05$). (Fig.9(B)) This indicates that for the species *M. decactis*, there is a significant decrease in the odds of expressing granular GFP compared to the *M. senaria* and *M. pharensis*. (Fig 9(A))

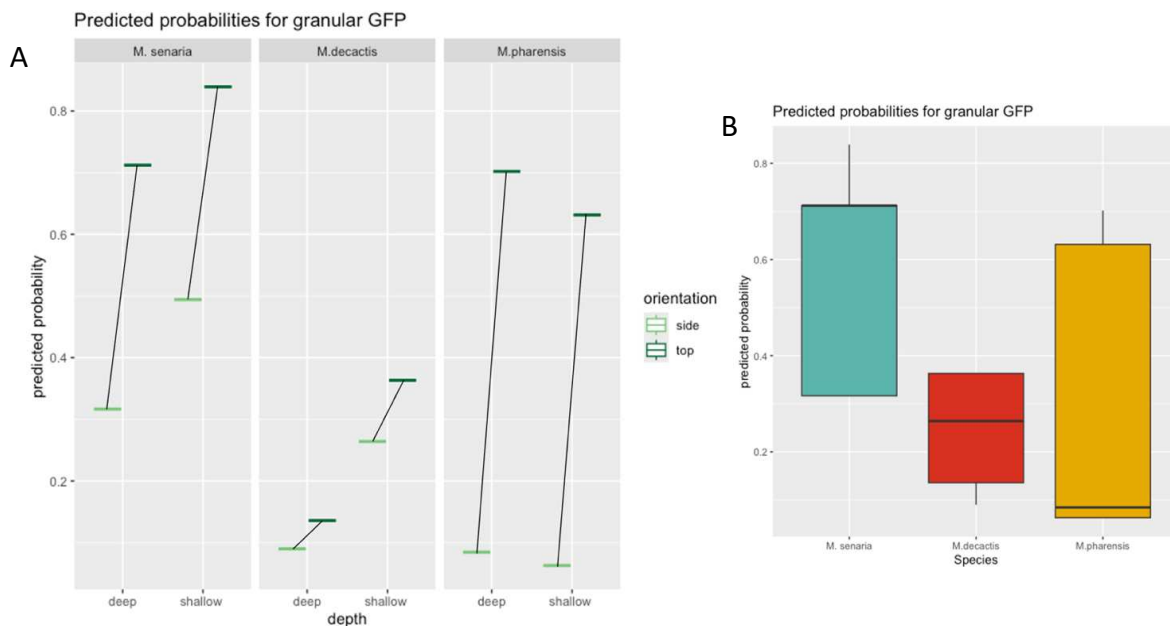


Figure 9. Predicted probabilities for the occurrence of granular GFP for different species and treatments (A) The predicted probability for top samples to express granular GFP is higher, when compared to side samples. (OR 5.34, 95% CI: [2.72-10.47], $p < .05$); shallow samples have a higher probability to express granular GFP (OR 2.11, 95% CI: [1.08, 4.12], $p < 0.05$) (B) Predicted probabilities for the occurrence granular GFP in *M. decactis* are significantly lower(OR of 0.21, 95% CI: [0.06, 0.67], $p < 0.05$) compared to *M. senaria* and *M. pharensis*.

Occurrence of CFP. The significance of each predictor variable was assessed using the likelihood ratio χ^2 -test. Orientation was found to be a significant predictor for the occurrence of CFP ($\chi^2 = 22.678$, $df = 1$, $p < 0.001$). The inclusion of depth ($\chi^2 = 16.350$, $df = 1$, $p < 0.001$) and species ($\chi^2 = 6.180$, $df = 2$, $p = 0.045$) as a predictor variable demonstrated a significant improvement in the models fit. Light also showed a significant effect, on its own ($\chi^2 = 32.33$, $df = 1$, $p < 0.001$) and in an interaction with species ($\chi^2 = 6.180$, $df = 2$, $p = 0.045$). (Fig.10) The model with light, species, depth, orientation and interactions between species and light as a predictor provides a significantly better fit to the data than other tested models. (Hosmer-Lemeshow Test $p > 0.05$; no evidence of lack of fit in the model). The model's pseudo-R² value of 0.8922 suggests that approximately 89.22% of the variability in the CFP outcome is accounted for by the model.

Differences in the occurrence of CFP signatures in %

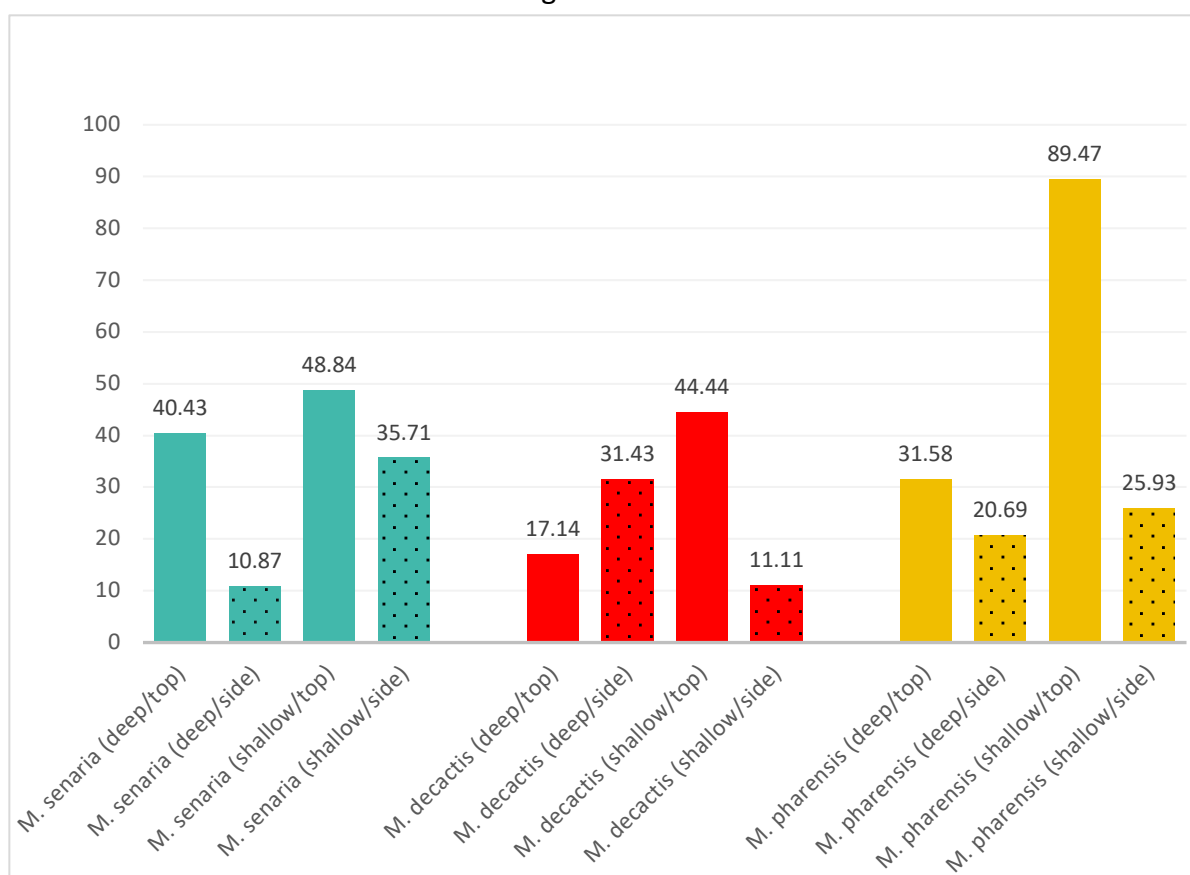


Figure 10. Occurrence of granular CFP in the three target species and two different treatments (orientation & depth). The assessment involved the calculation of the occurrence of CFP in percent (%) within the set of images per species and treatment. The Logistic regression analyses revealed that orientation ($\chi^2 = 22.678$, $df = 1$, $p < 0.001$), depth ($\chi^2 = 16.350$, $df = 1$, $p < 0.001$), species ($\chi^2 = 6.180$, $df = 2$, $p = 0.045$), light ($\chi^2 = 32.33$, $df = 1$, $p < 0.001$) and light in an interaction with species ($\chi^2 = 6.180$, $df = 2$, $p = 0.045$) as significant predictors for the occurrence of CFP.

For top samples, there is a significant increase in the odds of expressing CFP with an odds ratio (OR) of 3.63 (95% CI: [2.22, 5.92], $p < 0.05$). Also, shallow corals, have a significant increase in the odds of expressing CFP with an OR of 2.77 (95% CI: [1.75, 4.40], $p < 0.05$). (Fig. 11(A)) For species, the analysis yielded an OR of 0.44 for *M. decactis* (95% CI: 0.26, 0.76], $p < 0.05$). This indicates that for *M. decactis* samples, there is a significant decrease in the odds of expressing CFP compared to other species. (Fig. 11(B))

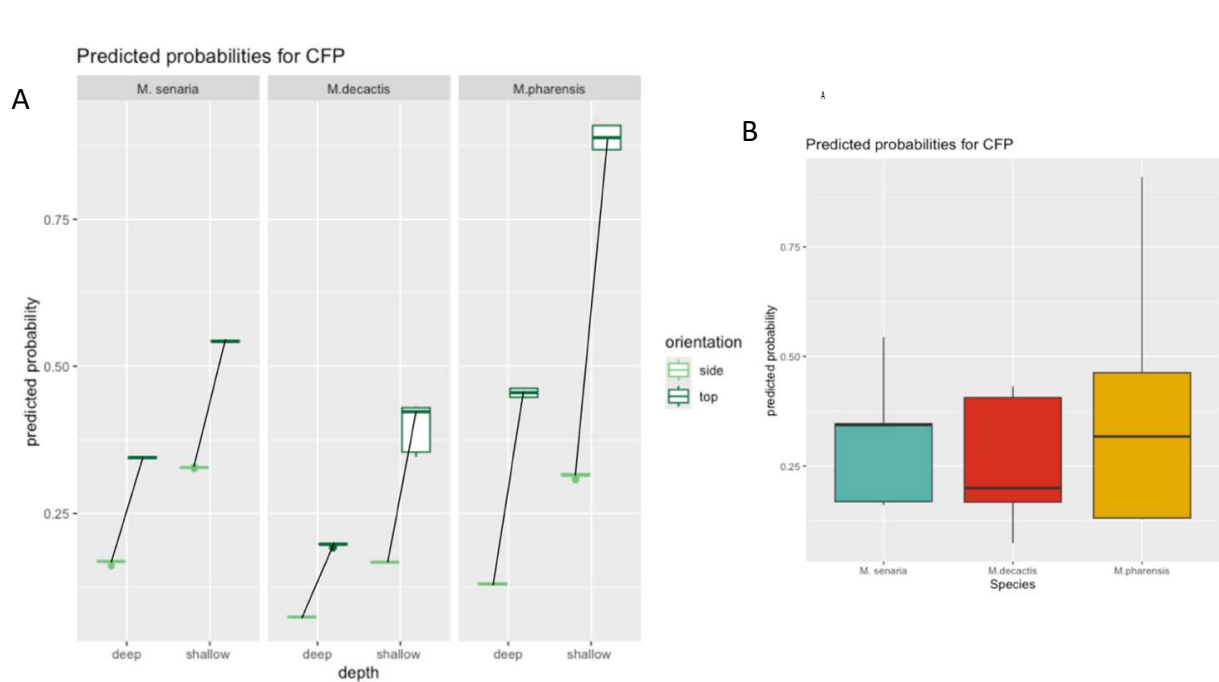


Figure 11. Predicted probabilities for the occurrence of CFP for different species and treatments
(A) The predicted probability for top samples to express CFP is higher, when compared to side samples. (OR 3.63, 95% CI: [2.22, 5.92], $p < .05$); shallow samples have a higher probability to express CFP (OR 2.77, 95% CI: [1.75, 4.40], $p < 0.05$)
(B) Predicted probabilities for the occurrence granular GFP in *M. decactis* are significantly lower (OR 0.44, 95% CI: 0.26, 0.76], $p < 0.05$). This indicates that for *M. decactis* samples, there is a significant decrease in the odds of expressing CFP compared to other species

Occurrence of YFP. The significance of each predictor variable was assessed using the likelihood ratio χ^2 -test. Species was found to be a significant predictor of the outcome ($\chi^2 = 27.0263$, $df = 2$, $p < 0.001$). (Fig.12)

The model with species and depth as a predictor provides a significantly better fit to the data than other tested models. (Hosmer-Lemeshow Test $p > 0.05$; no evidence of lack of fit in the model). The model's pseudo-R² value of 0.9141 suggests that approximately 91.41% of the variability in the YFP outcome is accounted for by the model. (Fig.13 (A))

Differences in the occurrence of YFP signatures in %

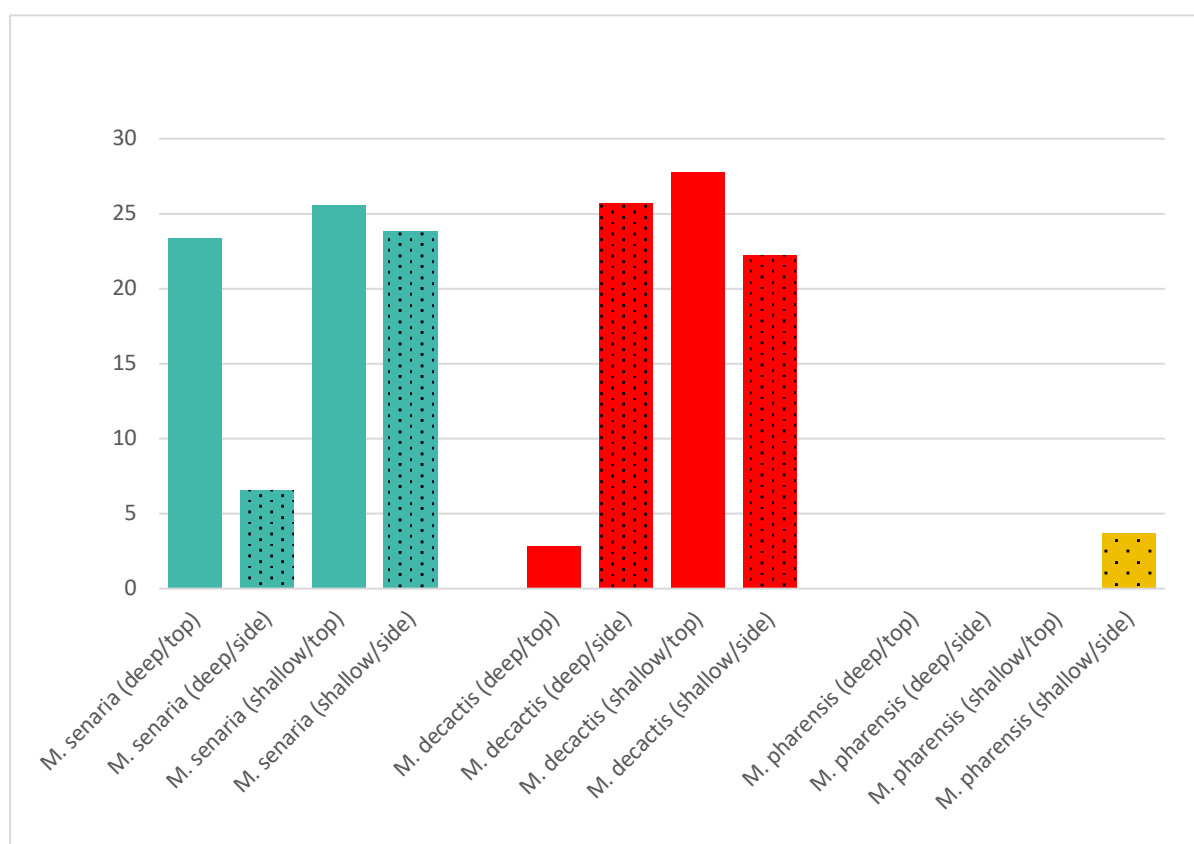


Figure 12. Occurrence of granular YFP in the three target species and two different treatments (orientation & depth). The assessment involved the calculation of the occurrence of YFP in percent (%) within the set of images per species and treatment.

The Logistic regression analyses revealed species ($\chi^2 = 27.0263$, $df = 2$, $p < 0.001$) as significant predictors for the occurrence of YFP.

For shallow samples, there is a significant increase in the odds of expressing YFP with an odds ratio (OR) of 1.68 (95% CI: [0.96, 2.96], $p > 0.05$). (Fig.13 (B)) For species, the analysis yielded an OR of 0.93 for *M. pharensis* (95% CI: [0.53, 1.63], $p < 0.05$) This indicates that for *M. pharensis* samples, there is a significant decrease in the odds of expressing YFP compared to other species. (Fig.13 (C))

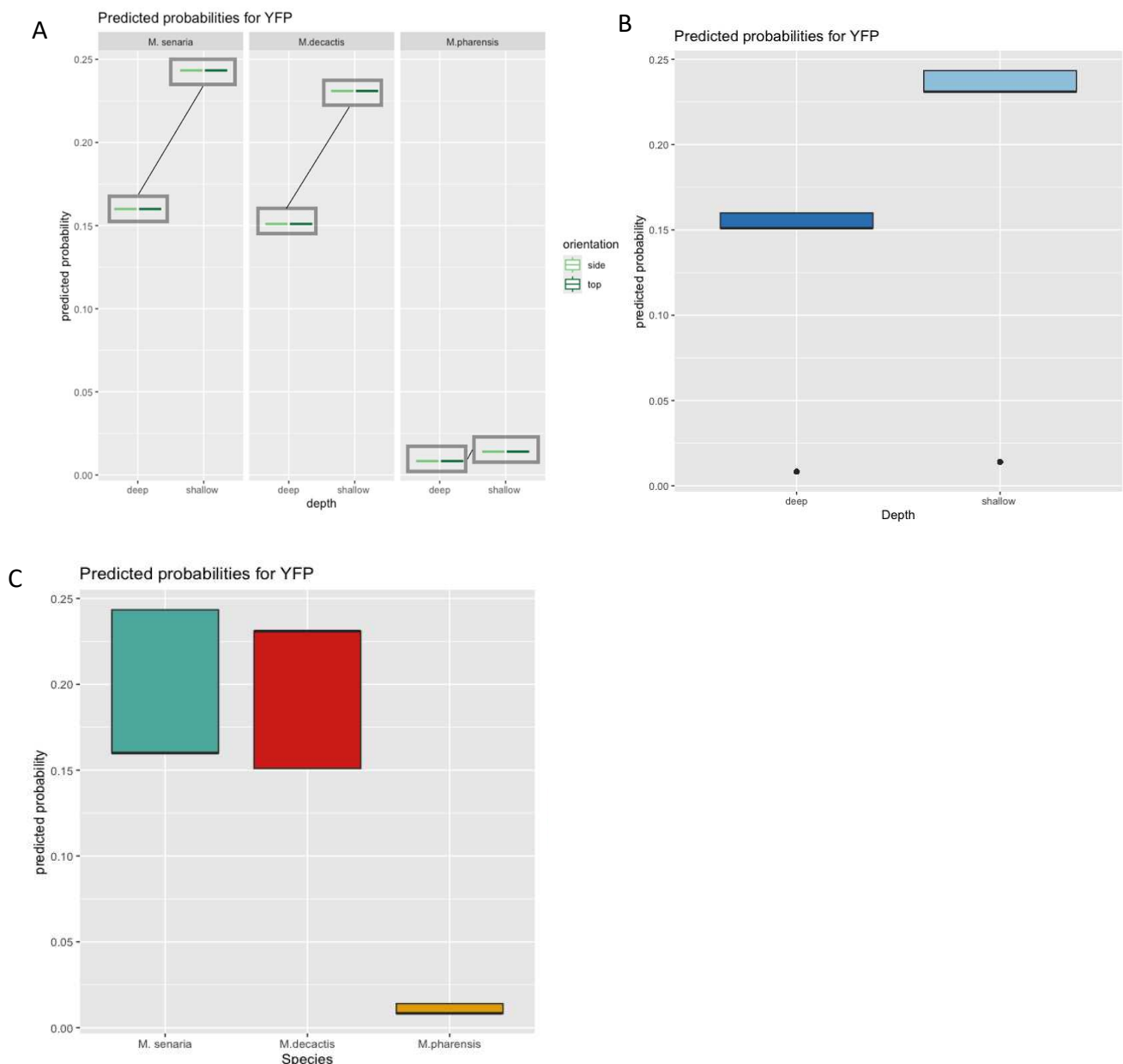


Figure 13. (A) Predicted probabilities for the occurrence YFP for three target species and two treatments (orientation & depth) (B) Predicted probabilities for the occurrence YFP for shallow samples are significantly higher (OR 1.68, 95% CI: [0.96, 2.96], $p > 0.05$.) (C) Predicted probabilities for the occurrence YFP in *M. pharensis* are significantly lower (OR 0.93, 95% CI: 0.53, 1.63], $p < 0.05$). This indicates that for *M. pharensis* samples, there is a significant decrease in the odds of expressing YFP compared to other species.

Undefined structures. Some structures (named yellow (not from tissue) found during confocal microscopy, which mostly appeared in yellow and had a filamentous shape, where found to not be part of the actual host tissue. During lab work it was tried to analyze those structures separately, which was not successful. Due to the shape, it is assumed that it might be filamentous algae which appear in the confocal due to their fluorescing phycoerythrin. The structures have not been further included into the analyses.

2.4.4 Ratios of different fluorescence signals across species

Green fluorescent protein : Red chlorophyll fluorescence (GFP:RCF across species)

Shallow samples express a higher GFP:RCF ratio compared to deeper samples (Kruskal-Wallis rank sum test: $\chi^2 = 19.771$, Df=1, $p < 0.001$) (Fig. 15(A)) and top oriented samples showed a higher ratio than side oriented samples (one-way ANOVA: Df=1, $F = 10.86$, $p < 0.001$) (Fig. 15(B)). Ratios did not differ between species. (Fig.14)

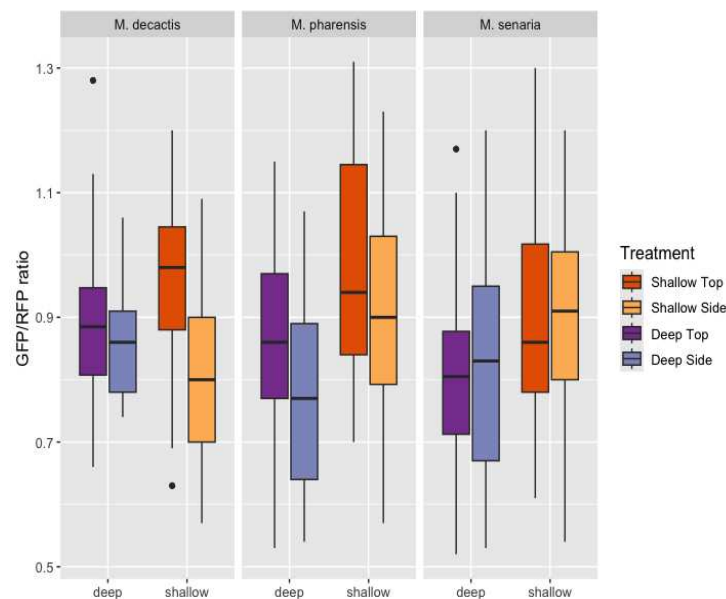


Figure 14. GFP:RCF across species

(A) GFP:RCF ratio of in different species and treatments; shallow samples express a higher ratio (Kruskal-Wallis rank sum test: $\chi^2 = 19.771$, Df=1, $p < 0.001$) same for top oriented samples (one-way ANOVA: Df=1, $F = 10.86$, $p < 0.001$).

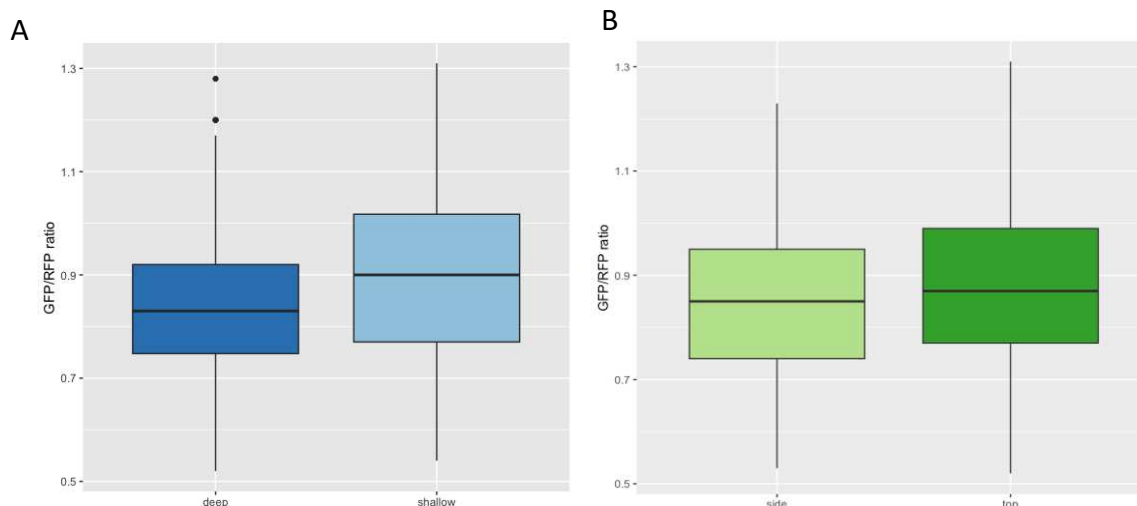


Figure 15. GFP:RFP ratio of in different treatments (A) shallow samples express a higher ratio (Kruskal-Wallis rank sum test: $\chi^2 = 19.771$, Df=1, $p < 0.001$) (B) same for top oriented samples (one-way ANOVA: Df=1, F= 10.86, $p < 0.001$).

