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New insights into coral reef threats and restoration perception: a case study in the Republic of Maldives

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Abstract

Coral reef is one of the most diverse ecosystem on Earth, yet one of the most exposed to natural and anthropogenic disturbances. Threats to coral reefs include macro and micro-plastic, global warming and coral predators. Understanding the dynamics of coral reef and the interactions with anthropogenic activities is fundamental to prevent the loss of the reef biodiversity. Such priority is essential for isolated and remote areas, such as the Republic of Maldives, which is highly sensitive to changes in environmental conditions, yet, barely investigated. Thus, this study explores a trio of interrelated topics: impacts of corallivory, anthropogenic marine debris (AMD) and coral restoration perception. The main focus regards the ecology and impacts of three corallivores on maldivian coral reef. The thesis then deal with a novel approach to quantify and detect AMD accumulating on maldivian shore and, at the end, the restoration topic is addressed through the perception of reef users. Specifically, we investigated the population structure, feeding preferences, distribution and density variation of the three major corallivores occurring in the Maldives (Acanthaster planci, Culcita spp. and Drupella spp.). The results highlighted the role of corallivores in decreasing coral cover by direct predation and co-occurrence with temperature-induced coral mortality. Especially for A. planci which showed a severe outbreak. While Culcita spp. showed a specific possible role in delaying coral recovery, since resulted to focus predatory pressure on coral recruits. Drupella spp. showed a population collapse compared to previous studies in Maldives, reducing the predatory pressure during the recovery phase of the reef. Thus, the interaction between corallivores and anthropogenic disturbances intesify mortality of corals. Yet, not all disturbances are known, often for lacking of standard methodology for quantification. Among those, a further disturb not known in details is represented by AMD. The purpose here was to develop an effective and time-saving method to automatically quantify AMD using a commercial Unmanned Aerial Vehicle (UAV) and Artificial Intelligence (AI) with a deep-learning based software (PlasticFinder). The high data resolution (0.5 cm/pix) allowed to detect more than 87% of AMD on the shores and the analysis from PlasticFinder reached a Positive Predictive Value of 94%, which overcame the limits highlighted in previous AI algorithm used in the literature. The use of drone resulted in a time saving tool to survey AMD accumulation even in remote areas such as uninhabited islands. The degradation of the maldivian coral reef caused by anthropogenic and natural disturbances is a trigger for active strategies to enhance recovery through coral restoration. However, assessment tools of restoration effectiveness and development are still restricted to few ecological factors, such as coral survival and growth. Very few studies included factors such as socio-economic assessment. The involvement of the community of reef users is as important as factors related directly to corals, since users' satisfaction may drive allocation of funding to improve restoration success. Results revealed weak points and ways to improve coral restoration programs management in two resorts in the Maldives. The use of satisfaction analysis revealed the need for intervention dedicated to active restoration rather than artificial reef. Further, women and Maldivians resulted to be the most willing to be involved in coral restoration project. Thus, suggesting that training session by the resort may be organized for such categories to build team of volunteers for improving local restoration programs. In conclusion, this work provide a baseline for future research on the ecology of coral predators and, additionally, for the understanding of further anthropogenic disturbances and for improving strategies to prevent the loss of maldivian coral reef biodiversity.