Green fluorescent protein : Blue tissue fluorescence (GFP:BTF across species)

The GFP : BTF showed significant results for shallow samples which showed a higher GFP:BTF compared to deep samples (Fig. 17(B)) (Kruskal-Wallis rank sum test: $\chi^2 = 4.60$, Df=1, $p=0.031$) and species (Kruskal-Wallis rank sum test: $\chi^2 = 8.35$, Df=2, $p= 0.0153$). (Fig 16) A Post Hoc test revealed that the ratio is significantly higher in *M. pharensis* when compared to *M. senaria*. (Fig. 17(A))

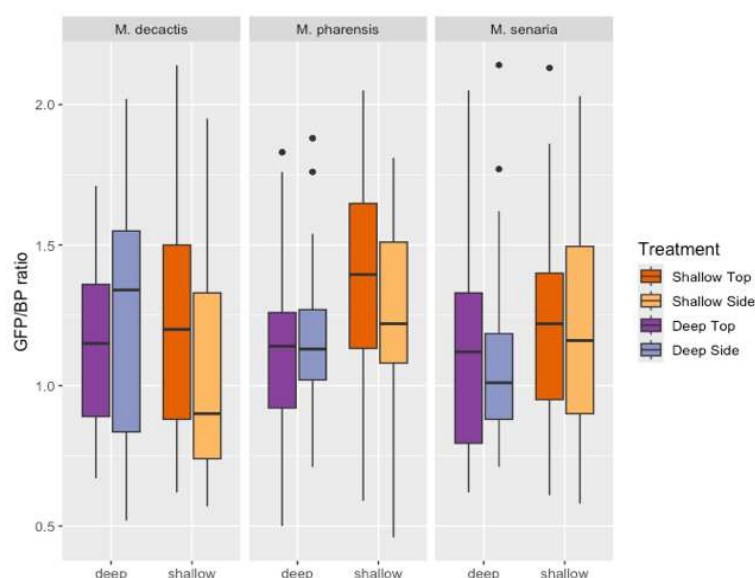


Figure 16. GFP:BTF ratio of in different species and treatments; shallow samples express a higher ratio (Kruskal-Wallis rank sum test: $\chi^2 = 19.771$, Df=1, $p < 0.001$) same for top oriented samples (one-way ANOVA: Df=1, F= 10.86, $p < 0.001$).

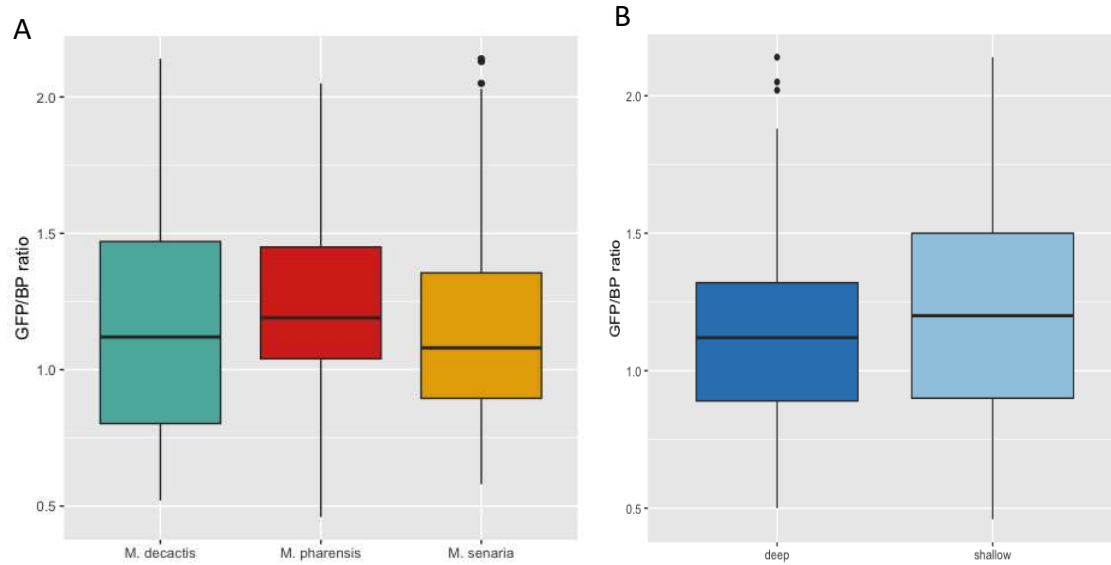


Figure 17 GFP:BP ratio of in different treatments (A) shallow samples express a higher ratio (Kruskal-Wallis rank sum test: $\chi^2 = 4.60$, Df=1, $p=0.031$) (B) species showed a significant result (Kruskal-Wallis rank sum test: $\chi^2 = 8.35$, Df=2, $p= 0.0153$). A Post Hoc test revealed a significant difference between *M. pharensis* and *M. senaria*, with *M. pharensis* showing a higher ratio.

M. senaria. The analysis of *M. senaria* revealed side samples show a higher GFP mean intensity (one-way ANOVA: Df=1, F= 7.85, p= 0.005) (Fig.18(A)).

Deep samples show a higher RCF mean intensity (one-way ANOVA: Df=1, F= 6.41, p = 0.012) (Fig.20). The YFP expression was found to be higher in shallow samples (one-way ANOVA: Df=1, F = 5.03, p= 0.031)(Fig.18(B)). GFP : RCF exhibited significant difference between depths (one-way ANOVA: Df=1, F= 11.81, p= 0.001)(Fig. 19(B)) as did GFP:BTf with both being higher in shallow samples (one-way ANOVA: Df=1, F = 4.96,p = 0.027)(Fig.19(A)).

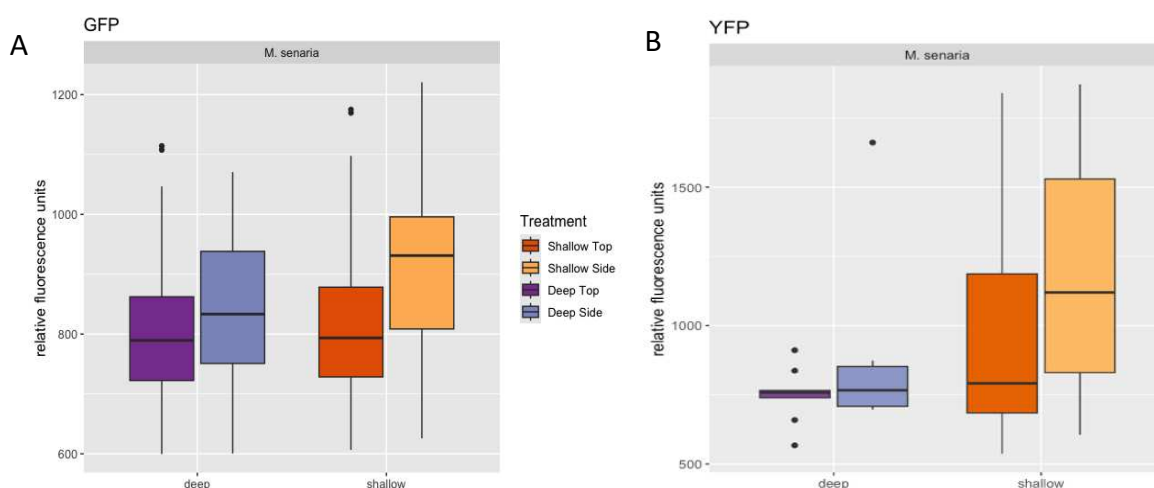


Figure 18. (A) differences in GFP mean intensity (relative fluorescent units) of *M. senaria* between different depths and orientation; side samples showed the highest GFP mean intensity (one-way ANOVA: Df=1, F= 7.85, p= 0.005) (B) differences in YFP mean intensity (relative fluorescent units) of *M. senaria* between different depths and orientation; shallow samples showed the highest YFP mean intensity (one-way ANOVA: Df=1, F = 5.03, p= 0.031)

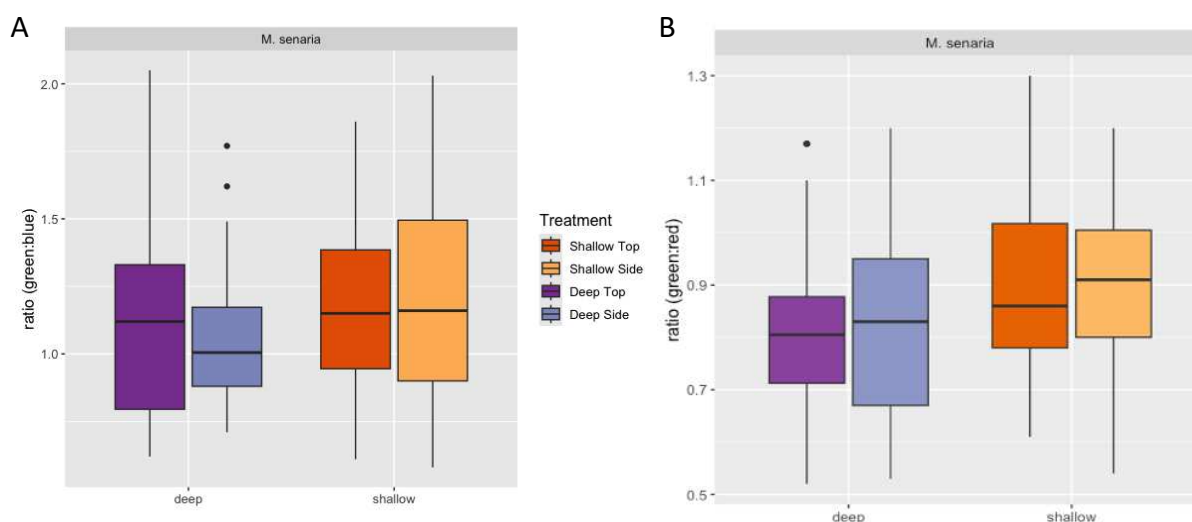


Figure 19. (A) GFP:BTf ratio in *M. senaria* and different treatments; shallow samples express a higher ratio in GFP to area (BTf) (one-way ANOVA: Df=1, F = 4.96,p = 0.027) (B) GFP:RCF ratio in *M. senaria* and different treatments; shallow samples express a higher ratio in GFP to chlorophyll (GFP:RCF) (one-way ANOVA: Df=1, F= 11.81, p= 0.001)

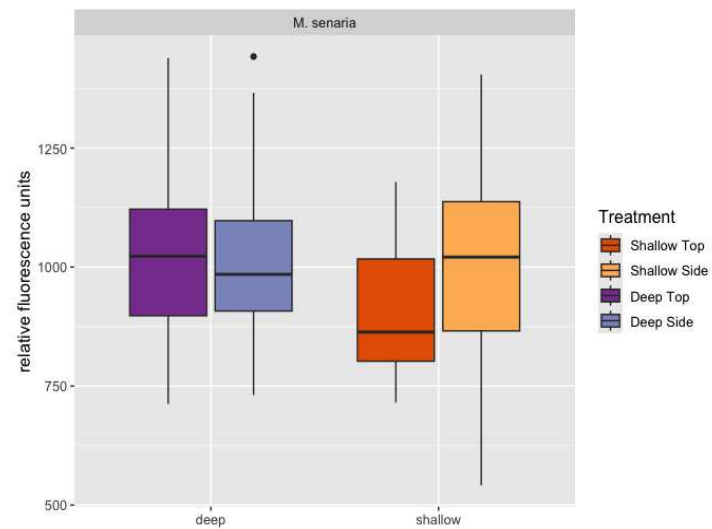


Figure 20. differences in RCF mean intensity (relative fluorescent units) of *M. senaria* between different depths and orientation; deep samples showed the highest GFP mean intensity (one-way ANOVA: Df=1, F= 6.41, p = 0.012)

M. pharensis The analysis for *M. pharensis* revealed significantly higher GFP intensity in shallow samples (one-way ANOVA: Df=1, F= 7.16, p= 0.008)(Fig. 21(A)). Differences in RCF were found between orientations (one-way ANOVA: Df=1, F= 5.33, p= 0.022) and depth (Kruskal-Wallis rank sum test: $\chi^2 = 20.378$, Df=2, p value < 0.001) for which deep and side samples showed a higher expression of RCF mean intensity.(Fig. 22(B))

The YFP mean intensity was found to be significantly higher in the shallows (Kruskal-Wallis rank sum test: $\chi^2 = 3.96$, Df=1, p= 0.046)(Fig. 22(A))

GFP: RCF was found to be higher in shallow samples (one-way ANOVA: Df=1, F= 26.12, p < 0.001) as well as top oriented samples (one-way ANOVA: Df=1, F= 6.65, p= 0.011).

(Fig.23(B)) The GFP : BTF ratio differed only between depths (Kruskal-Wallis rank sum test: $\chi^2 = 7.61$, Df=1, p= 0.005). (Fig 23(A))

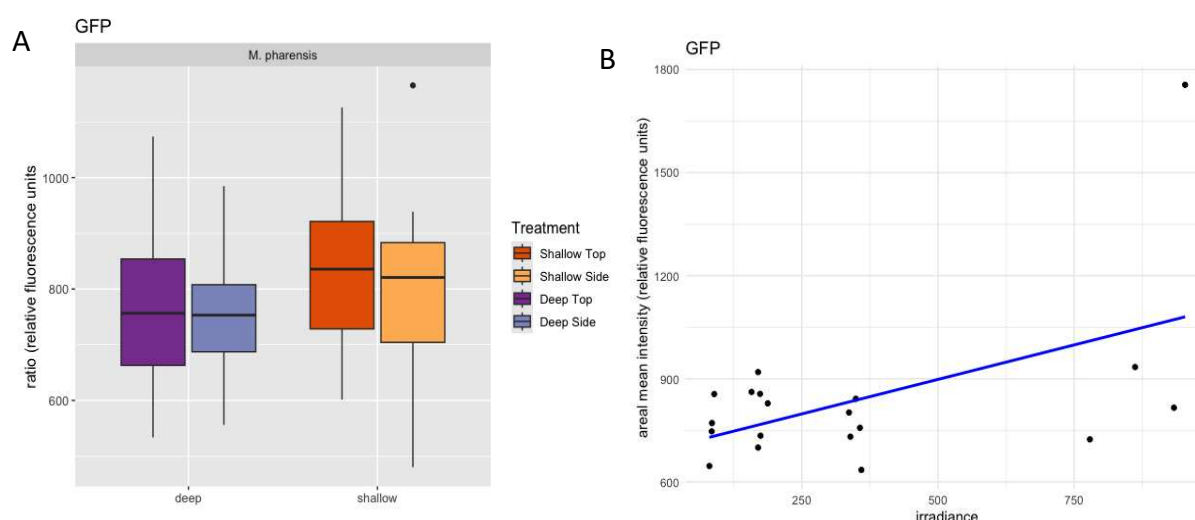


Figure 21. (A) differences in GFP mean intensity (relative fluorescent units) of *M. pharensis* between showed the highest GFP mean intensity in shallow samples (one-way ANOVA: Df=1, F= 7.16, p= 0.008) (B) The linear regression model for the GFP indicate a statistically significant relationship between GFP mean intensity and light, (p= 0.026)

The linear regression model for the GFP indicate a statistically significant relationship between green intensity and light, with a p-value of 0.026, suggesting that light has a significant effect on green intensity (Fig. 21(B)). The regression coefficient estimate for light is 0.4015, indicating that for every unit increase in light, the green intensity is expected to increase by approximately 0.4015 units. The model's R^2 value is 0.2584, implying that approximately 25% of the variance in green intensity can be explained by the variation in light. The ANOVA table confirms the significance of the relationship, with a p-value of 0.026 for the light variable. This suggests that light significantly contributes to the model's explanatory power regarding green intensity.

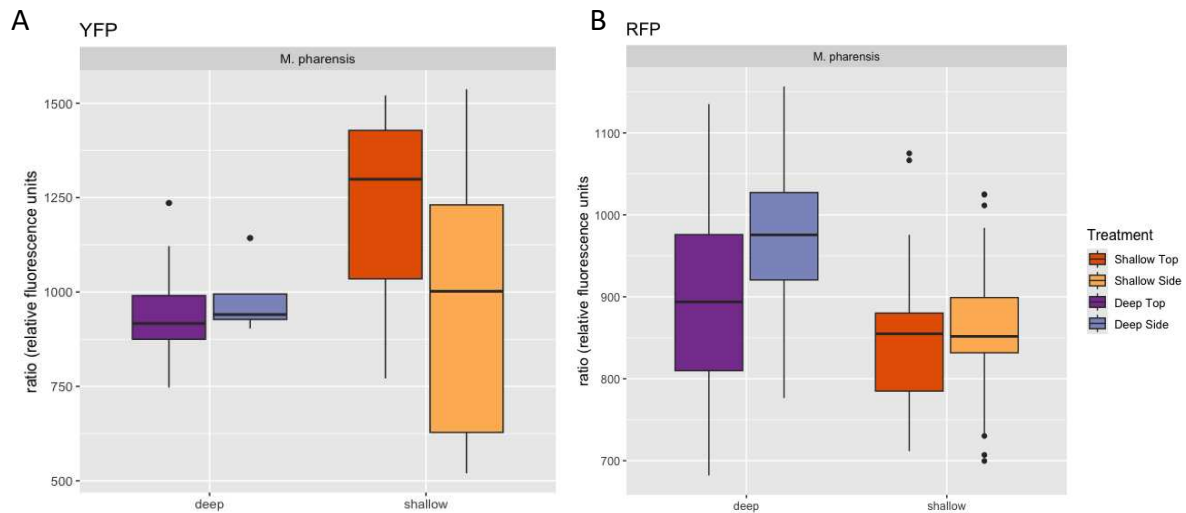


Figure 22. (A) differences in YFP mean intensity (relative fluorescent units) of *M. pharensis* was found to be significantly higher in the shallow. (Kruskal-Wallis rank sum test: $\chi^2 = 3.96$, Df=1, $p = 0.046$) (B) differences in RCF mean intensity; side samples exhibited a higher mean intensity (one-way ANOVA: Df=1, $F = 5.33$, $p = 0.022$) as well as deep samples (Kruskal-Wallis rank sum test: $\chi^2 = 20.378$, Df=2, p value < 0.001)

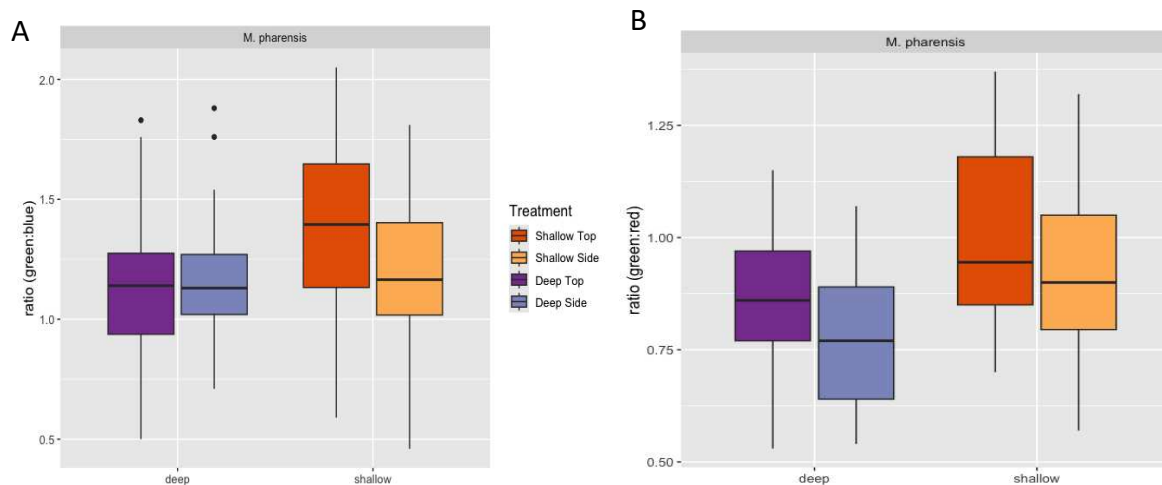


Figure 23. (A) GFP:BTF ratio in *M. pharensis* and different treatments; shallow samples express a higher ratio in GFP to area (BTF) (Kruskal-Wallis rank sum test: $\chi^2 = 7.61$, Df=1, $p = 0.005$) (B) GFP:RCF ratio in *M. pharensis* and different treatments; shallow samples express a higher ratio in GFP to chlorophyll (one-way ANOVA: Df=1, $F = 26.12$, $p < 0.001$) as well as top oriented samples (one-way ANOVA: Df=1, $F = 6.65$, $p = 0.011$).

M. decactis. The analysis for *M. decactis* revealed no significant differences in GFP intensity. Differences were found for RCF intensity between deep and shallow oriented samples (one-way ANOVA: Df=1, F= 7.47, p= 0.007) as well as differences in RCF between top and side (one-way ANOVA: Df=1, F= 23.5, p < 0.001). Side oriented samples as well as shallow samples show a higher mean intensity of RCF. (Fig 24(A))

The YFP mean intensity was found to be higher in deep samples (one-way ANOVA: Df=1, F= 44.35, p< 0.001). (Fig. 24(B)) When analyzing the ratios, GFP to RCF ratio differed between orientation (one-way ANOVA: Df=1, F= 14.04, p < 0.001) being higher in the top. (Fig 25(A)) GFP to BTF and RCF to BTF ratio did not give any results. The regression analysis revealed that zooxanthellae density significantly influences green intensity (p = 0.003), while the effect of depth, particularly shallow depths, was not statistically significant (p = 0.208). The model accounted for approximately 50.8% of the variance in green intensity. Additionally, the ANOVA results confirmed the significance of zooxanthellae density (p = 0.004) but not depth in explaining the variability in green intensity. The analysis of red intensity revealed that the orientation factor significantly influences it (Fig. 25(B)) (p = 0.010), with a coefficient of -89.00 for the "top" orientation. The model accounted for approximately 47.45% of the variance in red intensity. The ANOVA results further confirmed the significance of orientation (p = 0.01) but not depth in explaining the variability in red intensity.

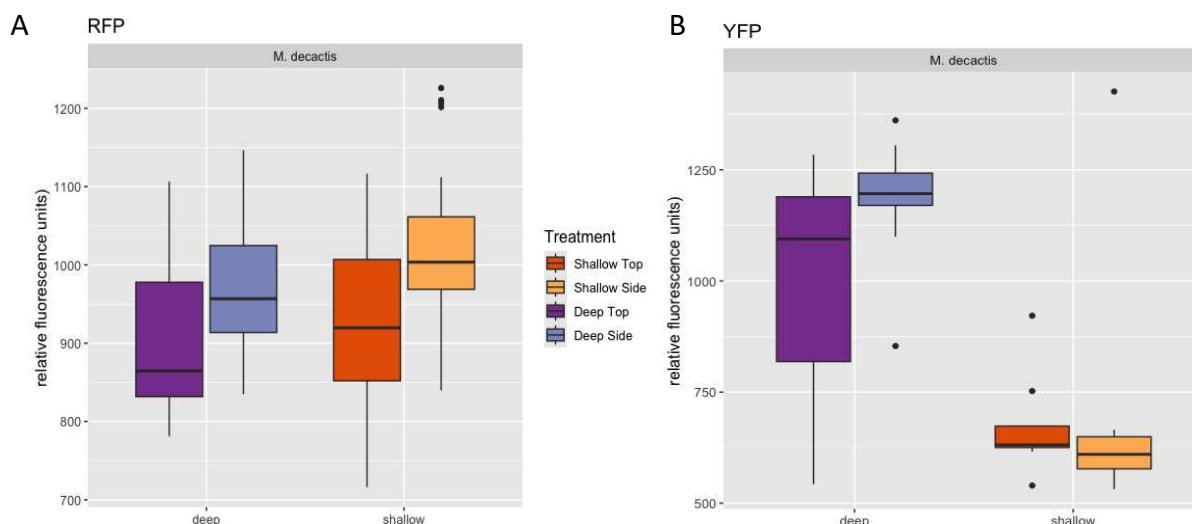


Figure 24. (A) RCF mean intensity (relative fluorescent units) of *M. decactis* was found to be significantly higher in shallow samples (one-way ANOVA: Df=1, F= 7.47, p= 0.007) as well as side oriented samples (one-way ANOVA: Df=1, F= 23.5, p < 0.001) (B) YFP mean intensity (relative fluorescent units) of *M. decactis* was found to be significantly higher in deep samples (one-way ANOVA: Df=1, F= 44.35, p< 0.001))

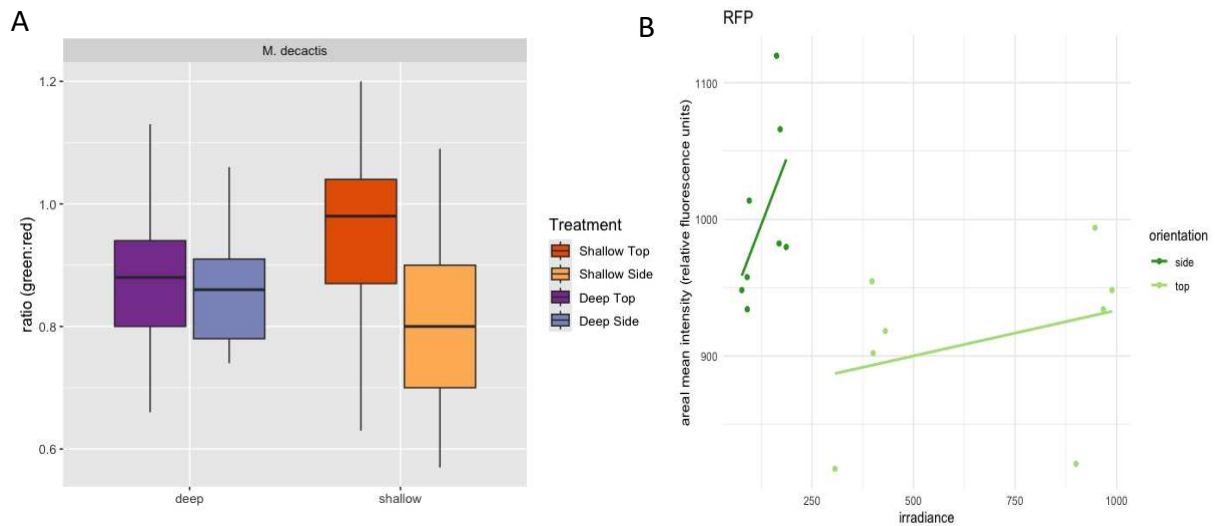


Figure 25. (A) GFP:RCF ratio in *M. decactis* and different treatments; top oriented samples showed a higher ratio in GFP:RCF (one-way ANOVA: Df=1, F= 14.04, $p < 0.001$) (B) The linear regression model for the RCF indicate a statistically significant relationship between RCF mean intensity and orientation, ($p = 0.010$) with a coefficient of -89.00 for the "top" orientation

2.4.6 Symbiont and photobiology analyses

PCA analysis on the symbiont data (zooxanthellae density per area, areal and cellular chlorophyll *a* and *c*₂, and yield) revealed that the first three principal components explain 81% of the total variance in the data. PC1 explains 32%, PC2 explains 26%, and PC3 explains 23% of the variance. PC1 is positively correlated with chlorophyll *c*₂ per area and per cell, yield and chlorophyll *a* per cell and area (Fig.26(A). Furthermore, PC1 is negatively correlated with zooxanthellae density per area, whereas PC2 appears to be positively correlated with zooxanthellae density per area. Chlorophyll *a* per cell and yield both are negatively correlated with light, which is also visually represented in Figure 26(A). The PCA analyses suggested that species and depth may not explain the variation in symbiont and photobiology parameters measured. Regarding their differences in symbiont and photobiology (Fig. 26,B&C) but top and side may differ (Fig.26(D)). This was later confirmed through more statistical testing.

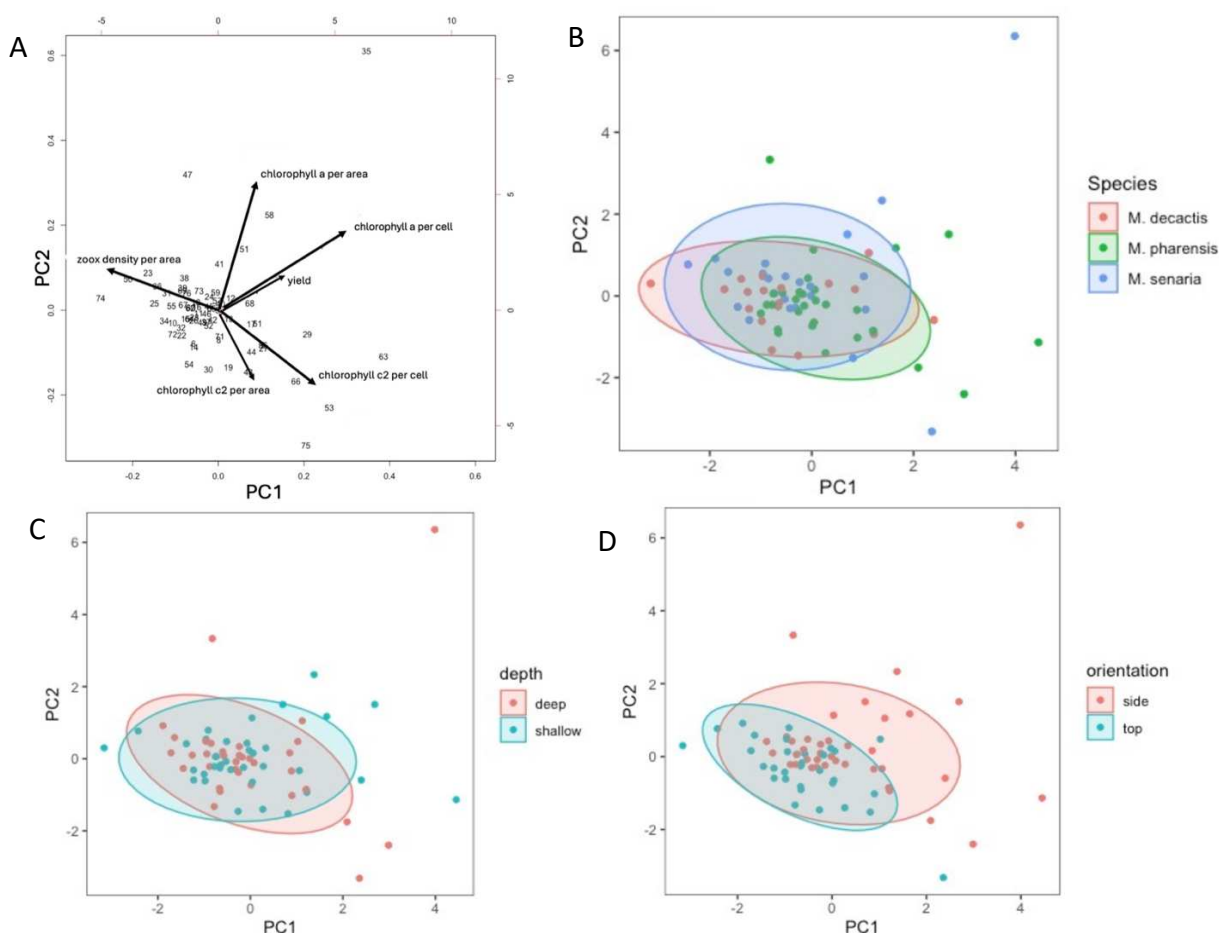


Figure 26. (A) correlation of the different variables in relation to PC1 and PC2 (B-D) relationships between symbiont physiology and the three different treatments, being species, depth, and orientation in the reduced-dimensional space.

Chlorophyll a content

There was no significant difference found between depths and orientation regarding the chlorophyll a (cellular and areal) content. Only a negative linear relationship between cellular chlorophyll a and light intensity. (see results “Effect of light on symbiont photobiology”)

Areal chlorophyll c₂

Chlorophyll c₂ content per area showed significant differences between colony orientation (Fig.27(A&B)). Top-oriented samples of the colonies across species showed a higher concentration in chlorophyll c₂ per cm² than side-oriented samples with 4.18 ± 2.95 pg/cm² vs. 2.83 ± 2.27 pg/cm² for top and side (one-way ANOVA: Df=1, F value= 4.968, p value= 0.029).

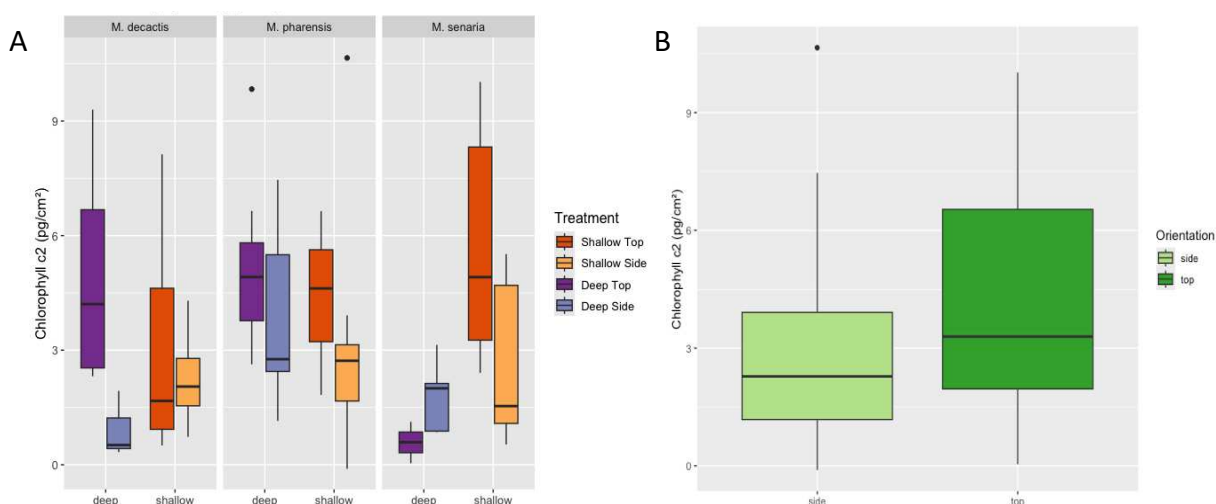


Figure 27. (A) Areal chlorophyll c₂ of different target species (*M. decactis*, *M. pharensis*; *M. senaria*) and treatments (B) Areal chlorophyll c₂ content of two different colony orientations (side; top) across species, top samples showed a significantly higher content in areal chlorophyll c₂ (one-way ANOVA: Df=1, F value= 4.968, p = 0.029)

Cellular chlorophyll c₂,

There was a significant difference in cellular c₂, content when tested among species (Fig. 28(A)) with *M. senaria*: 1.22 ± 0.141 (pg/cell); *M. pharensis*: 2.23 ± 1.15 (pg/cell) and *M. decactis* 0.73 ± 1.99 (pg/cell), (one-way ANOVA: Df=2, F value= 5.489, p=0.006). A post-hoc test (Tukey's HSD) was conducted to determine the specific group differences. *M. pharensis* shows a significantly higher cellular c₂, content compared to *M. senaria* (Fig.28(B))(p = 0.007). Cellular chlorophyll c₂, did not significantly differ between depths and colony orientation levels.

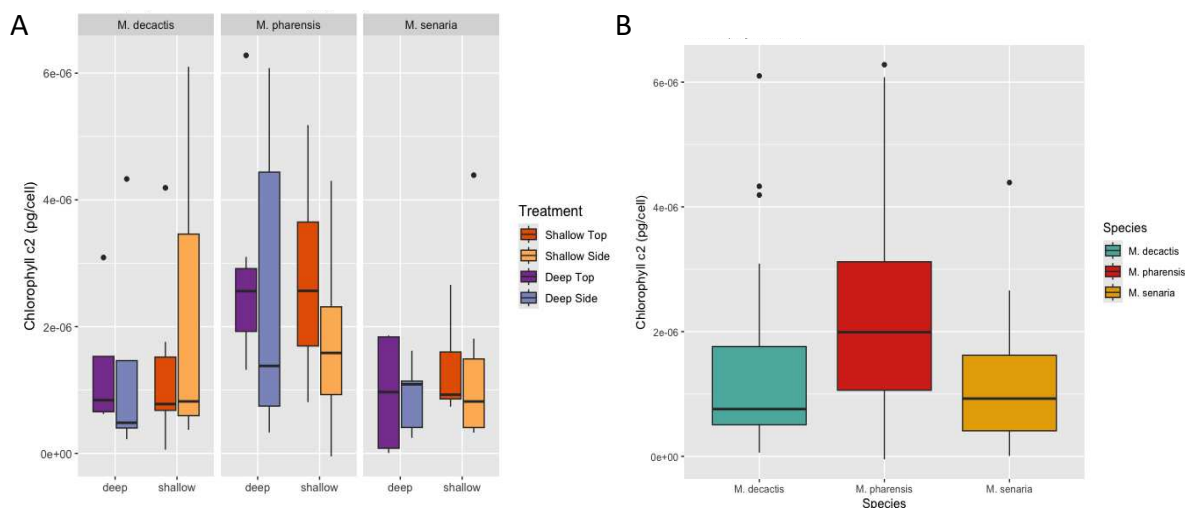


Figure 28. (A) Cellular chlorophyll c_2 , of different target species (*M. decactis*, *M. pharensis*; *M. senaria*) and treatments. (B) Differences in cellular chlorophyll c_2 , content between *M. senaria*, *M. pharensis* and *M. decactis*, *M. pharensis* showed the highest cellular chlorophyll c_2 , content and significantly higher than *M. senaria* one-way ANOVA: Df=2, F value= 5.489, $p=0.006$)

Zooxanthellae density

The highest zooxanthellae densities were obtained in the top oriented samples of the colony with $3.46 \times 10^6 \pm 2.63 \times 10^6$ cells/cm² and the lowest in the side-oriented samples of the colony with $2.24 \times 10^9 \pm 1.54 \times 10^6$ cells/cm² (Fig.29(A&B)), (one-way ANOVA: Df=1, F= 5.348, $p=0.023$). The differences in zooxanthellae densities per cm² were not significant between species and did not differ between deep and shallow samples.

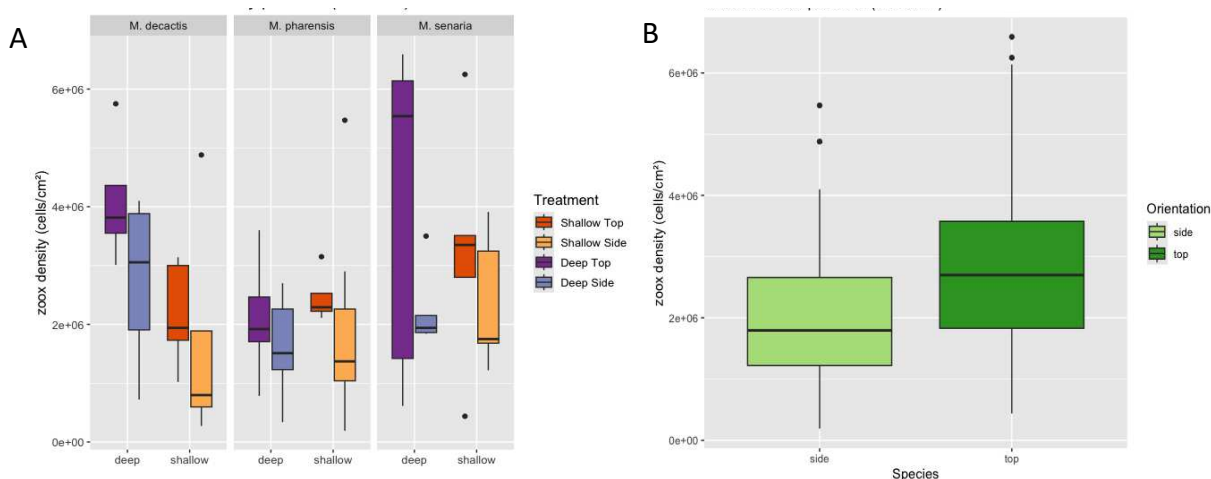


Figure 29. (A) zooxanthellae densities of different target species (*M. decactis*, *M. pharensis*; *M. senaria*) and treatments. (B) Differences in zooxanthellae between top and side-oriented samples of the colony with top oriented samples showing a higher density ($p=0.023$).

Quantum yield of photosynthesis

A notable contrast in photochemical yield emerged between top-oriented and side-oriented

samples within the colony. Side-oriented samples demonstrated significantly higher yields compared to their top-oriented counterparts, with values of 0.617 ± 0.033 and 0.517 ± 0.060 , respectively (one-way ANOVA: Df = 1, F= 79.58, $p < 0.001$). Fig.30 (A-B) illustrates the clear difference between top and side. The yield exhibited no discernible variance across species, nor did it vary between deep and shallow habitats.

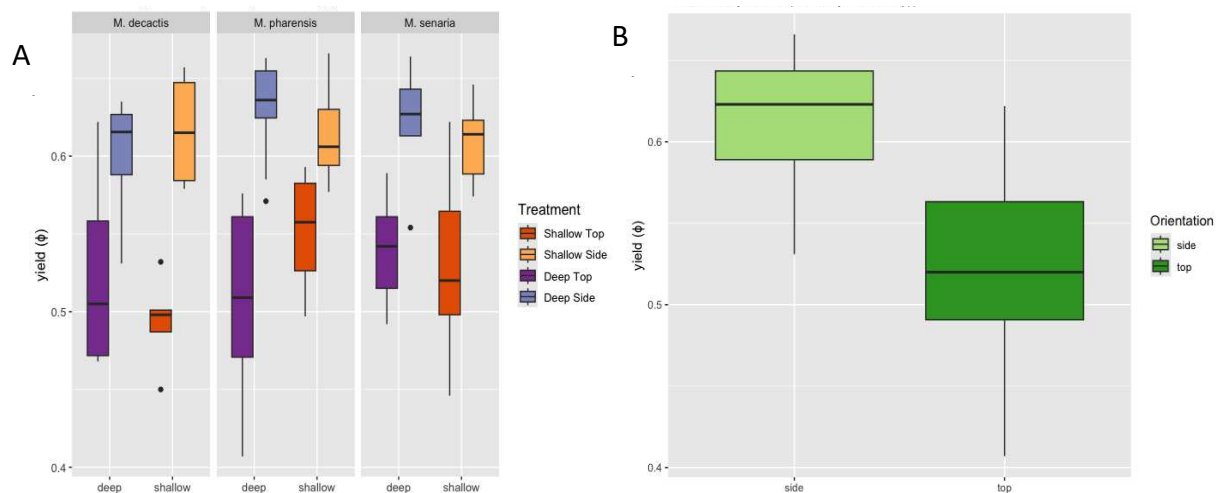


Figure 30. (A) The quantum yield of photosynthesis (ϕ) of different target species (B) and between top and side, with side samples showing a significantly higher quantum yield of photosynthesis (one-way ANOVA: Df = 1, F= 79.58, $p < 0.001$).

Effect of light on symbiont photobiology

The analysis unveiled a robust negative linear relationship between light and the quantum yield of photosynthesis, with a Pearson correlation coefficient of $r = -0.7$ and $p < 0.001$.

(Fig.31) Additionally, we observed a negative linear relationship between cellular chlorophyll a and light intensity, as indicated by a Pearson correlation coefficient of $r = 0.23$ and $p < 0.05$. (Fig.32)

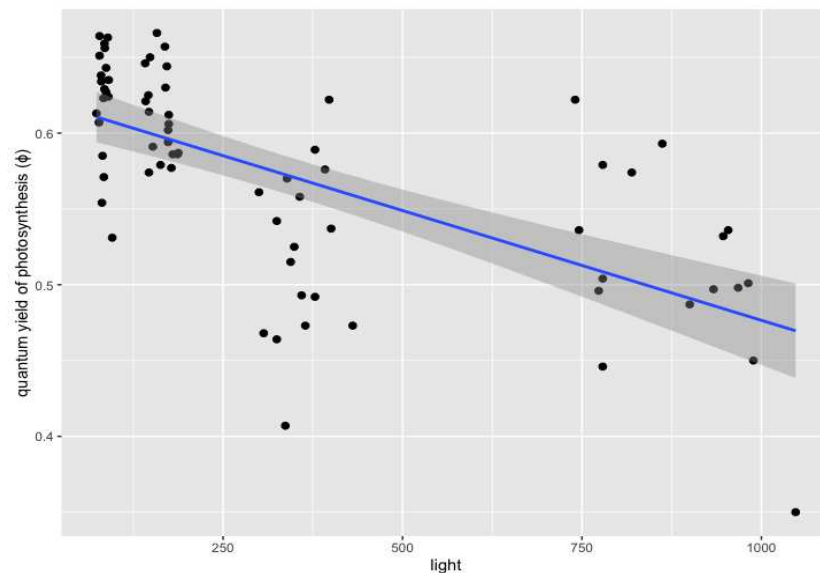


Figure 31. The quantum yield of photosynthesis (ϕ) of different target species and treatments showing a negative correlation between light and the quantum yield of photosynthesis ($p < 0.001$)

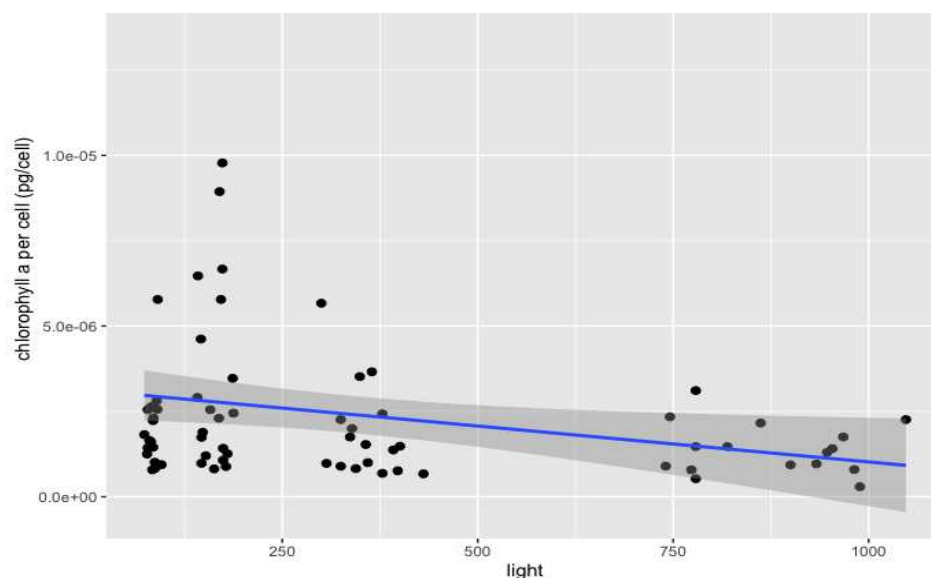


Figure 32. Negative linear relationship between cellular chlorophyll a and light intensity ($p < 0.05$)

2.5 [

Using three species in the coral genus *Madracis*, this study investigated the variation in fluorescence emission intensity of different FPs within coral tissue and the photobiological characteristics of symbionts as indicators of coral acclimation to their surrounding light

environment. Three different species from two different depths (deep and shallow) have been compared as well as samples from two different parts of each coral colony (top and side). The conclusions are based on the results of the image analyses (occurrence of different FP signatures), the analysis of the fluorescent mean intensity (based on the emission peaks) and the analysis of the symbiont photobiology.

2.5.1 Irradiance

The side-oriented samples from the shallow colonies experience similar light intensities as top-oriented colony parts from the deeper colonies here sampled. This highlights the importance of considering both the colony's depth and the varying environmental conditions experienced by different parts of the colony when examining light as an environmental factor.

2.5.2 Fluorescent proteins in corals

2.5.2.1 Occurrence of distinct FP signatures

Green Fluorescent Protein. The first FP this study focused on was the GFP. It was found in all three target species. The analyses of distinct FPs signatures showed that diffused GFP and aggregations of GFP (granular GFP) were to be found in all three species through all depths and colony orientations.

The conducted logistic regression analyses showed that corals in the shallow category as well as top oriented samples showed the highest occurrence of granular GFP, indicating, that the factor of total PAR irradiance might play a role in the probability of expressing granular GFP. Previous studies evaluating the role of GFP observed inconsistent results. One study conducted by Lynd by *et al.* (2016) states that as the density of light scattering granular GFP increases, local tissue scalar radiation also increases, subsequently increasing the chance of light absorption and heating by the coral. However, their results also showed that heating saturated at high levels of green fluorescence, suggesting that the effect of FPs on light absorption and heating of coral becomes nonlinear at high irradiance intensity. According to other studies it is possible that GFP plays a role in a coral's ability to cope with environmental stressors such as increased irradiance of ultraviolet and visible radiation as well as with elevated temperature (Mazel *et al.*, 2002). Still, it is important to mention that these physiological processes are not yet understood completely.