Riassunto

L'ecosistema delle scogliere coralline presenta una elevatissima diversità di specie, tuttavia è anche esposto a numerosi disturbi naturali ed antropici. Le minacce includono macro e micro-plastica, riscaldamento globale e predazione. La comprensione delle dinamiche ecologiche della scogliera corallina e le interazioni con attività umane è di primario interesse per evitare la perdita di biodiversità. Questa priorità è fondamentale per aree isolate e remote come la Repubblica delle Maldive, che risulta essere sensibile ai cambiamenti ambientali, anche se è un'area ancora poco investigata. Perciò, questo studio intende esplorare tre argomenti inter-connessi: l'impatto di predatori del corallo, rifiuti marini di orgine antropica e percezione del restauro delle scogliere coralline. L'obiettivo principale riguarda l'ecologia di tre predatori di corallo e il loro impatto sulla scogliera corallina maldiviana. La Tesi tratta poi un nuovo approccio nel quantificare rifiuti sulle spiagge e, alla fine, l'importanza della restaurazione corallina è affrontato attraverso l'analisi della percezione degli utenti della scogliera stessa. In particolare, la struttura di popolazione, le preferenze alimentari e le variazioni di densità dei tre corallivori principali delle Maldive (Acanthaster planci, Culcita spp. and Drupella spp.) sono stati studiati. Questi corallivori hanno un ruolo nel deteriorare la copertura di corallo tramite predazione diretta e interazione con altri disturbi di origine antropica come l'aumento delle temperature delle acque. Ciò è specialmente valido per A. planci che ha sviluppato un'esplosione demografica nell'area di studio intensificando la pressione predatoria. Mentre Culcita spp. ha mostrato un ruolo potenzialmente specifico nel rallentare il recupero della scogliera corallina, poiché focalizza la predazione sulle reclute dei coralli. Al contratio, Drupella spp. ha mostrato un collasso della popolazione dopo un evento di mortalità dei coralli, riducendo la pressione predatoria senza apparentemente influenzare la fase di recupero. L'interazione tra corallivoria e minacce di origine antropica intensifica la mortalità dei coralli. Tuttavia non tutti i disturbi antropici sono conosciuti, spesso per mancanza di metodologie standard per quantificarli. Una minaccia aggiuntiva non ancora studiata in dettaglio è rappresentata dai detriti marini antropici (DMA). Perciò, un ulteriore obiettivo è stato quello di sviluppare un metodo efficace e rapido per monitorare e quantificare DMA sulle spiagge usando un drone e un sistema di intelligenza artificiale (IA) basato su un programma di apprendimento artificiale (PlasticFinder). L'alta risoluzione (0.5 cm/pix) dei dati ha permesso di rilevare l' 87% degli oggetti presenti sulla spiaggia rappresentando uno strumento utile a monitorare rapidamente il tasso di accumulo di DMA anche in aree remote e disabitate. Negl'ultimi decenni le scogliere coralline hanno subito una elevata perdita di corallo vivo spingendo allo sviluppo di tecniche per il restauro della scogliera corallina. Tuttavia, la valutazione dell'efficacia delle strategie di restauro sono limitate a pochi fattori, come sopravvivenza e crescita del corallo. Il coinvolgimento delle comunità di utenti delle scogliere coralline è stato dimostrato essere fondamentale. L'analisi della soddisfazione degli utenti ha rivelato il bisogno di intervenire con attività di restauro attivo invece che con l'uso di strutture artificiali. Inoltre, donne e locali si sono dimostrati i più interessati a dedicarsi a progetti di restauro, suggerendo che l'investimento nella formazione di queste categorie può risultare in una maggiore efficienza dell'attività di restauro. In conclusione, questo lavoro può rappresentare un utile spunto per futuri studi sull'ecologia dei corallivori, e in più, per la comprensione di ulteriori impatti e strategie per salvaguardare la biodiversità delle scogliere coralline maldiviane.

- CHAPTER 1 -

1.1 General Introduction

1.1.2 The Republic of Maldives

The Republic of Maldives is composed of coral reefs and reefs islands representing one of the most diverse ecosystem on the planet. It consists of a double chain structure located in the middle of the Indian Ocean and centered upon the Laccadive-Maldives-Chagos ridge. The Maldivian archipelago stretches along 860 km from 7°6'35"N to 0°42'24"S laying between 72°33'19"E and 73°46'13"E. The chain structure is divided in 26 geographical regions (20 administrative atolls) consisting of submerged reefs and around 1192 natural coral islands. The coral islands are generally small, going from 0.1 to 5 km². There are almost 900 uninhabited islands, where urbanization is absent or low, followed by around 200 inhabited islands which accommodate village for local people and the remaining islands are dedicated to tourists.