The results of this study showed the highest occurrence of granular GFP in those samples, which have likely been exposed to higher total PAR irradiance (shallow and top). It can therefore be assumed that GFP plays a protective role against high light levels; this finding aligns with previous findings (Bollati *et al.*, 2020; Gittings *et al.*, 2015; Salih *et al.*, 2000).

Other than the granular GFP, the diffused GFP only gave a significant result for *M. senaria*, which out of the three species showed the highest occurrence in diffused GFP, this may be explained by the differences in the species distribution, which will be discussed later.

Cyan Fluorescent Protein. Overall, the analyses of CFP showed similar results to those of GFP. They showed that shallow and top oriented samples had a higher occurrence of CFP and a higher likelihood to express it. It seems possible that these results might again be explained by the role of total PAR irradiance, since those corals that are likely exposed to higher light levels, also showed the highest occurrence in CFP.

These findings may be somewhat limited because it was not possible to quantify the mean intensity of CFPs based on their emission peak using the confocal microscope. This limitation arose because I could not detect and measure the CFP mean intensity separately from the GFP mean intensity. Therefore, the analysis was based solely on presence-absence data.

Yellow Fluorescent Protein. The current investigation found that the predicted possibility for YFP to occur is higher in shallow samples compared to deep samples. The analysis for YFP mean fluorescence intensity aligns with this finding as two out of three species (*M. senaria* and *M. pharensis*) also showed a higher YFP mean fluorescence intensity in shallow colonies, once again pointing towards the hypothesis of protection from higher irradiance. (Bollati *et al.*, 2020; Gittings *et al.*, 2015; Salih *et al.*, 2000).

Linear regression analyses have shown that the likelihood of expressing FPs is influenced by a combination of factors, including species, depth, and orientation, rather than a single predictor. Total PAR irradiance alone was never selected as a factor in the best model, suggesting that light quantity by itself does not fully characterize the shallow environment.

2.5.2.2 FP mean intensities

While visual observation of FPs (i.e., checking for presence versus absence) provides valuable initial insights, it does not fully capture the specific patterns and characteristics of individual corals' FPs. Therefore, the second part of this study focused on analyzing the mean fluorescence intensity for each species separately. This approach allows for the identification of distinct differences and better characterization of the FPs in each coral species.

Emission peaks. The conducted method to identify different FPs associated to three species of *Madracis* by their distinct emission peaks (based on Alieva *et al.*, 2008) was successful. The green, fluorescent color is the most common in corals (Alieva *et al.*, 2008). The emission peaks varied between species. *M. decactis* exhibited an emission peak at 521nm, while *M.*

pharensis and *M. senaria* exhibited an emission peak at 516 nm.

The CFPs have been identified with an emission peak of at 501-504 nm. Typically, CFPs will emit light between 485–495 nm, but may occasionally exhibit more blue-shifted variants, with emission peaks as low as 477 nm. Occasionally there are borderline cases such as aacuGFP1, which has a narrow “green-like” emission peak at 502 nm. According to Alieva *et al.* (2008), CFPs, share the same chromophore as GFPs but have undergone different evolutionary adaptations within corals. “Non-green FPs, such as cyan and red, evolved under the pressure of positive natural selection” (Alieva *et al.*, 2008). This leads to the assumption that the diverse colors must serve some essential function. It underscores the necessity to classify CFPs distinctly, suggesting a specialized yet undefined role in coral physiology. YFP was observed in all three target species with an emission peak at 572 nm.

Differences within species. Two out of three species (*M. pharensis* and *M. senaria*) showed higher levels of GFP and YFP mean intensity in shallow colonies; this finding again points towards the conclusion, that the exposure to high light plays a role (Roth *et al.*, 2008; Bollati *et al.*, 2020; Salih *et al.*, 2000; Gittins *et al.*, 2015).

In addition, GFP mean intensity in *M. pharensis* showed a positive correlation with total PAR irradiance indicating an increase in the expression of GFP in higher light environments. Hence these findings support the likely existence of a connection between a high total PAR irradiance and the increased expression of GFP. This relationship has already been reported in several prior studies (Smith *et al.*, 2017) which suggest FPs as a protection strategy against high irradiance. In the reef corals are subject to gradients in both the spectral composition and intensity of light. This is due to attenuation by the water column (Kirk *et al.*, 1994). In line with their suggested photoprotective role, the expression of FPs in numerous shallow-water corals is controlled by light intensity, especially within the blue spectrum (D’Angelo *et al.*, 2008b, 2012; Smith *et al.*, 2013). Strongly pigmented colour morphs of corals are frequently found in the shallower habitats. This is suggesting a selective advantage of pigmentation in light exposed environments (Salih *et al.*, 2000, 2006; Smith *et al.*, 2013). However, different color morphs also often show an overlap in their distribution (Salih *et al.*, 2006; Leutenegger *et al.*, 2007a) and conspecific corals with different concentrations of the same FPs are found coexisting in highly light-exposed habitats (Gittins *et al.*, 2015). This suggests that FPs provide different advantages to enable corals to acclimate to a broader range of conditions or survive extreme stress events (Smith *et al.*, 2013) but they are not essential for survival (Gittins *et al.*, 2015).

M. decactis colonies did not differ between deep and shallow considering their GFP intensity. Regarding its YFP, the species *M. decactis* showed higher intensities in deeper colonies. The presence of FPs in colonies from low-light habitats indicates that FPs fulfill functions beyond photoprotection (Salih *et al.*, 2000; Leutenegger *et al.*, 2007b; Dove *et al.*, 2008).

Another important factor that needs to be considered is that the three species have distinct depth distributions (Vermeij and Bak 2003; Frade *et al.*, 2008). *Madracis* coral population structure varies significantly and is strongly influenced by species-specific traits (Vermeij *et al.*, 2003). The different distribution patterns among species, suggest that they have adapted uniquely to a variety of interacting environmental factors, such as light, sedimentation, water movement, and temperature, across different depth gradients.

M. senaria and *M. pharensis* can be found across all depth between 5 and 60 m whereas *M. decactis* occurs from 5 down to 40m. (Fig. 33; taken from Frade *et al.*, 2008)

For *M. senaria* and *M. pharensis*, a study by Vermeij *et al.* (2002) highlights that both species predominantly occupy a narrow light range, with most colonies found in low light conditions (5 to 60 $\mu\text{mol photons m}^2/\text{s}$) and the morphology of both species is influenced by small-scale, isobathic (same depth) light differences (Vermeij *et al.*, 2002). The study also discusses the challenges in distinguishing between *M. pharensis* and *M. decactis* as separate species. They have overlapping distributions on the reef slope and may not be distinct species but rather represent a species complex with different morphs adapted to varying light environments (Vermeij *et al.*, 2002).

Figure 33. *Madracis* spp. distribution over the reef slope at study site Buoy One. I, *M. pharensis*; II, *M. senaria*; III, *M. decactis*; IV, *M. mirabilis*; V, *M. carmabi*; VI, *M. formosa*. Data from Vermeij & Bak (2003) and P.R. Frade (2008)

It is important to bear in mind that the maximum sampling depth in this study was around 20 m for all the species. Neither of them has been sampled at their distribution maximum. More differences could have been revealed between species, if each of them would have been

sampled at their distribution maximum.

It can be argued that *M. decactis*, out of the three species, was sampled closest to its distribution maximum, potentially experiencing environmental conditions that are not optimal anymore for ideal light harvesting. The elevation in YFP intensity in deep *M. decactis* colonies, might be a reaction to the lower light conditions (Dove *et al.*, 2008; Wiedenmann and Nienhaus, 2006).

Comparing GFP to tissue area establishes a relationship between GFP expression and coral tissue area across different species. It was observed that the GFP:tissue ratio is higher in shallow water samples. Regression analysis of GFP in relation to depth suggests that light may have a significant positive effect on areal abundance of GFP. This finding suggests that coral colonies in shallow waters exhibit higher GFP intensity relative to their tissue area. Comparatively, *M. pharensis* displays a higher GFP intensity in relation to its tissue area than *M. senaria*. Within species, both *M. senaria* and *M. pharensis* demonstrate greater GFP expression intensity in relation to tissue area in shallow samples. This finding aligns with previous results, reinforcing the notion that corals in shallow water environments express more GFP relative to their surface area as a protection strategy against UV. (Smith *et al.*, 2017)

2.6 Symbionts and photobiology

Across species and across depth, the highest areal zooxanthellae density (per cm²) was found in the top oriented samples of the colony. It can be assumed that this is most likely a mechanism to acclimatize to different light conditions over the colonies surface. Low-light corals are known to show lower Symbiodiniaceae cell density but a higher chlorophyll concentration within the symbiont cells (Mass *et al.*, 2007). Since irradiance does not only differ between depths but also shows variation over the coral colonies surface, I am assuming that the symbiont density is adjusted accordingly depending on the position within the colony. Previous studies evaluating corals, and their symbionts adaptive mechanisms observed the consistent result of corals increasing their chlorophyll to acclimatize to less light availability. An elevation in chlorophyll can be achieved by elevating symbiont density. This study further confirms this claim as the measurements of red chlorophyll fluorescence revealed that deeper and side-oriented samples exhibited higher intensities of RCF. Another expected finding is that the cellular chlorophyll a content was higher towards lower light environments. The increase in cellular chlorophyll a as a response to decreasing total PAR irradiance is an indicator for a higher light-harvesting capacity under conditions of light limitation and higher photosynthetic production in the presence of light. This supports a broader absorption spectrum of the phototrophic component to maximize photosynthesis

(Frade *et al.*, 2008). The increase of chlorophyll concentration in dinoflagellates is well-known response to low light levels. This appears to be a universal response to more shaded conditions and the need to harvest more light (McCloskey and Muscatine, 1984).

Shallow samples also express a higher GFP:RCF ratio compared to deeper samples and top oriented samples showed a higher ratio than side-oriented samples. These results are again in line with previous findings, showing that the intensity of GFP in relation to the intensity of the chlorophyll fluorescence increases in shallower corals indicating that the GFPs act as UV protection. While cellular chlorophyll *c2* did not differ between depths, the mean intensity of GFP was higher in shallow. This can be interpreted that shallow corals express a higher intensity of GFP in relation to chlorophyll, which stayed the same. Since the GFP:RCF ratio was also higher in the top samples the the higher GFP:RCF ratio results from an increase in GFP, not a decrease in chlorophyll.

Differences between species.

For *M. senaria* and *M. pharensis* the GFP:RCF ratio was found to be higher in shallow water colonies. *M. pharensis* and *M. decactis* also exhibited a higher ratio in top oriented samples. Over all these results suggest, that in colony areas associated with a higher symbiont density (top parts of the colony) and therefore also parts which are exposed to a higher UV irradiance, the coral host will increase the expression of GFP in order to shade its symbionts from excessive irradiance in shallow-water (Smith *et al.*, 2017).

Furthermore, our study confirms this by demonstrating a negative correlation between total irradiance and chlorophyll a content per cell and with deeper colonies exhibiting higher relative chlorophyll fluorescence (RCF) intensity. Consequently, deeper corals acclimatize to reduced light by augmenting chlorophyll content, indicating enhanced light-harvesting capacity and photosynthetic productivity under light limitation

As expected, the measured quantum yield of photosynthesis (ϕ) is highest in the side-oriented part of the colony. This is a form of shade acclimatization The negative correlation between high irradiance and quantum yield of photosynthesis (ϕ) further support this conclusion. The increasing efficiency in light-use in deeper habitats or in more shaded parts of the colony has already been documented in several studies (Gorbunov *et al.*, 2001; Warner *et al.*, 2006) and an indicates the process of photo acclimation.

Figure 34 represents all results of the symbiont photobiology.

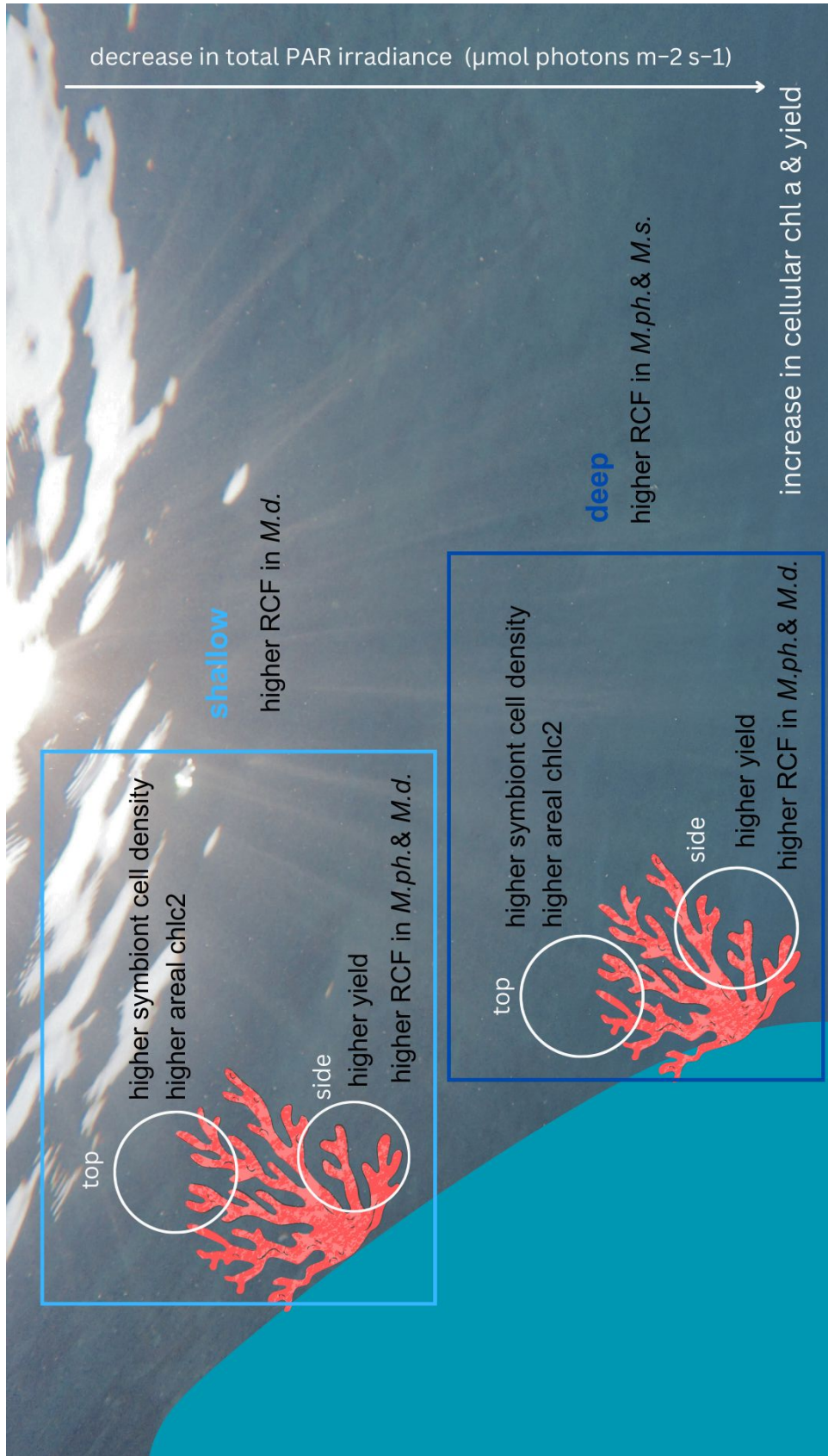


Figure 34. is giving a visual representation of symbiont and photobiology related findings when comparing two different depth groups with two different colony orientations over a light gradient for *M. senaria* (*M.s.*), *M. pharensis* (*M.ph.*) and *M. decactis* (*M.d.*)

2.7 Conclusion

This study underscores how complex the holobiont – consisting of the coral host and the zooxanthellae symbiont, as an organism is. Both symbiotic partners contribute to the physiological responses and acclimation/adaptation mechanisms. (Frade *et al.*, 2008; Roth *et al.*, 2008; Einbinder *et al.*, 2016) The present study was designed to shine light on two of these mechanisms: the expression of different FPs and the symbionts photobiology.

What makes this study unique is the multi-level approach that was used to get a better understanding of this dynamic. To set the hosts and the symbionts acclimation/adaptation mechanisms into relation, different parameters have been measured and analyzed such as symbiont cell density, chlorophyll concentration, photosynthetic performance, FP occurrence and FP mean intensities and then compared over a depth gradient and among three *Madracis* species. This gives a multi-dimensional view on the holobiont. (Fig.35)

Most striking was the substantial difference in the expression of FPs. Shallow samples and top oriented samples showed a higher occurrence and mean fluorescent intensity in FPs. These findings are in line with the hypothesis, that the expression of FPs in the coral tissue serve as protection from UV radiation and can dissipate excess light energy through fluorescence and light scattering. (Mazel *et al.*, 2002; ; Roth *et al.*, 2008) When looking at the GFP expression per tissue area, an increased expression of GFP was observed in colonies closer to the water surface, within species and across species. This further supports that FPs reduce the photo inhibitory effect of high levels of solar radiation, which in combination with heat stress could lead to bleaching.

Moving onto the next level, which is the symbionts, this studies results align with what is known in the literature. Within colony-mechanisms used to acclimate are the increase in zooxanthellae density per cm² in the top oriented parts of the colony and on the contrary the decrease in zooxanthellae numbers on the more shaded side parts which comes as an adaptation to minimize the effect of self-shading when irradiance is minimal. (Mass *et al.*, 2007). Moreover, increasing chlorophyll concentration within the symbiont cells appears to be a universal response to low light levels and the need to harvest more light (McCloskey and Muscatine, 1984; Mass *et al.*, 2007).

Another finding is that the quantum yield of photosynthesis (ϕ) was observed to be highest in the side-oriented parts of the colony. This, along with the negative correlation between high irradiance and photosynthetic quantum yield (ϕ), further supports the notion of the coral host's ability to acclimatize to varying conditions across its surface.

The study provides a new insight into the relationship between the coral host and the zooxanthellae symbiont and the complex mechanisms that happen to ensure optimal light

harvesting and UV protection at the same time.

Overall, fluorescence in corals is influenced by light, leading to acclimation responses that are evident through changes at both interspecific and intraspecific levels. The results are in line with similar studies reporting a correlation between bleaching resistance and the concentration of FPs within the tissue (Roth *et al.*, 2008; Salih *et al.*, 2000). The findings have been attributed to light scattering property of FPs which is an important factor linked to their sun-screening function. (Salih *et al.*, 2000) The multivariate statistical analysis highlights the central role of irradiance but also reveals that the expression of FP underlies not only one factor but a distinct sets of variables.

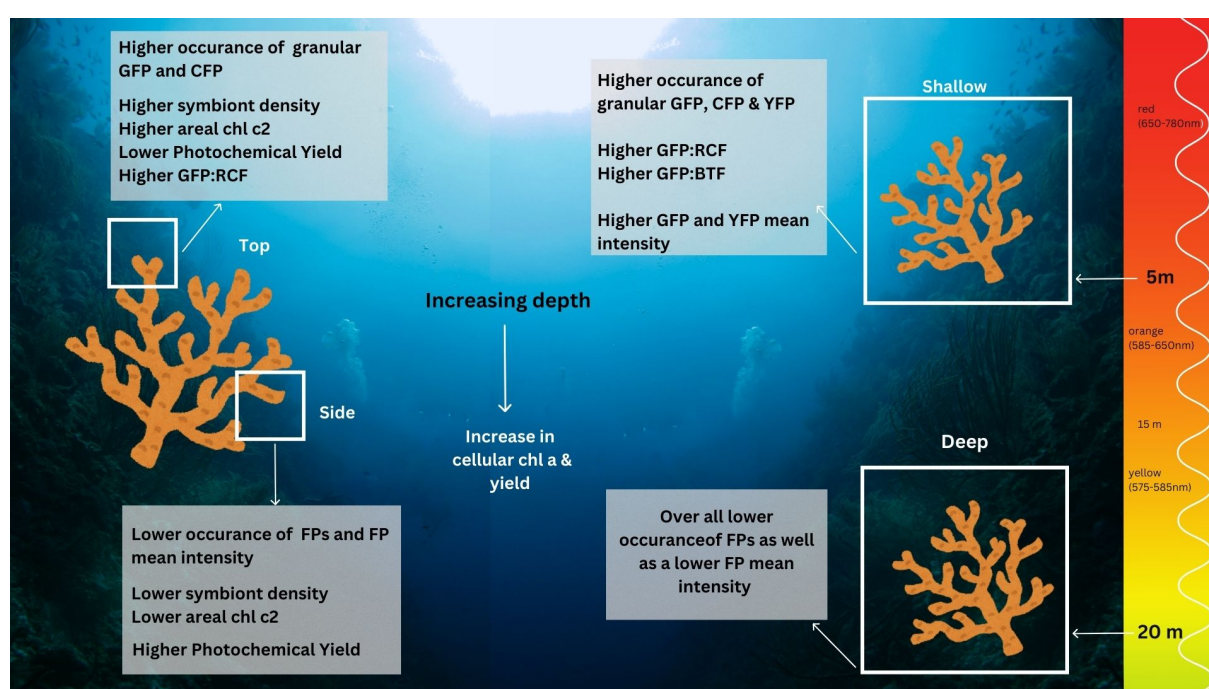


Figure 35. Is giving a visual overview of this study's findings.

3 Limitations and recommendations for future studies

The project comprised three key objectives: (1) the differentiation and quantification of FPs, (2) the localization of FPs within coral tissue, and (3) symbiont-specific analysis. While objectives (1) and (3) were successfully accomplished, the localization of FPs within the tissue did not go as planned.

During this investigation, I have encountered several challenges that I wish to address, aiming to enhance future research endeavors in this area. The primary issue was the quality of the tissue sections. Although some slices allowed for the localization and identification of FPs within specific parts of the coral tissue, this was not the case for the majority. Despite the sectioning process being carried out with precision and care, obtaining a clean tissue slice and placing it on the glass slide without rupturing or damaging it proved challenging. Consequently, in most confocal images, it was difficult to determine the exact part of the tissue being observed, especially for someone who is not an expert in coral histology. Maybe it could be beneficial to try using a different blade in the slicing process or even a different microtome. It could also be the case, that the species I was working with were especially fragile and that, when doing the same process with a different, more robust species with bigger polyps, would have given tissue slices of better quality and easier to analyze regarding their histology.

This study compared three distinct species, with samples originating from different environmental conditions, being shallow waters (5m), deeper waters (20m) and different positions on the colony (top and side). Within each species, there were four to five replicate samples for each treatment. The rather small replicate number was due to limited time under water and a rather short sampling time of two weeks. I contend that this limited number of replicates may not have been adequate for robustly comparing treatments within the same species. Future investigations in this field could benefit from increasing the number of replicates for each treatment. Given the significant heterogeneity among coral colonies, and even among orientations within the same colony, obtaining more samples from more different individual colonies could enhance the representativeness of the findings.

For the identification and quantification of FPs and their mean intensities, appropriate laser settings are crucial. During the microscopy analysis, I faced challenges and uncertainties regarding the laser settings to use. To enhance future studies, it would be beneficial to develop methods for distinguishing between GFP and CFP and quantifying their mean intensity individually. This may be done by using different lasers or confocal settings.

Specifically, by narrowing the filters of the channels for the CFP, GFP and YFP, it would have been beneficial to if I would have done Lambda scans as a first step to see the exact emission peaks of each protein and then base the channels on these emission peaks. When starting the confocal work, I assumed that the channels needed to be defined based on the available pre-defined settings of the confocal. However, it would be better to do the settings based on the fluorescent signals found in the lambda scans. Because of this, I could only discern visually between these two fluorescent proteins (GFP and CFP) in the images that were taken and then quantify them through the conducted image analysis (presence vs. absence). Since these occurrence analyses yielded some significant results, it can be assumed that quantifying the intensity of the CFP and GFP separately would have provided meaningful additional insights. In addition, identifying laser settings that are universally applicable to all species would enable comparisons of fluorescence intensities across different species. Because of the heterogeneity and the great differences between species, regarding their tissue characteristics and how they appear in the microscope, I doubt that this can be easily done.

Every answered research question will leave new open questions. This study is no exception. An interesting question is what exactly happens on a single-cellular level. Does one cell mostly produce one specific FP, or a mix of FPs? Can different FPs co-occur in the same cell and to different type of cells harbor FPs? What about the relative position of the proteins in relation to the symbionts? A possible approach to enhance this research and to investigate some of these open questions would be to localize the fluorescent proteins (FPs) within the coral tissue at a higher resolution. One potential method is to stain the cell nucleus with DAPI. This would allow for the differentiation of individual cells and provide better spatial orientation and make it easier to see where exactly FPs are within the coral tissue and in relation to the symbiont-harboring cells. Our research team is already looking into this approach.

4 General discussion

Fluorescent proteins in corals and coral reefs present an intriguing field of study, with much potential yet to be unlocked. Despite the progress made, there remains a significant gap in our understanding of their complex functions and roles within coral physiology. More comprehensive research is needed to unravel these mysteries and harness their potential for coral conservation.

Studies have shown a connection between coral fluorescence and heat resistance, as well as a positive effect of fluorescent proteins on bleaching resistance and recovery. This makes fluorescent proteins a fascinating subject to study in the context of coral reef conservation and restoration. An idea worth exploring (yet with many ethical issues that need consideration) is the breeding of genetically altered coral species that express more FPs, potentially making them more resistant to climate change and better adapted to warmer sea temperatures. However, one also has to consider the fact that we do not know yet, if corals that express more FPs are then maybe investing less energy in their reproduction and growth. There might be energetic trade-offs, which should be considered and need to be explored.

We often talk about the fragility of nature, biodiversity, and ecosystems, and how their survival depends on us. But if we are honest, it is us, humans, who truly depend on healthy ecosystems. Humanity's survival heavily relies on the benefits of healthy ecosystems (marine and terrestrial) which provide clean freshwater, fertile soil for food production, clean air and many other benefits. Coral reefs specifically offer protection and act as natural shields for our coasts. By supporting rich biodiversity, they are providing food and income for about 1 billion people and are potential sources for new pharmaceutical drug discoveries.

It is time to step back and recognize that we are not separate from this system; we are a part of it. Right now, we are on a path that's destroying the very system that keeps us alive. We must raise awareness and acknowledge our deep connection with nature to protect and preserve it—for our own survival and for future generations.

Healthy reefs are vital at multiple levels, from biodiversity preservation to supporting coastal communities and global economies. Raising public awareness about the significance of reefs and the threats they face is crucial. Enhanced science communication is necessary to engage the public, policymakers, and stakeholders, fostering a collective effort to protect and preserve these invaluable ecosystems. Protecting coral reefs necessitates a comprehensive approach encompassing local, national, and global efforts. Mitigating climate change by reducing carbon emissions is crucial, as this leads to ocean acidification and elevated SST

which then leads to coral bleaching. Some steps which should be taken, e.g., implementing sustainable fishing practices and prohibiting destructive techniques, are essential to maintaining ecological balance; coastal development should be managed sustainably to prevent habitat destruction and pollution, while protecting mangroves and seagrasses, which support reef ecosystems. Another important thing is pollution control through improved waste management and reduced agricultural runoff. This is vital to minimize nutrient pollution. Supporting coral reef restoration projects and investing into research to help develop a better understanding of corals stress resilience can aid in reef recovery as well as establishing and effectively managing marine protected areas (MPAs) to provide safe havens for reefs to thrive. Public awareness and education initiatives can engage local communities and promote responsible eco-tourism and global cooperation and participation in international agreements, coupled with financial and technical support for developing countries, are essential for comprehensive reef conservation. (Fig. 36)



Figure 36. Step by step Action Plan – Important steps in the Conservation and protection of coral reefs. Panels summarize published research on the different topic. (Literature sources used include: <https://www.coralreef.noaa.gov>; Hein *et al.*, 2021)

Although much of the damage already done may or may not be undone, we know that Nature is resilient and that ecosystems have a significant potential for recovery. Our focus should be on creating conditions that allow ecosystems to recover on their own, point 4 and 5 (Fig. 36) should be seen as additional measures. This includes investing in research that enhances ecosystem resilience while simultaneously addressing the root causes of climate

change. By doing so, we can foster a healthier planet and ensure the long-term survival of coral reefs and other vital ecosystems.

Ultimately, public awareness and science communication are crucial. Engaging the public, policymakers, and stakeholders in meaningful dialogue about the importance of healthy reefs and the threats they face can foster a collective effort to protect and preserve these invaluable ecosystems. Through a combination of research, conservation, and climate action, we can work towards a sustainable future for coral reefs and all the other ecosystems and the myriads of lives they support.

Science is both a body of knowledge and a dynamic process; while it may sometimes seem like a collection of isolated facts, its true value lies in how we apply this ever-evolving understanding to make meaningful changes and act for a better future.

In conclusion, while this study contributes valuable insights into the mechanisms of coral acclimation and adaptation and is a small pixel in the bigger picture on the understanding of coral ecophysiology, it also highlights the need for ongoing research and public engagement. By advancing our understanding and fostering greater awareness, we can work towards more effective conservation strategies and ensure the resilience of coral reefs in a rapidly changing world.

5 Supplementary:

5.1 Materials and Methods

5.1.1 Sample collection & Target species

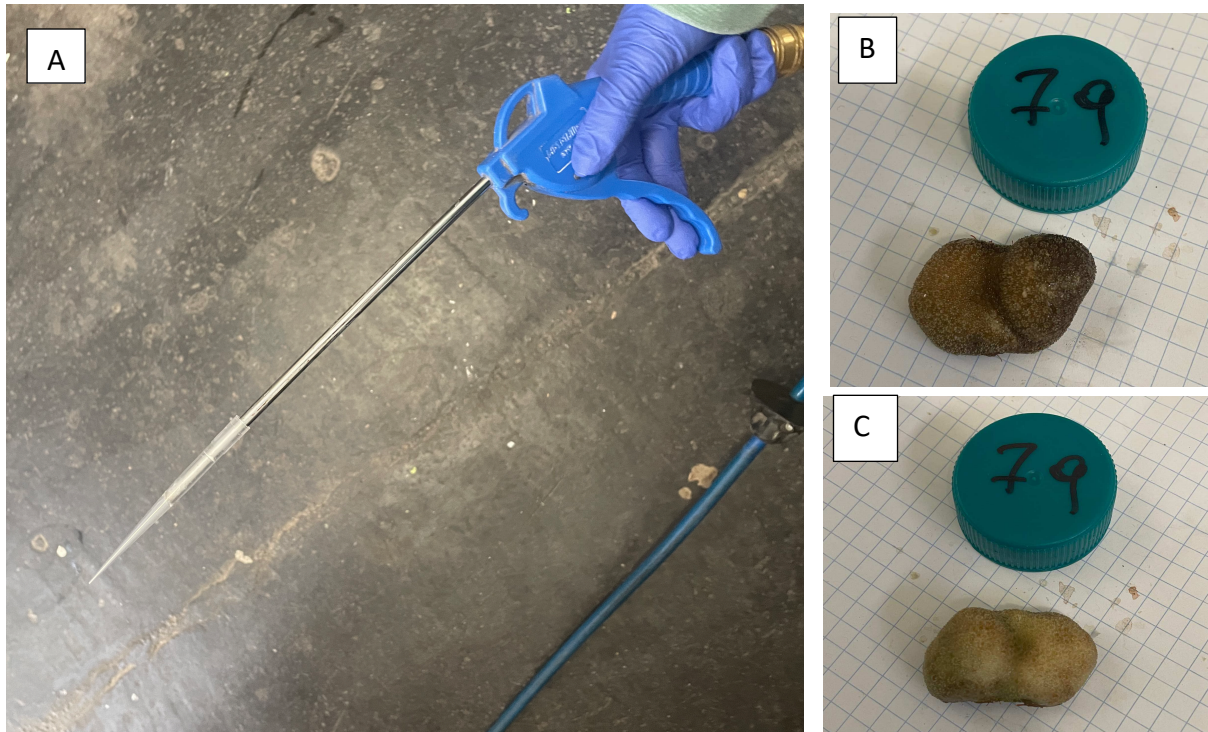
Samples of *Madracis pharensis*, *Madracis decactis* and *Madracis senaria* were collected in Snake Bay, Curaçao, in September 2022 to analyze host and symbiont physiological parameters described in the following sections. Samples were collected at two different depths (approximately 5 and 20 m) and two different colony orientations (top and side of the colony), and only light-exposed colonies were included in the study. All samples were collected under permit 2012/48584.

Before sampling, all samples were photographed under blue light (for colony fluorescence) and ambient light. In addition, corals in the field were measured for photosynthetic activity (efficiency), reflectance, and fluorescence using a pulse amplitude modulated (PAM) fluorometer (Diving-PAM; Walz GmbH, Germany). Samples (n = 4-5 replicates per species, depth, and colony orientation for a total of n=82) were divided into three fragments: one for tissue analysis under confocal microscopy, one for analysis of symbiont physiological parameters, and one extra for possible analysis of gene expression (not analyzed in this study). Briefly, coral fragments for confocal microscopy were brought to the laboratory in seawater after sampling in the field and fixed in paraformaldehyde (PFA) solution 4% for 6 h, transferred to phosphate-buffered saline (PBS) 1x and stored at 4°C. On the other hand, coral fragments destined for symbiont physiology and gene expression analysis were snap-frozen in liquid nitrogen immediately after leaving the water in the field. All samples were returned to Vienna in liquid nitrogen or dry ice. Samples for symbiont physiology and gene expression were then stored at -80°C and samples for confocal microscopy were stored in a cold room at 4°C until further analysis.

5.1.2 Symbiont photobiology

The first step was to remove the tissue from the skeleton. This was done using an air-jet with a pipette tip attached. The coral piece was gently held in a ziplock bag which was filled with 10 ml PBS 1x and the tissue was brushed off the coral skeleton from one side to the other while inside the ziplock bag. The tissue sample was then processed and prepared for further analysis. To characterize the host physiology, the zooxanthellae were counted using a hemocytometer. A minimum of 8 squares and minimum of 200 cells per sample were established to ensure a robust and precise counting of zooxanthellae. It was aimed for a

coefficient of variation below 10%. The cell density per coral surface area was then calculated accordingly. Surface area was determined using the aluminum foil method (Marsh 1970).



A: Airbrushing gun, with a pipette tip attached to it, which was used for airbrushing off the tissue
 B: Coral sample before airbrushing (tissue is still on the skeleton)
 C: Coral sample after airbrushing (bare skeleton without tissue showing)

For chlorophyll a concentration per area and per cell, the quantification was done using a spectrophotometer. Absorbance was measured at 630, 663, and 750 nm using a BioTek™ microplate reader. The Jeffrey & Humphrey (1975) formulas were used to calculate chlorophyll a and c2.

Jeffrey & Humphrey (1975) for 100% acetone:

$$Chl\ a\ [\mu g\ mL^{-1}] = 11.43 \times A_{663} - 0.64 \times A_{630}$$

$$Chl\ c2\ [\mu g\ mL^{-1}] = 27.09 \times A_{630} - 3.63 \times A_{663}$$

Conversion from concentration to absolute amount of extracted chlorophyll:

$$Chl\ [\mu g] = Chl\ [\mu g\ mL^{-1}] \times V_{\text{extraction}}\ [mL]$$

5.1.3 Fluorescent Proteins

5.1.3.1 *Sample preparation and Laser Scanning Confocal Microscopy (LSCM)*

Decalcification

Fragments collected for LSCM and previously fixed in PFA (4%) were transferred to a 15ml Falcon tube with Ethylenediaminetetraacetic acid (EDTA 10%) for decalcification. The decalcification process took approximately one to three weeks, depending on the size of the sample, with regular changes of the EDTA solution every four to six days. During decalcification the tubes were kept on a swivel plate. After decalcification, the remaining tissue samples were extracted from the tube and transferred to a 30% sucrose medium for overnight rehydration and then stored at -20°C until further processing.

Embedding

To further prepare the samples for slicing, the rehydrated tissue was embedded in OCT in small plastic molds. A thin layer of OCT was put into the mold, then the tissue sample was placed on top of the layer with its surface facing upwards. The tissue was placed evenly in the mold and then covered in more OCT and placed on dry ice until it was completely frozen. After the embedding the samples were stored in a -20° freezer until further processing.

Cryosectioning

Tissue samples were sectioned using a cryostat. A slice thickness of at 20µm was then defined and kept the same for all samples. For each sample, 90 tissue slices have been made. The tissue slices were placed on regular glass slides (6 tissue slices on one glass slide). The slices were all positioned with the same orientation on the glass slides. After finishing the slicing left over samples, were put back to the plastic molds and brought back to the -20°C freezer as well as the glass slides with the tissue samples on it.

Mounting the slides

Slides containing tissue samples were washed with PBS 1x to rehydrate the samples and to remove the OCT compound. The excess of PBS 1x was then carefully removed with paper. In the next step, approximately 80-100 µl of Mowiol was placed in the center of the slides, followed by gently placing a coverslip on the slides. Samples were then allowed to dry for an hour, and then ready for confocal microscopy .

LSCM – Laser scanning confocal microscopy

The slides were visualized with Confocal Light Microscopy. To image the fluorescence Zeiss LSM 780 and Zen Black processing software (2011 v14.0.16.201) were being used. Different excitation lasers have been applied at specific wavelengths ($\lambda=405\text{nm}$, $\lambda=488\text{nm}$, $\lambda=546\text{nm}$ and $\lambda=633\text{nm}$) of determined fluorescent compounds.

Settings In the smart set up four lasers have been chosen: Dapi blue, AF 488 green, AF 546 yellow, AF647 red. The frame size was then set 1024x1024 and the speed t to MAX. The averaging number was set to 2, the bit depth to 12 (bit) and the direction to bidirectional. The the mode was then set to „line“ and for method the „mean“ was used. These were the standard settings for all samples. All the images were taken in 20x.

Taking an image First step was to find a good area on the tissue sample to image. To focus was to find an area that appeared bright and clear in focus.

Each channel was then separately chosen to set the range indicator to help chose a good setting with good exposure for each channel. The pinhole was the same for all settings.

When a good setting was found for each channel, all tracks were selected. The tile scan was set to: 3x3, Bidirectional, online stitching and the threshold was set to 0.70.

Next step was to take the image which was then saved for further analysis.

Linear Unmixing To quantify the mean intensity of the fluorescence, the linear unmixing function was used. The four target areas were: green, blue, red and yellow. The 4 colors were selected in each image and the mean intensity of each color was then quantified and saved. For each species, four to five samples have been analyzed for every treatment (four treatments). That makes 16-20 samples per species. Per sample, three tissue sections, from three different slides were analyzed. That makes 9 technical replicates per sample.

Within each of the three species, the settings were kept the same to be able to compare them. Between species, different settings had to be used.

Lambda Scan A typical spectral image lambda scan collected by a microscope consists of thousands or even millions of individual spectra, with each pixel representing one spectrum, depending on the image's dimensions. Each fluorophore has a unique spectral signature or emission fingerprint, despite spectral overlap with others. This signature can be independently identified and used to determine the contribution of each probe to individual pixels in a lambda stack. Linear unmixing techniques generate distinct emission fingerprints for each fluorophore (or excitation fingerprints if excitation spectra are used), allowing for accurate analysis of the specimen.

To acquire the emission spectrum of the Fluorescent Proteins (FPs) found in both the coral animal tissue and the zooxanthellae symbiont, a lambda scan procedure was implemented. This procedure was executed using each of the six available laser lines ($\lambda=405\text{nm}$, $\lambda=458\text{nm}$, $\lambda=488\text{nm}$, $\lambda=514\text{nm}$, $\lambda=561\text{nm}$, and $\lambda=633\text{nm}$). The Lambda Scan was done for each of the three species.

Sample selection For both *M. senaria* and *M. pharensis*, 20 samples have been analyzed in the confocal. (5x shallow-top, 5x shallow-side, 5x deep-top, 5x deep-side) For *M. decactis* it

was 16 (4x shallow-top, 4x shallow-side, 4x deep-top, 4x deep-side). Within the slicing process, 15 slides, each holding 6 tissue slices, have been prepared for each sample. Out of these 15 slides, three were selected for microscopy and 3 tissue slices, off each of the three slides have been analyzed. That makes 9 measurements (replicates) for each sample.

5.1.3.2 *Image analyses from confocal microscopy*

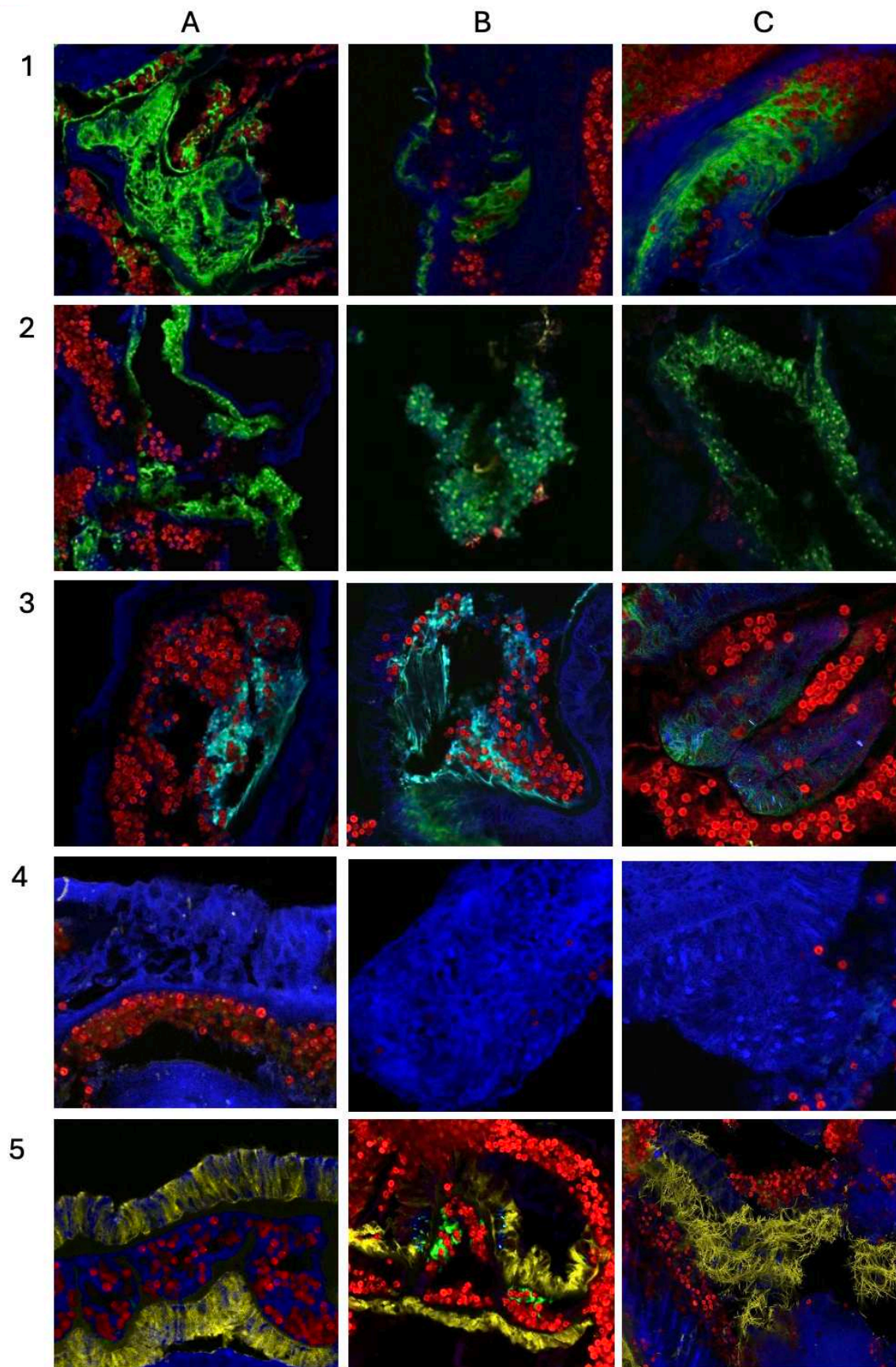
Another approach, besides FP intensity quantification in the confocal was to characterize FP patterns was the descriptive analyses of the images taken in the confocal. The following categories were scored by using a binary variable, with values of "1" indicating the presence of the category and "0" indicating its absence:

Granular green fluorescent protein (granular GFP), diffused green fluorescent protein (diffused GFP), granular blue fluorescent protein (granular BFP), diffused blue fluorescent protein (diffused BFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP) and also yellow structures, which are most likely not part of the host tissue (yellow not from tissue) (Figure 1). In total, 403 images have been coded.

During the imaging process, we identified structures that did not seemed to be part of the actual coral host tissue. These structures exhibited the same emission peak as the yellow originating from the coral tissue and were consequently quantified as yellow fluorescence by the LSCM. Given that our study focuses solely on analyzing host fluorescence, images containing non-identified structured labeled as "yellow not from tissue" were excluded from the intensity analyses. Additionally, images containing both, yellow from the tissue and yellow not from the tissue were also omitted from our analysis.

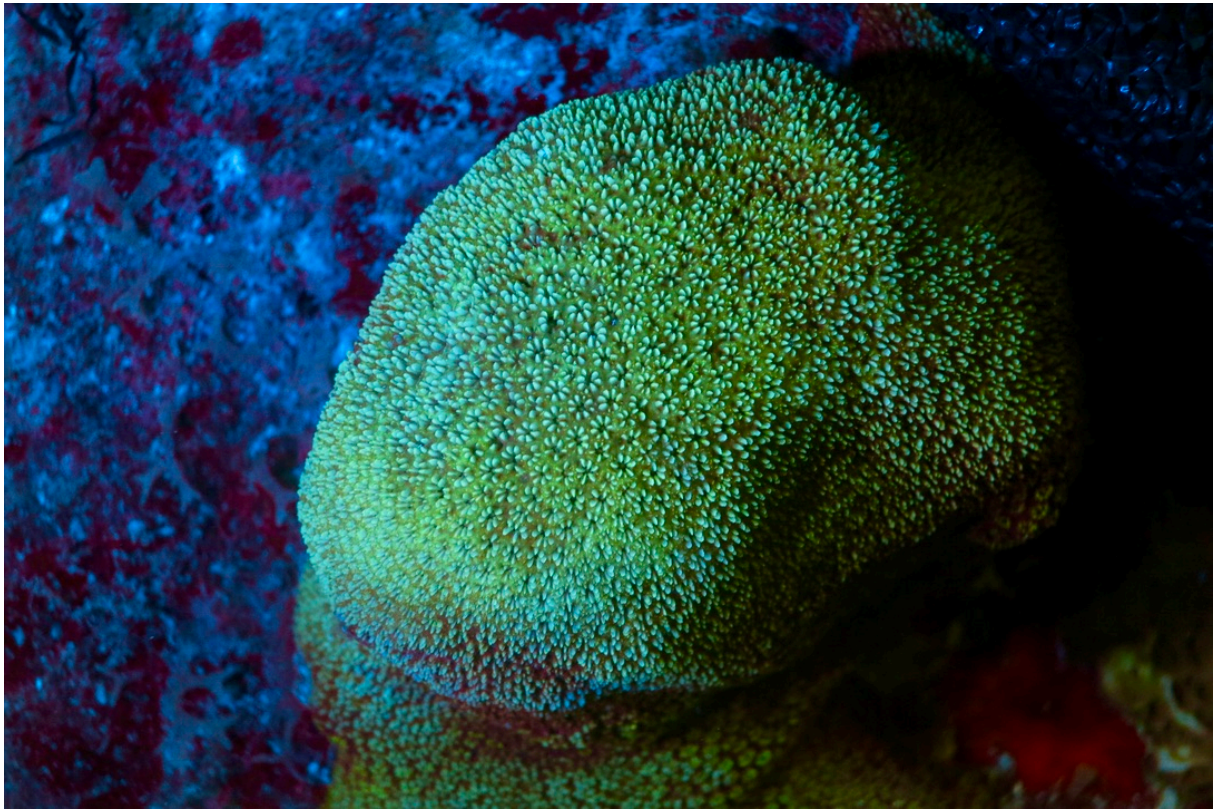
The figure below shows some examples of each analyzed category.