The Maldives has a tropical humid climate, dominated by two monsoons which alternate annually: the southwest monsoon from May to November and the northeast monsoon from January to March, with December and April being transitional periods (Anderson et al., 2011). The annual mean temperature is 28°C with a maximum of 31°C and a minimum of 24°C. The average annual rainfall of approximately 1994 mm is evenly distributed throughout the year (MEE, 2017). The Maldives cover an area of about 21.596 km² (including land and sea area) of which only 1% is composed by land (Naseer and Hatcher, 2004). The reef area cover around 4493 km² (Naseer and Hatcher, 2004) contributing up to 2.11% of the global reef area (Burke et al., 2011). The Maldivian atolls are composed of lagoons surrounded by reefs with the general feature described by Ciarapica and Passeri (1993) and Bianchi et al. (1997), such as the atoll rim, the oceanward margin and the lagoonward margin. The atolls rim may extend for several kilometers or it can be interrupted by oceanic passes which lead to oceanic water exchange promoting water circulation inside the lagoon. Each atoll is composed by an internal lagoon, in which other formation are located. These formations are circular-shaped reefs with an internal lagoon, patch reefs and islands. The islands are made of sand originated by the skeleton of corals, changing in coral rubble and few corals as the reef edge approach, to the slope where the development of corals is at its maximum.

Maldivian reefs represent an extraordinary reserve of marine biodiversity with around 250 species of scleractinian corals (Pichon and Benzoni, 2007), around 1200 species of fish (Rajasuriya et al., 2004), 23 species of marine mammals (Anderson et al., 2012), about 40 species of sharks (De Maddalena and Galli, 2017) and even more including echinoderms, mollusks, algae, reptiles, seagrasses and other phyla.

The Maldivian reef system and such high biodiversity provide important good and services to the local population including food, shoreline protection and income from recreational activity and tourism business (Burke et al., 2011).

However, the Maldivian reef system is highly exposed to several anthropogenic and natural threats being vulnerable to climate change, land reclamation and the increasing growing space dedicated to construction (Becken et al., 2011).

1.1.3 General Threats

Maldivian reefs are facing several disturbances that during last decades are increasing the degradation of the reef ecosystem (Khan et al., 2002; Jaleel, 2013; Stevens et al., 2019). Climatic anomalies, related to global warming, have triggered extended mass coral mortality event across the Maldives (Morri et al., 2015; Pisapia et al., 2016, Perry and Morgan, 2016). Such events act synergically with natural (storms, tsunami, crown-of-thorns seastar outbreaks, etc.) and anthropogenic (tourism, fishing, coral mining, anchoring, pollution, plastic littering, etc.) disturbances increasing coral mortality at a global scale (Kayal et al., 2012).

Local human induced disturbances are represented by direct exploitation of marine resources such us, professional and recreation fishery, coral and sand mining, damage at small scale, such as anchoring or snorkeling and diving inappropriate behavior (Shareef, 2010).

In the Maldives, corals are mined mainly for buildings and commercial purposes. Massive corals are the principal mined coral growth form, since they constitute perfect and solid bricks (Naseer, 1996). As a consequence, the 3D structure of the reef is lost, followed by a breakdown of diversity of corals and fish, and, further, leaving an unconsolidated substratum, which may trigger the erosion of close-by shorelines and reduce reef resilience by limiting space for recruits of corals settlement