- 1 (A-B): Diffused GFP
- 2 (A-B): Granular GFP
- 3 (A-B): Cyan, CFP
- 4 (A-B): Blue Tissue Fluorescence
- 4 C: granular BFP (not further analyzed)
- 5 (A-B): YFP
- 5 C: Yellow structure which is not from the coral host tissue

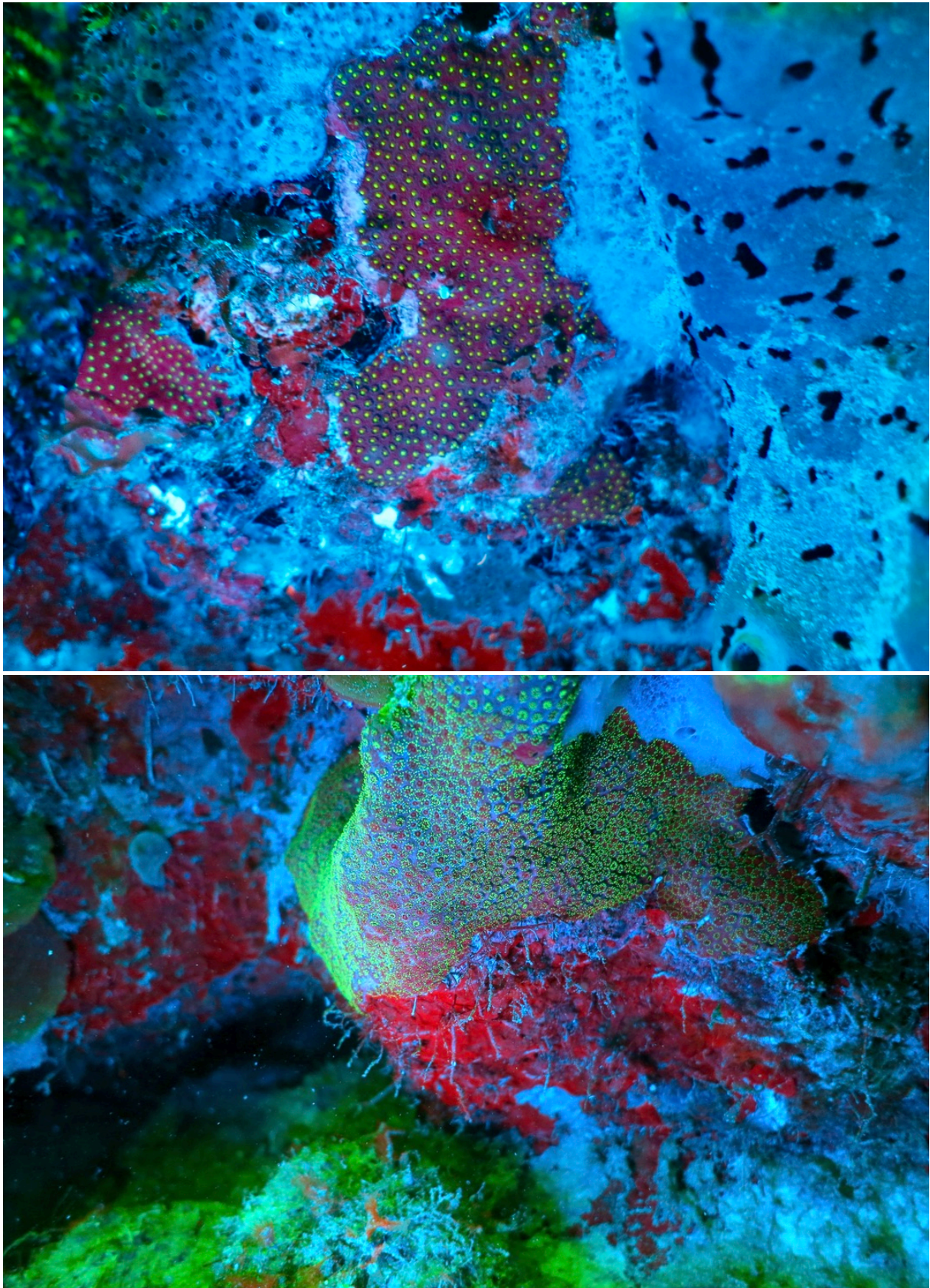


6 Gallery

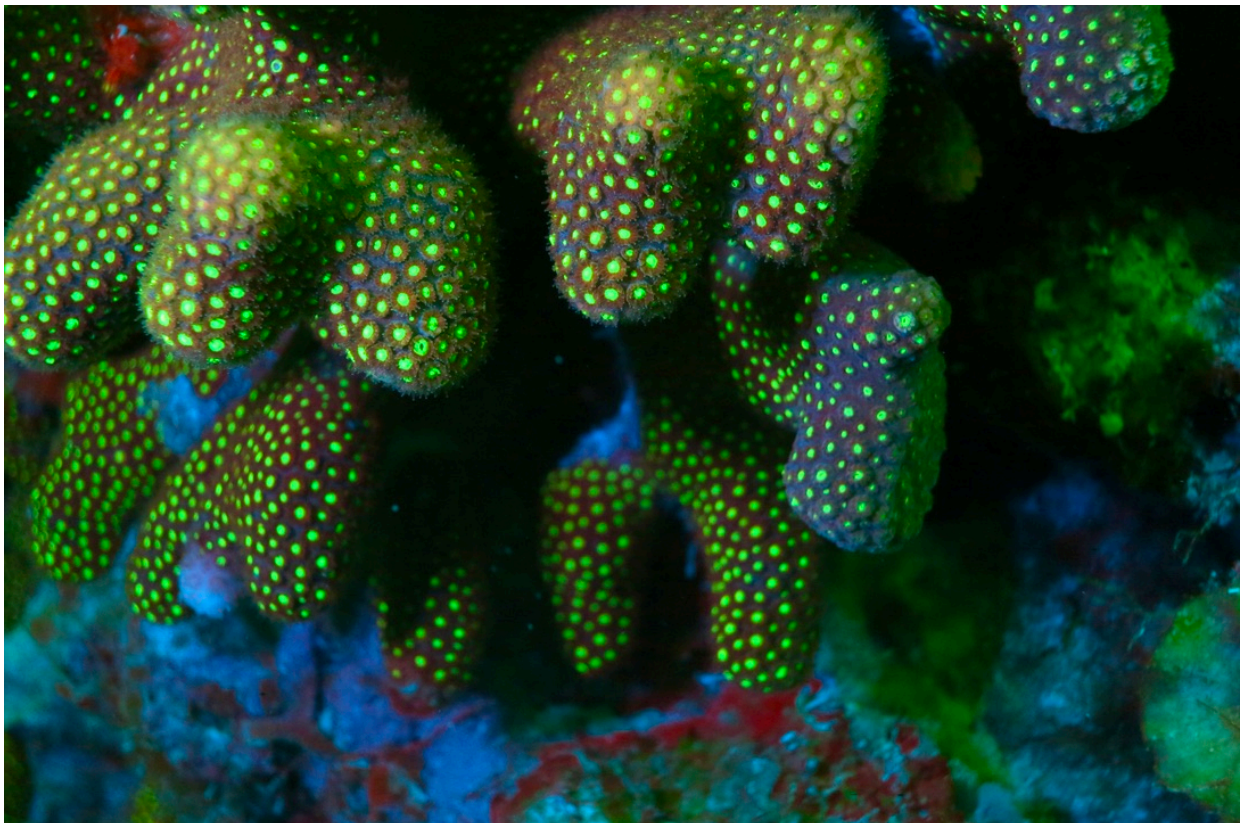
6.1 Photos taken during fieldwork



M. senaria; photographed in September 2022 in Curacao



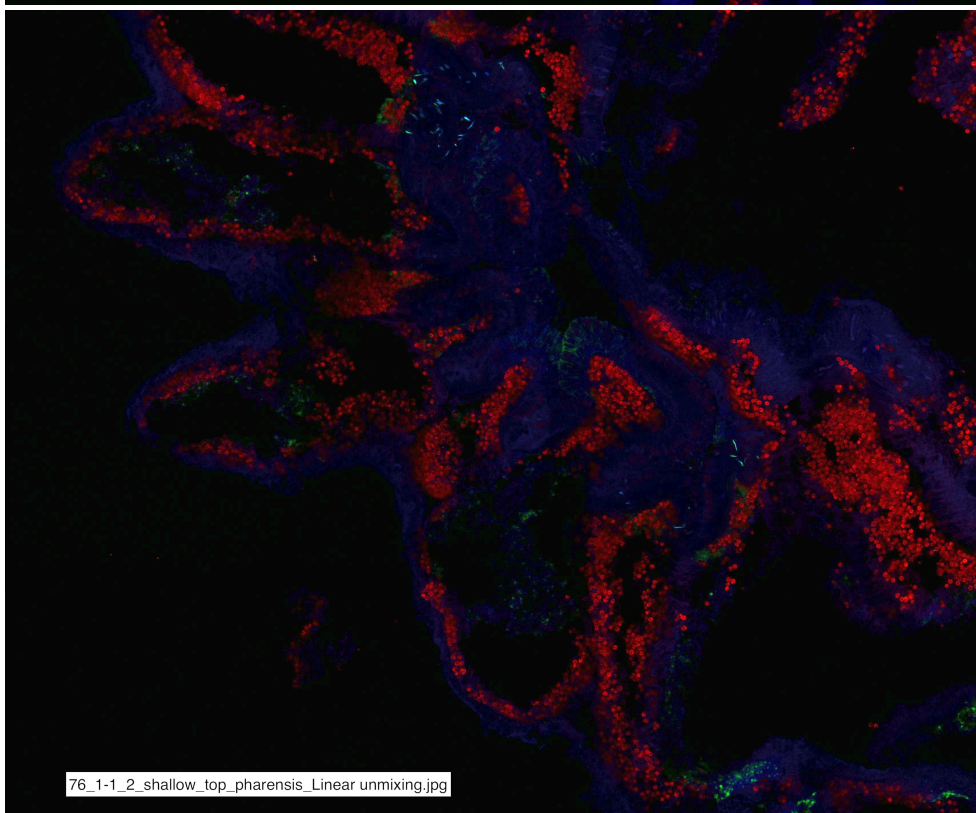
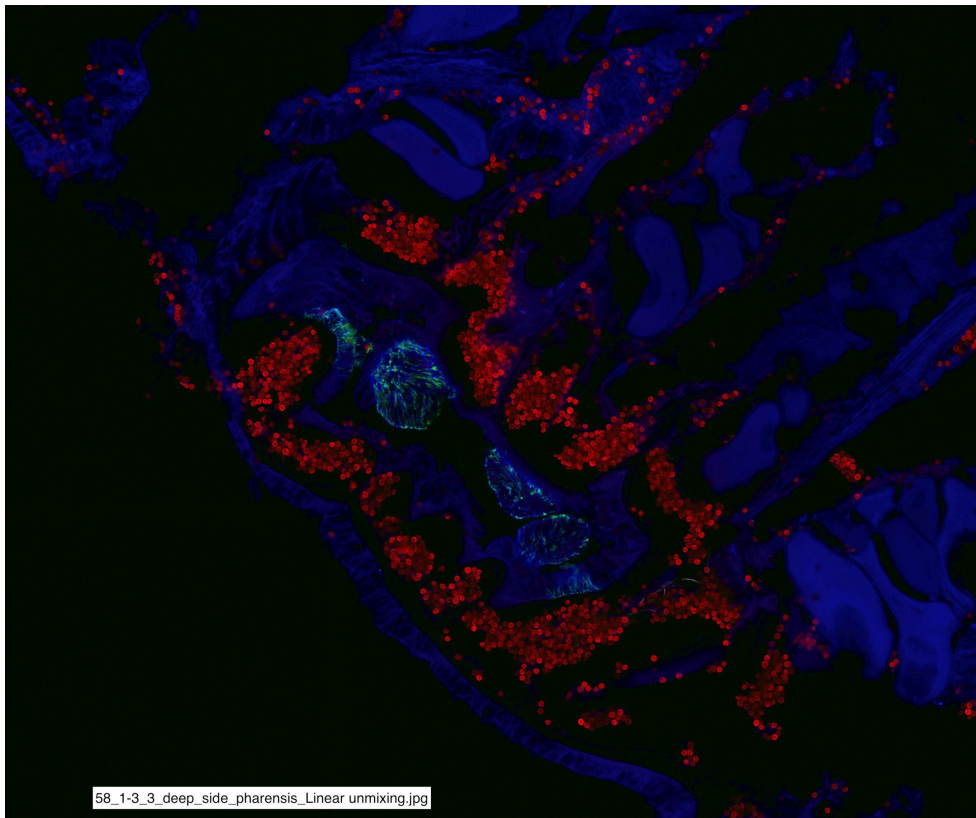
M. pharensis; photographed in September 2022 in Curacao

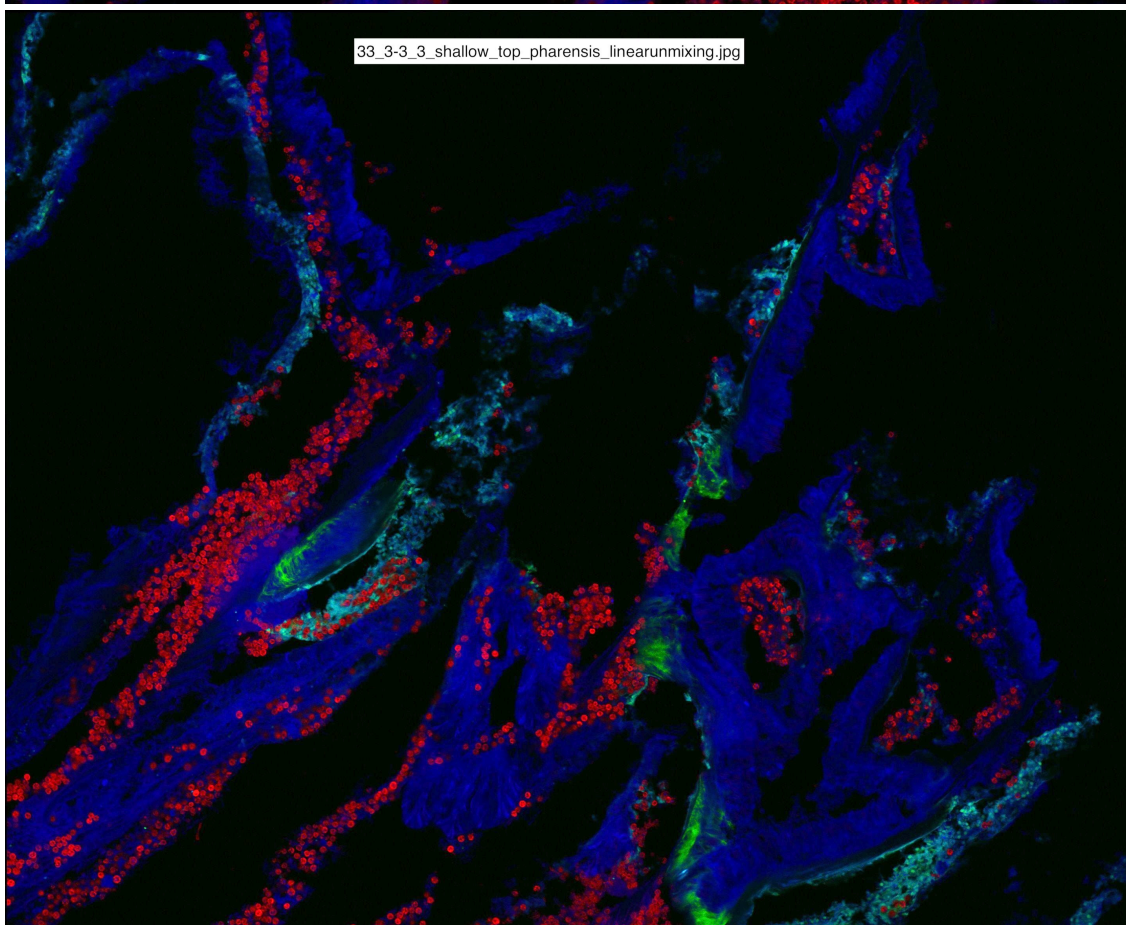
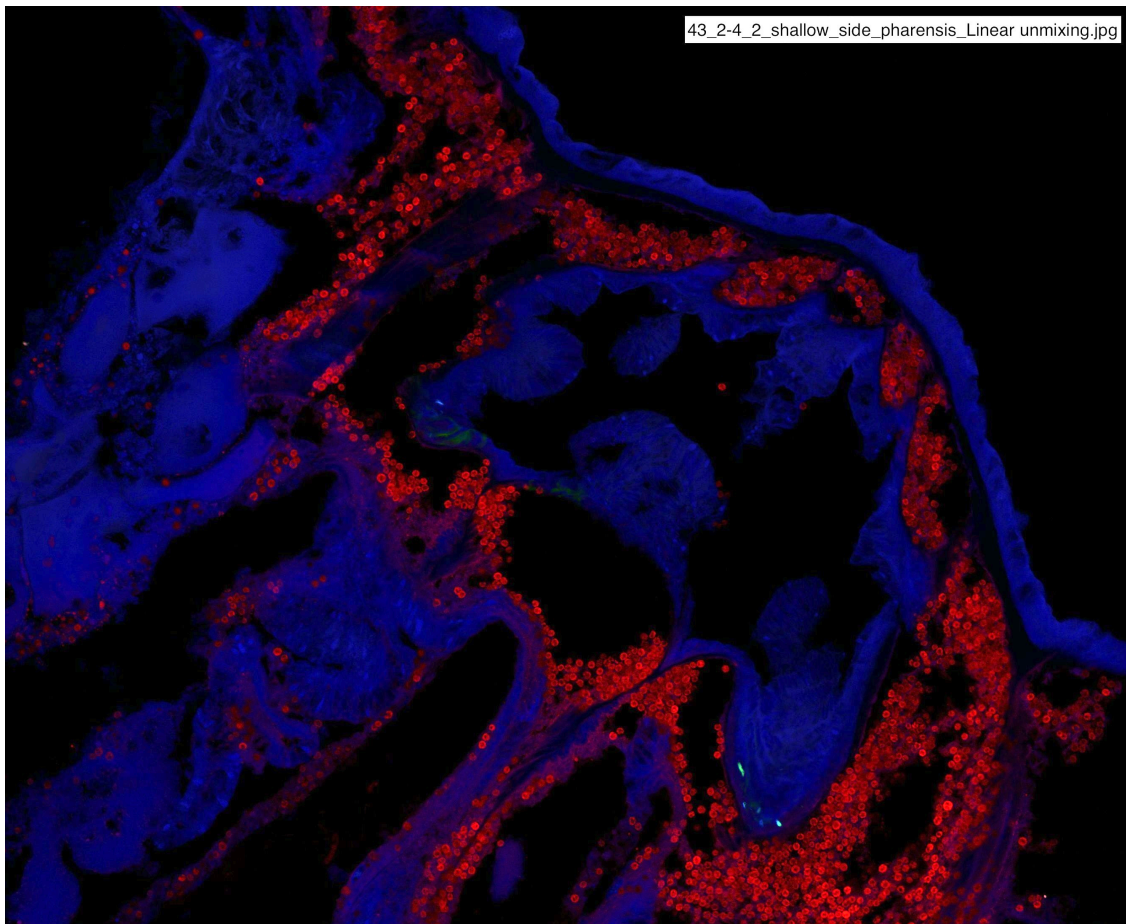


M. decactis; photographed in September 2022 in Curacao

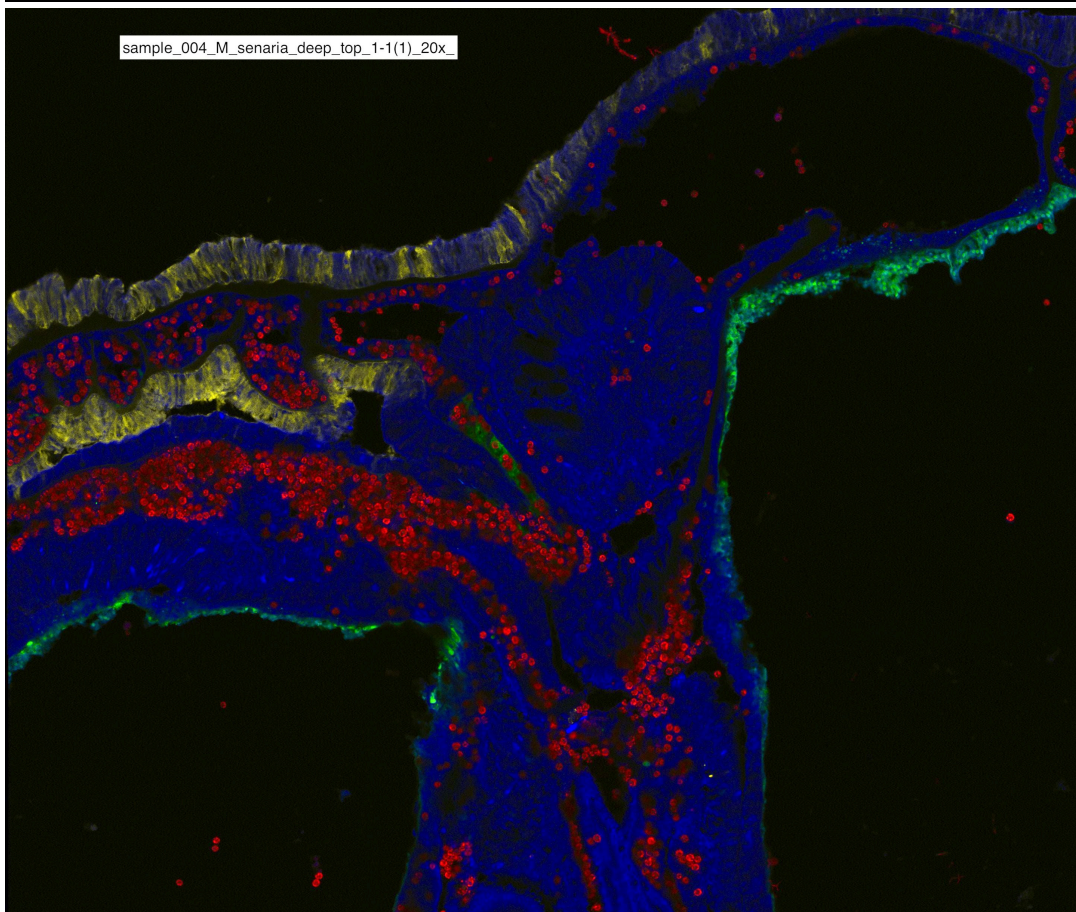
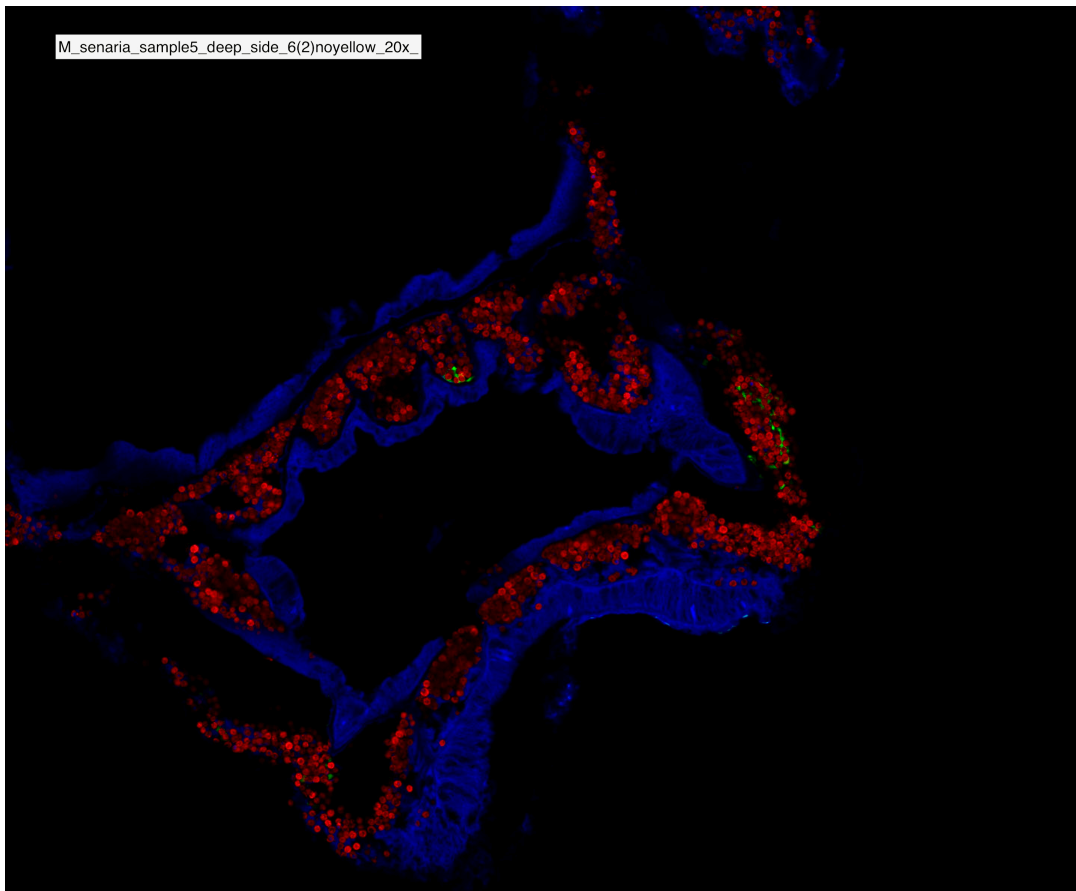
6.2 Original images taken in the LSCM

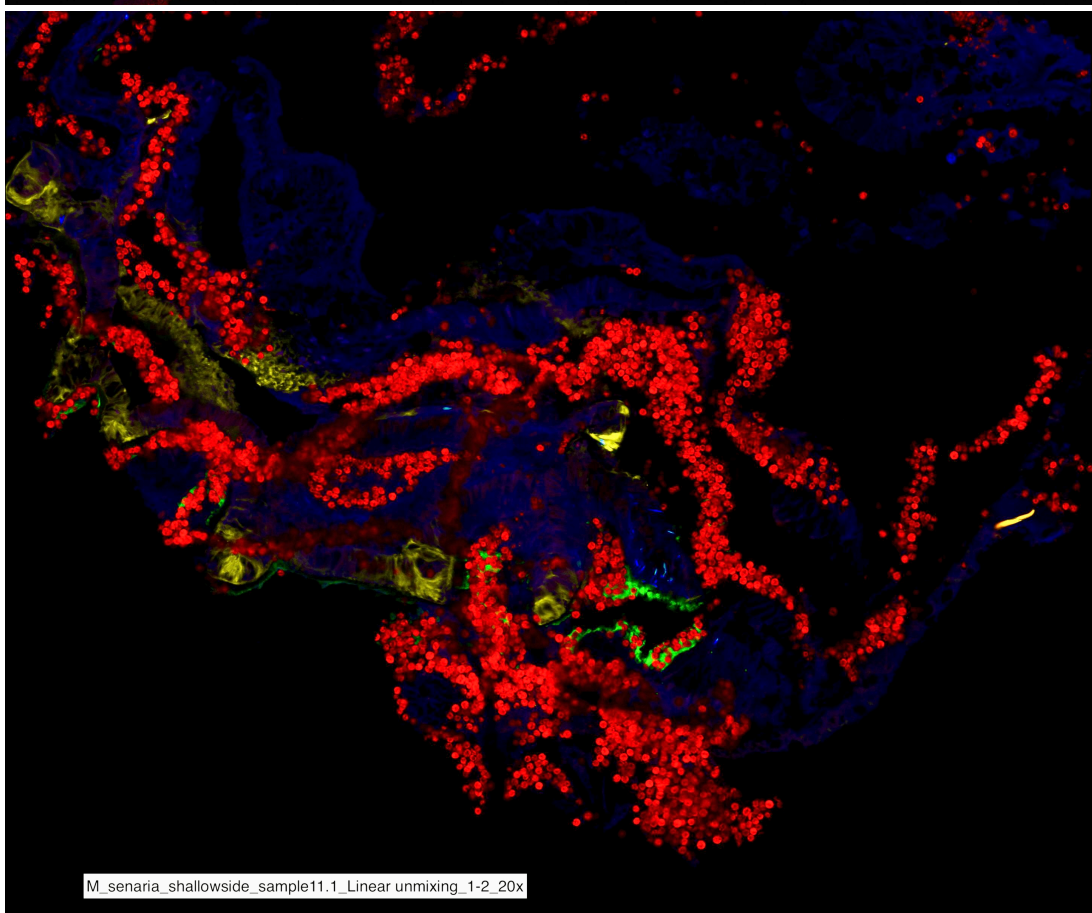
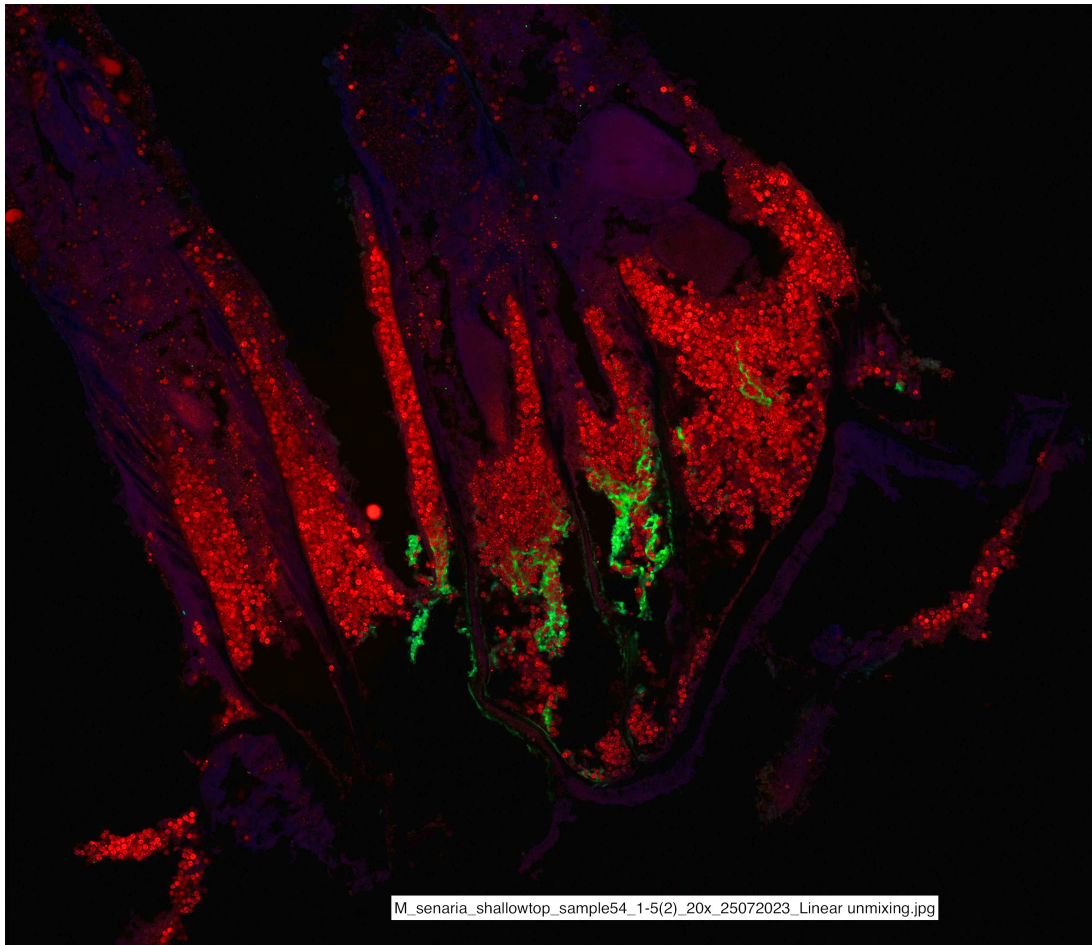
M. pharensis



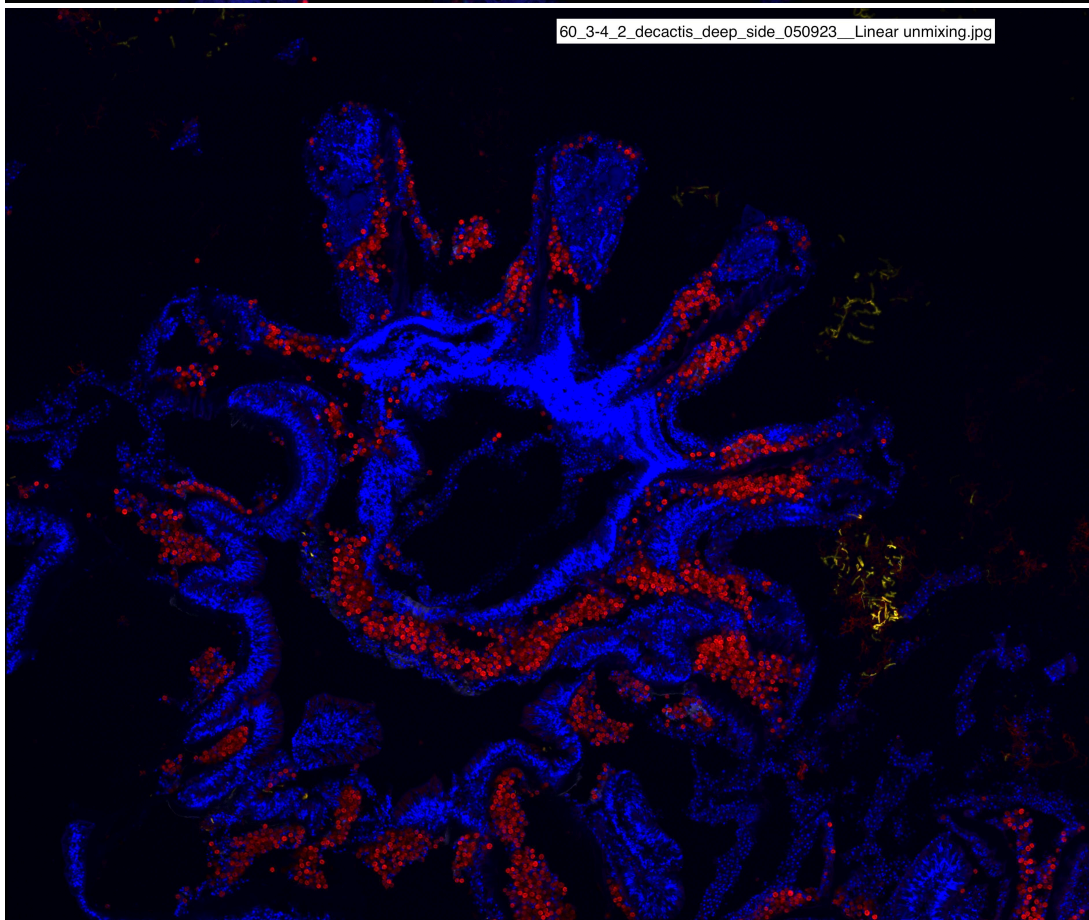
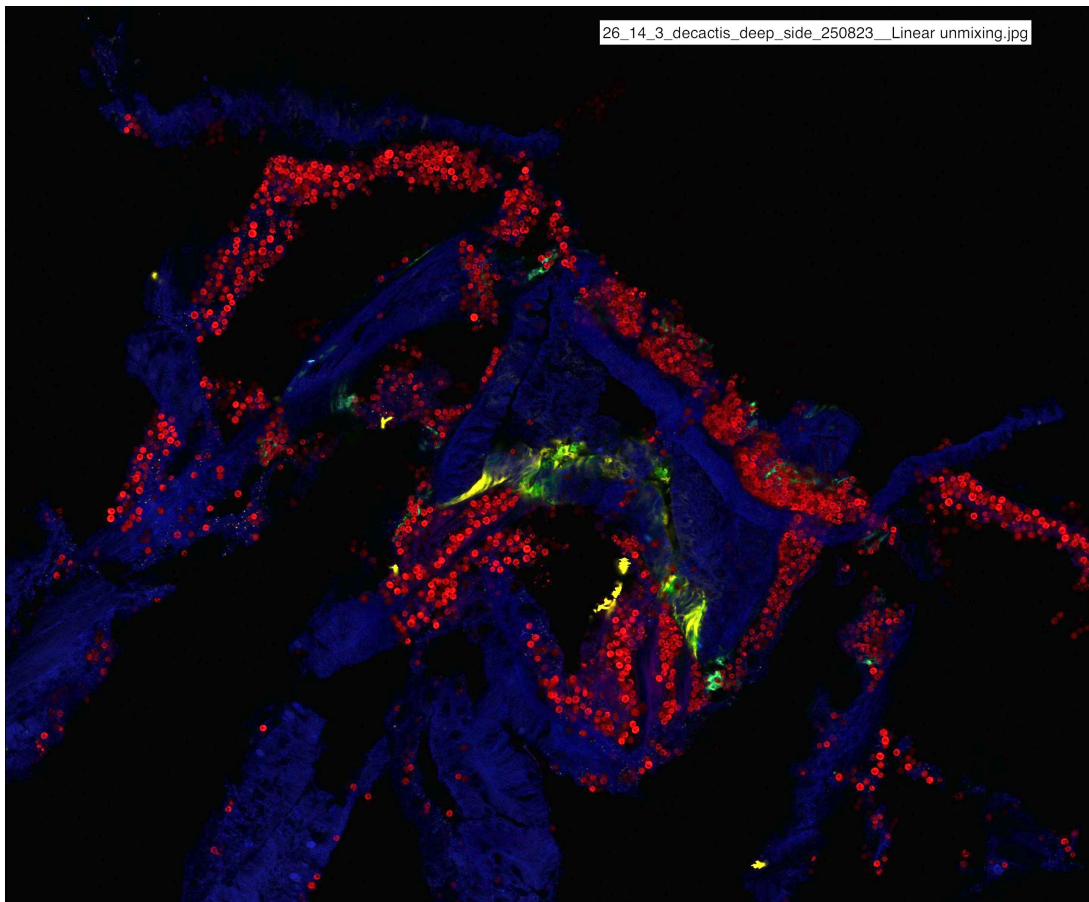


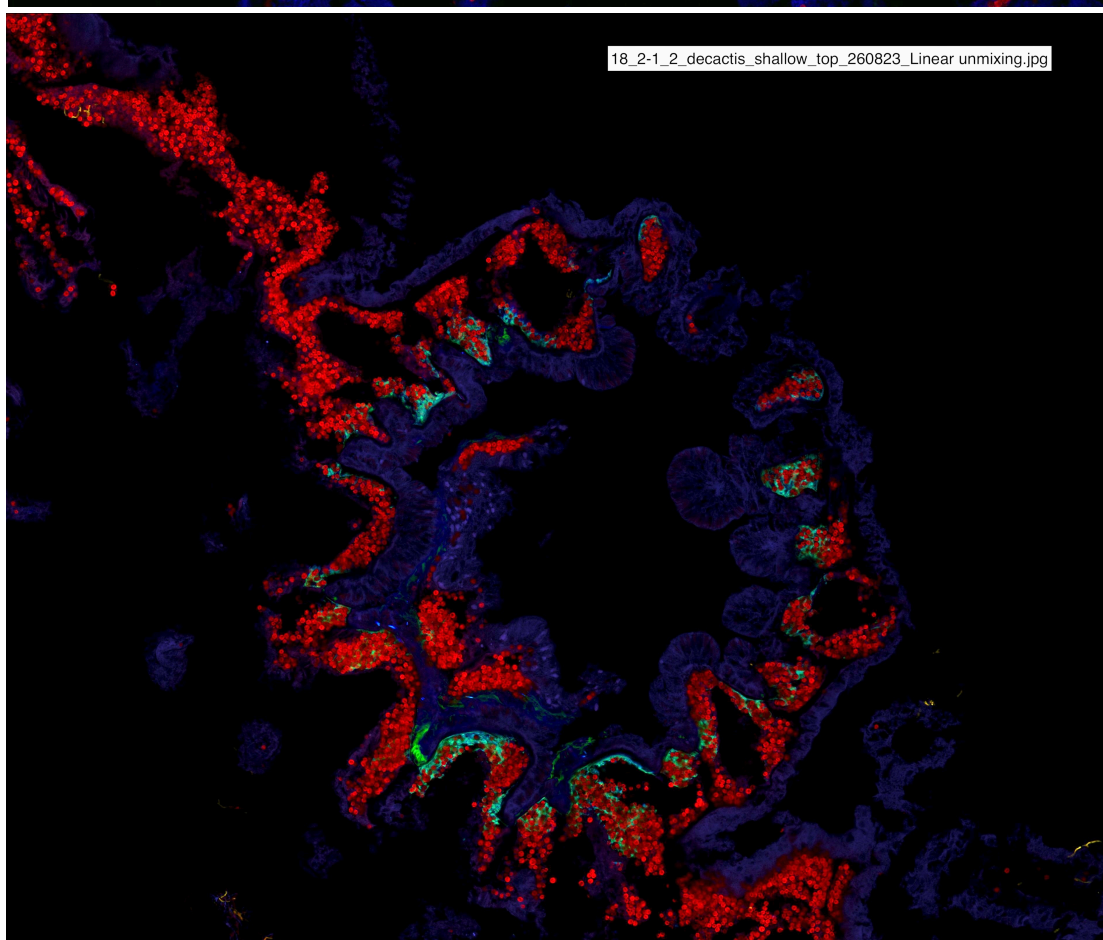
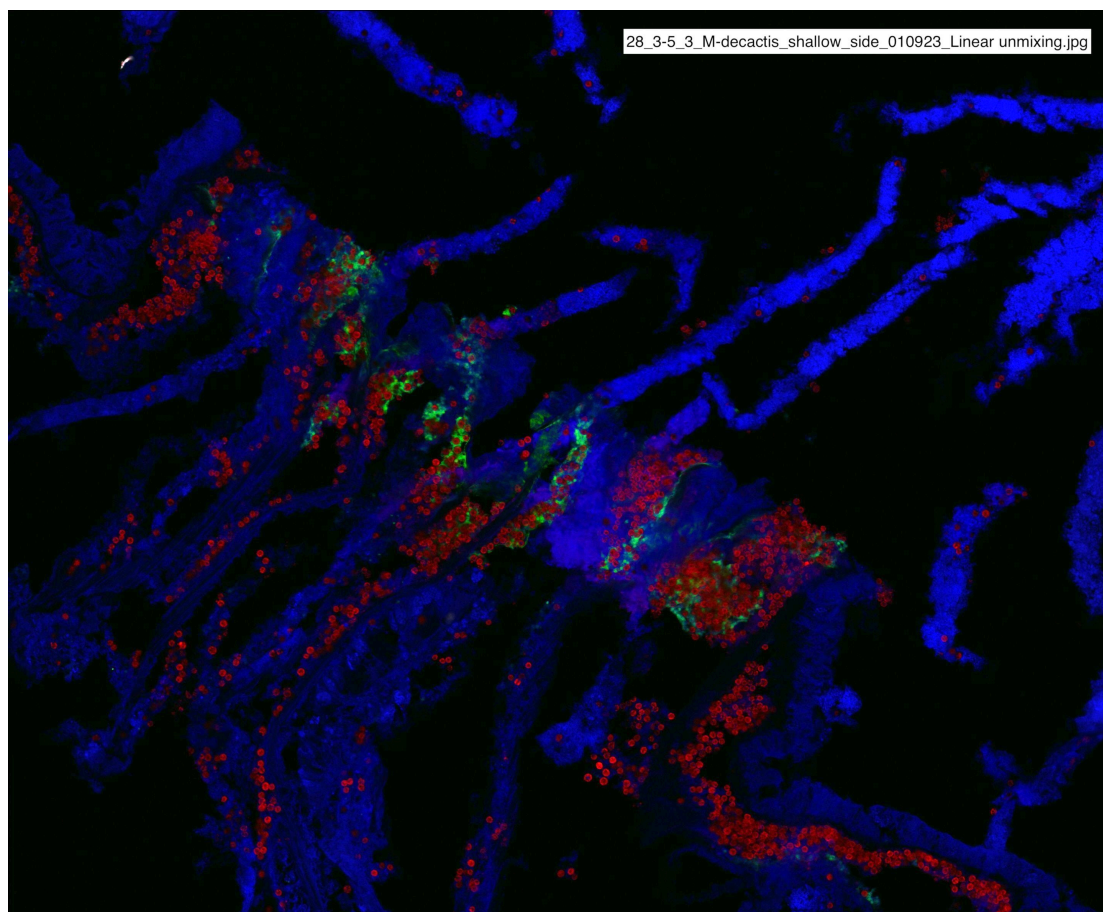
M. senaria





M. decactis





7 Literature

- Aguzzi, J., Thomsen, L., Flögel, S., Robinson, N. J., Picardi, G., Chatzievangelou, D., Bahamon, N., Stefanni, S., Grinyó, J., Fanelli, E., Corinaldesi, C., Del Rio Fernandez, J., Calisti, M., Mienis, F., Chatzidouros, E., Costa, C., Violino, S., Tangherlini, M., & Danovaro, R. (2024). New technologies for monitoring and upscaling marine ecosystem restoration in deep-sea environments. *Engineering (Beijing, China)*, 34, 195–211.
<https://doi.org/10.1016/j.eng.2023.10.012>
- Aihara, Y., Maruyama, S., Baird, A. H., Iguchi, A., Takahashi, S., & Minagawa, J. (2019). Green fluorescence from cnidarian hosts attracts symbiotic algae. *Proceedings of the National Academy of Sciences of the United States of America*, 116(6), 2118–2123.
<https://doi.org/10.1073/pnas.1812257116>
- Alieva, N. O., Konzen, K. A., Field, S. F., Meleshkevitch, E. A., Hunt, M. E., Beltran-Ramirez, V., Miller, D. J., Wiedenmann, J., Salih, A., & Matz, M. V. (2008). Diversity and evolution of coral fluorescent proteins. *PloS One*, 3(7), e2680.
<https://doi.org/10.1371/journal.pone.0002680>
- Angelo, D., Denzel, C., Vogt, A., Matz, A., Oswald, M. V., Salih, F., & Wiedenmann. (2008). Blue light regulation of host pigment in reef-building corals. *Marine Ecology Progress Series*, 364, 97–106.
- Apprill, A., Girdhar, Y., Mooney, T. A., Hansel, C. M., Long, M. H., Liu, Y., Zhang, W. G., Kapit, J., Huguen, K., Coogan, J., & Greene, A. (2023). Toward a New Era of coral reef monitoring. *Environmental Science & Technology*, 57(13), 5117–5124.
<https://doi.org/10.1021/acs.est.2c05369>
- Aronson, R. B., Precht, W. F., Macintyre, I. G., & Toth, L. T. (2012). Catastrophe and the life span of coral reefs. *Ecology*, 93(2), 303–313. <https://doi.org/10.1890/11-1037.1>
- Aronson, R., Cimino, J., Gambi, R., & Snelgrove, C. (2021). Van Dover Marine ecosystem restoration in a changing ocean. *Restor Ecol*, 29(S2).
- Bay, L. K., Ulstrup, K. E., Nielsen, H. B., Jarmer, H., Goffard, N., Willis, B. L., Miller, D. J., & Van Oppen, M. J. H. (2009). Microarray analysis reveals transcriptional plasticity in the reef building coral *Acropora millepora*. *Molecular Ecology*, 18(14), 3062–3075.
<https://doi.org/10.1111/j.1365-294X.2009.04257.x>
- Beger, M., Selkoe, K. A., Treml, E., Barber, P. H., von der Heyden, S., Crandall, E. D., Toonen, R. J., & Riginos, C. (2014). Evolving coral reef conservation with genetic information. *Bulletin of marine science*, 90(1), 159–185.
<https://doi.org/10.5343/bms.2012.1106>
- Ben-Zvi, O., Lindemann, Y., Eyal, G., & Loya, Y. (2022). Coral fluorescence: a prey- lure in deep habitats. *Communications Biology*, 5(1), 1–8.