(Brown and Dunne, 1988). Corals are also taken for commercial purposes, sold as jewelry or ornamental objects for tourists (Naseer, 1996). In addition, land reclamation is increasing in the last decades, it is mostly related to touristic buildings, such as resorts (MoT, 2015, 2018), but also to airport, expanding islands territory for agriculture or housing, harbors, etc. resulting in the degradation of natural fundamental habitat such as coral reefs and mangrove areas (Saleem and Nileysha, 2003). Above all, fishery represent the main source of food, employment and trade in the Maldives, although the overfishing pressure is increasing in recent years (Stevens et al., 2019). The fishery sector contributes to 1.3% of the GDP (NBS, 2015) with the tuna fishing being the primary export (Hemmings et al., 2014). The pole-and-line traditional tuna fishing methods has been accredited as sustainable in 2012 by the Marine Stewardship Council (Miller et al., 2017). However, an alarming 31% decline in the overall fish catch was recorded in between 2006 and 2014 pushing the government into a race for developing a proper management plan of the national tuna fishery (Adam 2010; MEE 2015). Other important sources of food and trade are reef fish and grouper fishery, although the pressure from local fishing and recreational touristic demand for fishing excursions is rapidly growing, thus increasing concern on the future survival of these populations (MEE, 2017).

In addition, the Maldives have been considered as one of the most vulnerable and least defensible country to climate change (Becken et al., 2011). In fact, climate change is a direct driven factor of changes in the Maldives through increasing sea surface temperatures, extreme weather conditions and sea level rise putting the biodiversity and local population at serious risk (Stevens et al., 2019). Coral bleaching, induced by increasing temperatures, is the most concerning events recorded in the last decades in the Maldives. Events of coral bleaching have been reported since 1977 (Morri et al., 2015; Ibrahim et al., 2016) with the most destructive being in 1998 dropping coral cover down to less than 3% from a prebleaching 40-60% (Morri et al., 2015). A consistent recover in live coral cover has been recorded by Pisapia et al. (2016) in the aftermath, although a second sever bleaching event took place in 2016. The latest event resulted in a 75% average bleaching across 11 atolls (Ibrahim, 2016; Muir et al., 2017; Perry and Morgan, 2017). Future prospectives appear to be not prosperous by recent modeling, suggesting an increase in frequency and intensity of bleaching events with a risk of delay in coral recovery (Van Hooidonk et al., 2016). Corals are also exposed to

predator's pressure, corallivores are organisms feeding on coral tissue which are normally in a low and sustainable density in a healthy reef (Rotjan and Lewis, 2008). However, the impact of corallivores may be substantial when an outbreak or an event of coral mortality appear, enhancing coral mortality and delaying the recovery. In Maldives, outbreak of the crown-of-thorns seastar (COTs, Acanthaster planci) has been considered one of the major cause of coral mortality (Saponari et al., 2014; Pisapia et al., 2016). In the same context, other corallivores, such as the seastar *Culcita* spp. and the snail *Drupella* spp., increased their pressure causing high mortality in the aftermath of the 2016 coral bleaching event (Bruckner et al., 2017, 2018). Generally, coral reefs are also impacted by plastic debris, one of the most important worldwide threat. The occurrence of plastic debris in the ocean could cause severe impacts on organisms at different trophic levels, both mechanical (Wright et al., 2013) and indirect, represented by harmful substances from microplastic (Browne et al., 2013). The Republic of Maldives is heavily impacted by plastic pollution from local sources and from neighboring countries caused by transportation driven by oceanic currents (MEE, 2017). The plastic debris pollution is scarcely investigated in Maldives (Barnes, 2004; Browne et al., 2011; van Sebille et al., 2015; Imhof et al., 2017; Saliu et al., 2018, 2019) calling for a further studies to clearly define accumulation pattern and impacts on biodiversity.

1.1.4 Coral Bleaching Events

The coral bleaching is a phenomenon triggered by several stressful events including diseases, tidal exposition, turbidity, lack of light, etc. (Glynn, 1993, 1996; Hoegh-Guldberg, 1999; Wilkinson, 2000). However, since 1980s, the world's tropical reefs are severely affected by the increasing of sea temperatures owing to global warming (Hughes et al., 2003; Baker et al., 2008; Spalding et al., 2015; Eakin et al., 2016; Heron et al., 2016). Thermal stress determine the whitening of corals due to the loss of the symbiotic algae and/or their pigments - the primary energy source for most reef corals (Brown, 1997). The prolonged exposition to this condition often leads to high level of coral mortality, loss of reef complexity and dramatic change in community composition (Loya et al., 2001; Stuart-Smith et al., 2018). Up to date, several episodes of bleaching have been reported since 1979, with an unprecedented bleaching event in 1997-1998 (Berkelmans and Oliver, 1999; Hoegh-