- Ben-Zvi, Or, Eyal, G., & Loya, Y. (2019). Response of fluorescence morphs of the mesophotic coral *Euphyllia paradivisa* to ultra-violet radiation. *Scientific Reports*, 9(1), 5245. <https://doi.org/10.1038/s41598-019-41710-3>
- Ben-Zvi, Or, Wangpraseurt, D., Bronstein, O., Eyal, G., & Loya, Y. (2021). Photosynthesis and bio-optical properties of fluorescent mesophotic corals. *Frontiers in marine science*, 8. <https://doi.org/10.3389/fmars.2021.651601>
- Berumen, M. L., & Pratchett, M. S. (2006). Recovery without resilience: persistent disturbance and long-term shifts in the structure of fish and coral communities at Tiahura Reef, Moorea. *Coral Reefs*, 25(4), 647–653. <https://doi.org/10.1007/s00338-006-0145-2>
- Bollati, E., D'Angelo, C., Alderdice, R., Pratchett, M., Ziegler, M., & Wiedenmann, J. (2020). Optical feedback loop involving dinoflagellate symbiont and scleractinian host drives colorful coral bleaching. *Current Biology: CB*, 30(13), 2433-2445.e3. <https://doi.org/10.1016/j.cub.2020.04.055>
- Bollati, E., Plimmer, D., D'Angelo, C., & Wiedenmann, J. (2017). FRET-mediated long-range wavelength transformation by photoconvertible fluorescent proteins as an efficient mechanism to generate orange-red light in symbiotic Deep Water corals. *International Journal of Molecular Sciences*, 18(7), 1174. <https://doi.org/10.3390/ijms18071174>
- Bongaerts, P., Frade, P. R., Hay, K. B., Englebert, N., Latijnhouwers, K. R. W., Bak, R. P. M., Vermeij, M. J. A., & Hoegh-Guldberg, O. (2015). Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coral-endosymbiont community. *Scientific Reports*, 5(1), 7652. <https://doi.org/10.1038/srep07652>
- Bou-Abdallah, F., Chasteen, N. D., & Lesser, M. P. (2006). Quenching of superoxide radicals by green fluorescent protein. *Biochimica et Biophysica Acta*, 1760(11), 1690–1695. <https://doi.org/10.1016/j.bbagen.2006.08.014>
- Brown, B. E., Dunne, R. P., Warner, M. E., Ambarsari, I., Fitt, W. K., Gibb, S. W., & Cummings, D. G. (2000). Damage and recovery of Photosystem II during a manipulative field experiment on solar bleaching in the coral *Goniastrea aspera*. *Marine ecology progress series*, 195, 117–124. <https://doi.org/10.3354/meps195117>
- Carballo-Bolaños, R., Denis, V., Huang, Y.-Y., Keshavmurthy, S., & Chen, C. A. (2019). Temporal variation and photochemical efficiency of species in Symbiodinaceae associated with coral *Leptoria phrygia* (Scleractinia; Merulinidae) exposed to contrasting temperature regimes. *PloS One*, 14(6), e0218801. <https://doi.org/10.1371/journal.pone.0218801>
- Caspers, H. (1979). Helmut Schuhmacher: Korallenriffe. Ihre Verbreitung, Tierwelt und Ökologie. – 205 Fotos (127 farbig), 28 Zeichnungen, 275 S. München – Bern – Wien: BLV Verlagsgesellschaft 1976. ISBN 3-405-11448-8. DM 28,–. *Internationale Revue Der Gesamten Hydrobiologie*, 64(4), 500–500. <https://doi.org/10.1002/iroh.19790640412>
- Characteristics of the light field in highly turbid waters: A Monte Carlo study* JT Kirk *Limnology and Oceanography*. (1994). Wiley Online Library.
- Coffroth, M. A., & Santos, S. R. (2005). Genetic diversity of symbiotic dinoflagellates in the genus *Symbiodinium*. *Protist*, 156(1), 19–34. <https://doi.org/10.1016/j.protis.2005.02.004>

- Coles, S. L., & Brown, B. E. (2003). Coral bleaching--capacity for acclimatization and adaptation. *Advances in Marine Biology*, 46, 183–223. [https://doi.org/10.1016/s0065-2881\(03\)46004-5](https://doi.org/10.1016/s0065-2881(03)46004-5)
- Da Ros, Z., Dell'Anno, A., Morato, T., Sweetman, A. K., Carreiro-Silva, M., Smith, C. J., Papadopoulou, N., Corinaldesi, C., Bianchelli, S., Gambi, C., Cimino, R., Snelgrove, P., Van Dover, C. L., & Danovaro, R. (2019). The deep sea: The new frontier for ecological restoration. *Marine Policy*, 108(103642), 103642. <https://doi.org/10.1016/j.marpol.2019.103642>
- D'angelo, C. D. (2012). Locally accelerated growth is part of the innate immune response and repair mechanisms in reef-building corals as detected by green fluorescent protein (GFP)-like pigments. *Coral Reefs*, 31, 1045–1056.
- D'Angelo, C., & Wiedenmann, J. (2014). Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival. *Current opinion in environmental sustainability*, 7, 82–93. <https://doi.org/10.1016/j.cosust.2013.11.029>
- Dela Cruz, D., & Harrison, P. L. (2022). *Methods for restoring damaged reefs using coral larval restoration*. Southern Cross University. <https://doi.org/10.25918/REPORT.233>
- DeSalvo, M. K., Voolstra, C. R., Sunagawa, S., Schwarz, J. A., Stillman, J. H., Coffroth, M. A., Szmant, A. M., & Medina, M. (2008). Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. *Molecular Ecology*, 17(17), 3952–3971. <https://doi.org/10.1111/j.1365-294X.2008.03879.x>
- Dizon, E. G. S., Da-Anoy, J. P., Roth, M. S., & Conaco, C. (2021). Fluorescent protein expression in temperature tolerant and susceptible reef-building corals. *Journal of the Marine Biological Association of the United Kingdom*. *Marine Biological Association of the United Kingdom*, 101(1), 71–80. <https://doi.org/10.1017/s0025315421000059>
- Douglas, A. E. (2003). Coral bleaching--how and why? *Marine Pollution Bulletin*, 46(4), 385–392. [https://doi.org/10.1016/S0025-326X\(03\)00037-7](https://doi.org/10.1016/S0025-326X(03)00037-7)
- Dove, S. G., Hoegh-Guldberg, O., & Ranganathan, S. (2001). Major colour patterns of reef-building corals are due to a family of GFP-like proteins. *Coral Reefs*, 19(3), 197–204. <https://doi.org/10.1007/pl00006956>
- Dove, Sophie G., Lovell, C., Fine, M., Deckenback, J., Hoegh-Guldberg, O., Iglesias-Prieto, R., & Anthony, K. R. N. (2008). Host pigments: potential facilitators of photosynthesis in coral symbioses. *Plant, Cell & Environment*, 31(11), 1523–1533. <https://doi.org/10.1111/j.1365-3040.2008.01852.x>
- Downs, C. A., Fauth, J. E., Robinson, C. E., Curry, R., Lanzendorf, B., Halas, J. C., Halas, J., & Woodley, C. M. (2005). Cellular diagnostics and coral health: declining coral health in the Florida Keys. *Marine Pollution Bulletin*, 51(5–7), 558–569. <https://doi.org/10.1016/j.marpolbul.2005.04.017>
- Einbinder, S., Gruber, D. F., Salomon, E., Liran, O., Keren, N., & Tchernov, D. (2016). Novel adaptive photosynthetic characteristics of mesophotic symbiotic microalgae within the reefbuilding coral, *Stylophora pistillata*. *Frontiers in Marine Science*, 3.
- Erratum: Fluorescent proteins from nonbioluminescent Anthozoa species. (1999). *Nature Biotechnology*, 17(12), 1227–1227. <https://doi.org/10.1038/70787>

- Ferreira, G., Bollati, E., & Köhl, M. (2023). The role of host pigments in coral photobiology. *Frontiers in marine science*, 10. <https://doi.org/10.3389/fmars.2023.1204843>
- Ferse, S. C. A., Hein, M. Y., & Rölfer, L. (2021). A survey of current trends and suggested future directions in coral transplantation for reef restoration. *PloS One*, 16(5), e0249966. <https://doi.org/10.1371/journal.pone.0249966>
- Field, S. F., Bulina, M. Y., Kelmanson, I. V., Bielawski, J. P., & Matz, M. V. (2006). Adaptive evolution of multicolored fluorescent proteins in reef-building corals. *Journal of Molecular Evolution*, 62(3), 332–339. <https://doi.org/10.1007/s00239-005-0129-9>
- Fox, H. E., Mous, P. J., Pet, J. S., Muljadi, A. H., & Caldwell, R. L. (2005). Experimental assessment of coral reef rehabilitation following blast fishing. *Conservation Biology: The Journal of the Society for Conservation Biology*, 19(1), 98–107. <https://doi.org/10.1111/j.1523-1739.2005.00261.x>
- Frade, P. R., Englebert, N., Faria, J., Visser, P. M., & Bak, R. P. M. (2008). Distribution and photobiology of Symbiodinium types in different light environments for three colour morphs of the coral *Madracis pharensis*: is there more to it than total irradiance? *Coral Reefs*, 27(4), 913–925. <https://doi.org/10.1007/s00338-008-0406-3>
- Frade, P. R., Reyes-Nivia, M. C., Faria, J., Kaandorp, J. A., Luttikhuisen, P. C., & Bak, R. P. M. (2010). Semi-permeable species boundaries in the coral genus *Madracis*: introgression in a brooding coral system. *Molecular Phylogenetics and Evolution*, 57(3), 1072–1090. <https://doi.org/10.1016/j.ympev.2010.09.010>
- Frade, Pedro R., Bongaerts, P., Englebert, N., Rogers, A., Gonzalez-Rivero, M., & Hoegh-Guldberg, O. (2018). Deep reefs of the Great Barrier Reef offer limited thermal refuge during mass coral bleaching. *Nature Communications*, 9(1), 3447. <https://doi.org/10.1038/s41467-018-05741-0>
- Franceschini, S., Meier, A. C., Suan, A., Stokes, K., Roy, S., & Madin, E. M. P. (2023). A deep learning model for measuring coral reef halos globally from multispectral satellite imagery. *Remote Sensing of Environment*, 292(113584), 113584. <https://doi.org/10.1016/j.rse.2023.113584>
- Gittins, J. R., D'Angelo, C., Oswald, F., Edwards, R. J., & Wiedenmann, J. (2015). Fluorescent protein-mediated colour polymorphism in reef corals: multicopy genes extend the adaptation/acclimatization potential to variable light environments. *Molecular Ecology*, 24(2), 453–465. <https://doi.org/10.1111/mec.13041>
- Goatley, C. H. R., & Bellwood, D. R. (2013). Ecological consequences of sediment on high-energy coral reefs. *PloS One*, 8(10), e77737. <https://doi.org/10.1371/journal.pone.0077737>
- Good, A. M., & Bahr, K. D. (2021). The coral conservation crisis: interacting local and global stressors reduce reef resiliency and create challenges for conservation solutions. *SN Applied Sciences*, 3(3). <https://doi.org/10.1007/s42452-021-04319-8>
- Hagedorn, M., Carter, V. L., Henley, E. M., van Oppen, M. J. H., Hobbs, R., & Spindler, R. E. (2017). Producing coral offspring with cryopreserved sperm: A tool for coral reef restoration. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-14644-x>

- Hein, M., Mcleod, I., Shaver, E., Tali, Mohamed, A. B., Einarsson, L., & Grimsditch, G. (2021). *CORAL REEF RESTORATION TO IMPROVE ECOSYSTEM SERVICES- POLICY BRIEF*.
- Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Marine & Freshwater Research*, 50(8), 839. <https://doi.org/10.1071/mf99078>
- Hoegh-Guldberg, O., Poloczanska, E. S., Skirving, W., & Dove, S. (2017). Coral reef ecosystems under climate change and ocean acidification. *Frontiers in marine science*, 4. <https://doi.org/10.3389/fmars.2017.00158>
- Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J. B. C., Kleypas, J., Lough, J. M., Marshall, P., Nyström, M., Palumbi, S. R., Pandolfi, J. M., Rosen, B., & Roughgarden, J. (2003). Climate change, human impacts, and the resilience of coral reefs. *Science (New York, N.Y.)*, 301(5635), 929–933. <https://doi.org/10.1126/science.1085046>
- Hughes, Terry P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., Babcock, R. C., Beger, M., Bellwood, D. R., Berkelmans, R., Bridge, T. C., Butler, I. R., Byrne, M., Cantin, N. E., Comeau, S., Connolly, S. R., Cumming, G. S., Dalton, S. J., Diaz-Pulido, G., ... Wilson, S. K. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, 543(7645), 373–377. <https://doi.org/10.1038/nature21707>
- Important Coral-Reef Builders Threatened by Pollution December 1995AMBIO A. (1995). *A Journal of the Human Environment*, 24(7), 502–505.
- Jones, H. P., Jones, P. C., Barbier, E. B., Blackburn, R. C., Rey Benayas, J. M., Holl, K. D., McCrackin, M., Meli, P., Montoya, D., & Mateos, D. M. (2018). Restoration and repair of Earth's damaged ecosystems. *Proceedings. Biological Sciences*, 285(1873), 20172577. <https://doi.org/10.1098/rspb.2017.2577>
- Kahng, S. E., Hochberg, E. J., Apprill, A., Wagner, D., Luck, D. G., Perez, D., & Bidigare, R. R. (2012). Efficient light harvesting in deep-water zooxanthellate corals. *Marine Ecology Progress Series*, 455, 65–77. <https://doi.org/10.3354/meps09657>
- Kawaguti, S. (1944). On the physiology of reef corals. VI. Study of the pigments. *Palao Trop Biol Stn Stud*, 2, 617–674.
- Kirk, J. T. O. (1975). A theoretical analysis of the contribution of algal cells to the attenuation of light within natural waters ii. Spherical cells. *The New Phytologist*, 75(1), 21–36. <https://doi.org/10.1111/j.1469-8137.1975.tb01367.x>
- Leeworthy, V. R., Maher, T., & Stone, E. A. (2006). A. Source: *Bulletin of Marine Science*, 78(1), 29–38.
- Lesser, M. P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. *Annual Review of Physiology*, 68(1), 253–278. <https://doi.org/10.1146/annurev.physiol.68.040104.110001>
- Leutenegger, A., D'Angelo, C., Matz, M. V., Denzel, A., Oswald, F., Salih, A., Nienhaus, G. U., & Wiedenmann, J. (2007). It's cheap to be colorful. Anthozoans show a slow turnover of GFP-like proteins: Anthozoans show a slow turnover of GFP-like proteins. *The FEBS Journal*, 274(10), 2496–2505. <https://doi.org/10.1111/j.1742-4658.2007.05785.x>

- Madin, E. M. P., Darling, E. S., & Hardt, M. J. (2019). Emerging technologies and coral reef conservation: Opportunities, challenges, and moving forward. *Frontiers in marine science*, 6. <https://doi.org/10.3389/fmars.2019.00727>
- Manzello, D., Warner, M., Stabenau, E., Hendee, J., Lesser, M., & Jankulak, M. (2009). Remote monitoring of chlorophyll fluorescence in two reef corals during the 2005 bleaching event at Lee Stocking Island, Bahamas. *Coral Reefs*, 28(1), 209–214. <https://doi.org/10.1007/s00338-008-0455-7>
- Mass, T., Einbinder, S., Brokovich, E., Shashar, N., Vago, R., Erez, J., & Dubinsky, Z. (2007). Photoacclimation of *Stylophora pistillata* to light extremes: metabolism and calcification. *Marine ecology progress series*, 334, 93–102. <https://doi.org/10.3354/meps334093>
- Mass, T., Kline, D. I., Roopin, M., Veal, C. J., Cohen, S., Iluz, D., & Levy, O. (2010). The spectral quality of light is a key driver of photosynthesis and photoadaptation in *Stylophora pistillata* colonies from different depths in the Red Sea. *The Journal of Experimental Biology*, 213(Pt 23), 4084–4091. <https://doi.org/10.1242/jeb.039891>
- Matz, M. V., Fradkov, A. F., Labas, Y. A., Savitsky, A. P., Zarausky, A. G., Markelov, M. L., & Lukyanov, S. A. (1999). Fluorescent proteins from nonbioluminescent Anthozoa species. *Nature Biotechnology*, 17(10), 969–973. <https://doi.org/10.1038/13657>
- Matz, Mikhail V., Marshall, N. J., & Vorobyev, M. (2006). Are corals colorful? *Photochemistry and Photobiology*, 82(2), 345–350. <https://doi.org/10.1562/2005-08-18-RA-653>
- Mazel, C. H. (1995). Spectral measurements of fluorescence emission in Caribbean cnidarians. *Marine ecology progress series*, 120, 185–191. <https://doi.org/10.3354/meps120185>
- Muscatine, L., R. McCloskey, L., & E. Marian, R. (1981). Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration1: Muscatine et al. *Limnology and oceanography*, 26(4), 601–611. <https://doi.org/10.4319/lo.1981.26.4.0601>
- Nagelkerken, I., van der Velde, G., Gorissen, M. W., Meijer, G. J., Van't Hof, T., & den Hartog, C. (2000). Importance of mangroves, seagrass beds and the shallow coral reef as a nursery for important coral reef fishes, using a visual census technique. *Estuarine, Coastal and Shelf Science*, 51(1), 31–44. <https://doi.org/10.1006/ecss.2000.0617>
- Nakaema, S., & Hidaka, M. (2015). Fluorescent protein content and stress tolerance of two color morphs of the coral *Galaxea fascicularis*. *Galaxea*, 17(1), 1–11. <https://doi.org/10.3755/galaxea.17.1>
- Obura, D. O., Aeby, G., Amornthammarong, N., Appeltans, W., Bax, N., Bishop, J., Brainard, R. E., Chan, S., Fletcher, P., Gordon, T. A. C., Gramer, L., Gudka, M., Halas, J., Hendee, J., Hodgson, G., Huang, D., Jankulak, M., Jones, A., Kimura, T., ... Wongbusarakum, S. (2019). Coral reef monitoring, reef assessment technologies, and ecosystem-based management. *Frontiers in marine science*, 6. <https://doi.org/10.3389/fmars.2019.00580>
- Osborne, B. A., & Kirk, J. T. O. (1995). Light and photosynthesis in aquatic ecosystems. *The journal of applied ecology*, 32(2), 448. <https://doi.org/10.2307/2405114>

- Palmer, C. V., Roth, M. S., & Gates, R. D. (2009). Red fluorescent protein responsible for pigmentation in trematode-infected *Porites compressa* tissues. *The Biological Bulletin*, 216(1), 68–74. <https://doi.org/10.1086/BBLv216n1p68>
- Polak, O., & Shashar, N. (2012). Can a small artificial reef reduce diving pressure from a natural coral reef? Lessons learned from Eilat, Red Sea. *Ocean & Coastal Management*, 55, 94–100. <https://doi.org/10.1016/j.ocecoaman.2011.10.006>
- Prouty, N. G., Cohen, A., Yates, K. K., Storlazzi, C. D., Swarzenski, P. W., & White, D. (2017). Vulnerability of coral reefs to bioerosion from land-based sources of pollution: Coral reef vulnerability. *Journal of Geophysical Research. Oceans*, 122(12), 9319–9331. <https://doi.org/10.1002/2017jc013264>
- Raymundo, L. J., Maypa, A. P., Gomez, E. D., & Cadiz, P. (2007). Can dynamite-blasted reefs recover? A novel, low-tech approach to stimulating natural recovery in fish and coral populations. *Marine Pollution Bulletin*, 54(7), 1009–1019. <https://doi.org/10.1016/j.marpolbul.2007.02.006>
- Reimer, J. D., Peixoto, R. S., Davies, S. W., Traylor-Knowles, N., Short, M. L., Cabral-Tena, R. A., Burt, J. A., Pessoa, I., Banaszak, A. T., Winters, R. S., Moore, T., Schoepf, V., Kaullysing, D., Calderon-Aguilera, L. E., Wörheide, G., Harding, S., Munbodhe, V., Mayfield, A., Ainsworth, T., Voolstra, C. R. (2024). The Fourth Global Coral Bleaching Event: Where do we go from here? *Coral Reefs*, 43(4), 1121–1125. <https://doi.org/10.1007/s00338-024-02504-w>
- Richmond, R. H. (1993). Coral reefs: Present problems and future concerns resulting from anthropogenic disturbance. *American zoologist*, 33(6), 524–536. <https://doi.org/10.1093/icb/33.6.524>
- Rosenberg, E., & Loya, Y. (Hrsg.). (2014). *Coral health and disease*. Springer.
- Roth, M. S., & Deheyn, D. D. (2013). Effects of cold stress and heat stress on coral fluorescence in reef-building corals. *Scientific Reports*, 3(1), 1421. <https://doi.org/10.1038/srep01421>
- Roth, M. S., Latz, M. I., Goericke, R., & Deheyn, D. D. (2010). Green fluorescent protein regulation in the coral *Acropora yongei* during photoacclimation. *The Journal of Experimental Biology*, 213(Pt 21), 3644–3655. <https://doi.org/10.1242/jeb.040881>
- Salih, A., Larkum, A., Cox, G., Kühl, M., & Hoegh-Guldberg, O. (2000). Fluorescent pigments in corals are photoprotective. *Nature*, 408(6814), 850–853. <https://doi.org/10.1038/35048564>
- Salvat, B. (1992). Coral reefs — a challenging ecosystem for human societies. *Global Environmental Change: Human and Policy Dimensions*, 2(1), 12–18. [https://doi.org/10.1016/0959-3780\(92\)90032-3](https://doi.org/10.1016/0959-3780(92)90032-3)
- Scholar Leutenegger, G. (2007). It's cheap to be colorful - Anthozoans show a slow turnover of GFP-like proteins. *FEBS J*, 274, 2496–2505.
- Schuhmacher, H. (1976). *Korallenriffe: ihre Verbreitung, Tier- welt und Oekologie*. BLV.
- Schwarz, A.-M., & Hellblom, F. (2002). The photosynthetic light response of *Halophila stipulacea* growing along a depth gradient in the Gulf of Aqaba, the Red Sea. *Aquatic Botany*, 74(3), 263–272. [https://doi.org/10.1016/s0304-3770\(02\)00080-3](https://doi.org/10.1016/s0304-3770(02)00080-3)

- Shantz, A. A., Ladd, M. C., & Burkepile, D. E. (2020). Overfishing and the ecological impacts of extirpating large parrotfish from Caribbean coral reefs. *Ecological Monographs*, 90(2). <https://doi.org/10.1002/ecm.1403>
- Sheppard, C., Dixon, D. J., Gourlay, M., Sheppard, A., & Payet, R. (2005). Coral mortality increases wave energy reaching shores protected by reef flats: Examples from the Seychelles. *Estuarine, Coastal and Shelf Science*, 64(2–3), 223–234. <https://doi.org/10.1016/j.ecss.2005.02.016>
- Shimomura, O., Johnson, F. H., & Saiga, Y. (1962). Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *Journal of Cellular and Comparative Physiology*, 59(3), 223–239. <https://doi.org/10.1002/jcp.1030590302>
- Smith, E. G., D'Angelo, C., Sharon, Y., Tchernov, D., & Wiedenmann, J. (2017). Acclimatization of symbiotic corals to mesophotic light environments through wavelength transformation by fluorescent protein pigments. *Proceedings. Biological Sciences*, 284(1858). <https://doi.org/10.1098/rspb.2017.0320>
- Sparks, J. S., Schelly, R. C., Smith, W. L., Davis, M. P., Tchernov, D., Pieribone, V. A., & Gruber, D. F. (2014). The covert world of fish biofluorescence: a phylogenetically widespread and phenotypically variable phenomenon. *PloS One*, 9(1), e83259. <https://doi.org/10.1371/journal.pone.0083259>
- Sully, S., Burkepile, D. E., Donovan, M. K., Hodgson, G., & van Woesik, R. (2019). A global analysis of coral bleaching over the past two decades. *Nature Communications*, 10(1), 1264. <https://doi.org/10.1038/s41467-019-09238-2>
- Teixeira, J. C., & Huber, C. D. (2021). The inflated significance of neutral genetic diversity in conservation genetics. *Proceedings of the National Academy of Sciences of the United States of America*, 118(10), e2015096118. <https://doi.org/10.1073/pnas.2015096118>
- Trench, R. K. (1993). Microalgal-Invertebrate Symbioses: A Review. *Endocytobiosis Cell Res*, 9, 135–175.
- Vermeij, M. J. A., & Bak, R. P. M. (2002). How are coral populations structured by light? Marine light regimes and the distribution of *Madracis*. *Marine ecology progress series*, 233, 105–116. <https://doi.org/10.3354/meps233105>
- Veron, J. (2000). *Corals of the world (3 vols)*. Australian Institute of Marine Science.
- Voolstra, C. R. (2020). Coral bleaching: A colorful struggle for survival. *Current Biology: CB*, 30(13), R768–R770. <https://doi.org/10.1016/j.cub.2020.05.008>
- Voolstra, C. R., Buitrago-López, C., Perna, G., Cárdenas, A., Hume, B. C. C., Rädicker, N., & Barshis, D. J. (2020). Standardized short-term acute heat stress assays resolve historical differences in coral thermotolerance across microhabitat reef sites. *Global Change Biology*, 26(8), 4328–4343. <https://doi.org/10.1111/gcb.15148>
- Wangpraseurt, D., Larkum, A. W. D., Ralph, P. J., & Kühl, M. (2012). Light gradients and optical microniches in coral tissues. *Frontiers in Microbiology*, 3, 316. <https://doi.org/10.3389/fmicb.2012.00316>
- Warner, M. E., Lesser, M. P., & Ralph, P. J. (2010). *Chlorophyll fluorescence in aquatic sciences: Methods and applications* (D. J. Suggett & O. M. A. Prasil, Hrsg.). Springer.

Wells, J. W. (1973). New and old scleractinian corals from Jamaica. *Bulletin of Marine Science*, 23(1), 16–55.

Wiedenmann, J., D'Angelo, C., Smith, E. G., Hunt, A. N., Legiret, F.-E., Postle, A. D., & Achterberg, E. P. (2013). Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, 3(2), 160–164. <https://doi.org/10.1038/nclimate1661>

Wooldridge, S. A. (2009). Water quality and coral bleaching thresholds: formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. *Marine Pollution Bulletin*, 58(5), 745–751. <https://doi.org/10.1016/j.marpolbul.2008.12.013>

Zaneveld, J. R., Burkepile, D. E., Shantz, A. A., Pritchard, C. E., McMinds, R., Payet, J. P., Welsh, R., Correa, A. M. S., Lemoine, N. P., Rosales, S., Fuchs, C., Maynard, J. A., & Thurber, R. V. (2016). Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nature Communications*, 7, 11833. <https://doi.org/10.1038/ncomms11833>

Zhao, H., Yuan, M., Stokal, M., Wu, H. C., Liu, X., Murk, A., Kroeze, C., & Osinga, R. (2021). Impacts of nitrogen pollution on corals in the context of global climate change and potential strategies to conserve coral reefs. *The Science of the Total Environment*, 774(145017), 145017. <https://doi.org/10.1016/j.scitotenv.2021.145017>