Gudlberg, 1999) and recently in 2016 (Perry and Morgan, 2017). Coral mortality was reported from many areas around the world with particularly severe bleaching and high mortality form the Indian Ocean (Sheppard, 1999; McClanahan, 2000; Sully et al., 2019). In the Maldives, the coral bleaching caused from 60 to 100% coral mortality in 1998 (Ciarapica and Passeri, 1999; Longo et al., 2000; Zahir, 2000) and around the 75% reduction in coral cover in 2016 (Ibrahim et al., 2016; Muir et al., 2017; Perry and Morgan, 2017). Branching corals were the most affected, especially those from the genera *Acropora*, *Montipora* and *Pocillopora* while only massive and submassive *Porites*, *Pavona* and *Astreopora* were reported to have survived best becoming dominant species (McClanahan, 2000; Zahir, 2000; Loch et al. 2002, 2004). Coral assemblages showed marked recovery in the aftermath of the 1998 bleaching event with >40% in coral cover in 2015 (Pisapia et al., 2016). After the coral bleaching in 2016, the most severely affected taxa was *Acropora* although even the less susceptible corals exhibited changes in populations structure (Pisapia et al., 2019).

Currently, the remnant coral will grow to affect recovery, although, the very low coral cover level and exposition to several stress including land reclamation, sedimentation and the risk of new bleaching events may substantially delay reef recovery (Pisapia et al., 2019).

1.1.5 Impact of Corallivory

Corallivory is the predation of coral tissue, mucus or skeleton by fish and invertebrates. Coral predators are common in coral reefs and encompass a wide range of taxa including fishes, echinoids, crustaceans, mollusks, and annelids (Rotjan and Lewis, 2008). Corallivores diversity is related to the feeding behaviour and strategies which can be divided in (1) mucus feeders, (2) browsers removing only the tissue, (3) scrapers removing tissue and a tiny layer of skeleton and (4) excavators or bioeroders removing large amount of coral skeleton (Rotjan and Lewis, 2008). Thus, corallivores play an important role in modelling coral community by acting directly or undirectly on coral growth and reproduction (Henry and Hart, 2005; Rotjan and Lewis, 2008). Corallivores may even cause widespread coral mortality as assessed for *Acanthaster* spp. which is considered one of the main reason for the reduction in coral cover in the Australian Great Barrier Reef from 1985 to 2012 (De'ath et al., 2012).

Corallivory may impact coral community dynamics with different direction and intensity of effects (Mumby, 2009). Thus, there is a critical need to understand predators biology and ecology and how they can interact with multiple source of stress in a changing environment era (Rotjan et al., 2008; Rice et al., 2019) to set proper management strategies.

The maldivian archipelago host a great diversity of life included some of the major corallivores, such as *Acanthaster planci* (Ciarapica and Passeri, 1998; Saponari et al., 2014). However, very few information are available on their diversity, biology, ecolgoy and impacts. The maldivian reef has suffered a recent coral bleaching in 2016, which eradicated more than 75% of live coral (Perry and Morgan, 2017). Such a reduction of source of food may represent a constraint for corallivores, with obligate corallivores at risk of local extinction and facultative corallivores obligated to switch to other source of food as documented in other locations (Pratchett et al., 2006; Hoeksema et al., 2013; Brooker et al., 2014; Bruckner et al., 2017, 2018). Three of the major corallivores have been documented in the Maldives being part of the degradation of the reef during and after the 2016 bleaching event. The seastar *Acanthaster* spp. (Echinodermata:Asteroidea), has been reported outbreaking just before the 2016 bleaching event in Ari Atoll (Saponari et al., 2014), while *Culcita schmideliana* (Echinodermata:Asteroidea), and *Drupella* spp (Mollusca:Gastropoda) have been documented to delay the reef recovery (Bruckner et al., 2017, 2018). Further information on the ecology of those species is required to fill the gap and set proper management strategies to lower the risk of reef degradation.

1.1.6 Anthropogenic Marine Debris

One of the most severe environmental contamination is generated by Anthropogenic Marine Debris (AMD) (Ryan, 2015). AMD is responsible of several ecological threats, although, the knowledge on the impact on human and ecosystem health is still poor and it needs to be properly quantified and understood (Thompson et al., 2009). It has been estimated that up to 13 million tonnes of litter enter the ocean each year (Geyer et al., 2017), of which plastic account for over 80% (Penca et al., 2018). Once it is introduced in the ocean it can be transported by surface currents and wind, accumulate by shorelines or degraded into microplastic (Kako et al., 2010, 2014; Cinner et al., 2018). Data showed

the occurrence of plastic everywhere in the ocean, from the surface to the deep seafloor (Van Cauwenberghe et al., 2013). However, the quantification of the total amount within diverse marine compartments has never been accurately studied. Among the compartments, shoreline accumulation is among the less studied, where only few local assessment are available (e.g. Ebbesmeyer et al., 2012; Andrades et al., 2016; Vlachogianni et al., 2018; Martin et al., 2018), but knowing the accumulation rate on beach would be a crucial information to refine global estimation of accumulation and dispersal mechanisms. The biggest problem is the unstandardized methodology which makes difficult data comparisons at spatial and temporal scale (e.g. Watts et al., 2017). Monitoring of beach litter is often time consuming if visual census is used along transects (Lavers and Bond, 2017), although recently, the use of aerial imagery is becoming more appropriate and time saving (Deidun et al., 2018). By combining the use of high quality cameras mounted on Unmanned Aerial Vehicles (UAVs) with Artificial Intelligence (AI) is possible to obtain a performing, less time-labour and automatic quantification of beach litters (Martin et al., 2018). Such methodology may be useful especially in remote and isolated areas, where information are absent or very scarce (Bergmann et al., 2015; van Sebille et al., 2015, Imhof et al., 2017). The Republic of Maldives represent the seventh largest coral reef in the world (Spalding et al., 2001) with an immense diversity of life. However, AMD and microplastic contamination may be a growing problem (Imhof et al., 2017; Saliu et al., 2018, 2019). It has been reported that a considerable amount of plastic is present in maldivian remote island (Imhof et al., 2017), particularly, contamination by microplastic has been documented for surface water, sediment and scleractinian corals (Saliu et al., 2018, 2019). Thus, the impact of AMD should be rapidly elucidated to assess firstly the accumulation pattern and, for a global overview, a standardized methodology should be urgently developed.

1.1.7 Perception of coral restoration

Coral reefs worldwide are at risk of high level of degradation due to anthropogenic and natural source of stress (Hughes et al., 2017). For this reason, efforts to increase reef resilience is growing rapidly to face species extinction and reef death (Grimsditch and Salm, 2006; Young et al. 2012).

Among the various methodologies, the concept of restoration is rapidly developing, especially, in the meaning of rehabilitation, i.e. assisting the recovery of an ecosystem which was damaged or destroyed (SER, 2004). The process consists of outplanting nursery-raised corals to damaged reefs to increase coral population and accelerates recovery at an ecological meanigfull scale (Epstein et al., 2001; Rinkevich, 2005; Young et al., 2012; Miller et al., 2016; Montoya-Maya et al., 2016). However, activities of rehabilitation are still in the infancy of understanding the long-term efficency and often only few parameters are considered, such as coral growth and survival rate (Hein et al., 2017; Ladd et al., 2019). In fact, a review of more than 80 coral restoration programs highlighted the need to expand the evaluation factors including socio-cultural, economic and governance indicators (Hein et al., 2017). These factors are useful to understand the relation between reef health and users satisfaction (Okubo and Onuma, 2015) in order to drive management actions. Such evaluation is particularly usefull for maldivian resorts managment since it can suggest how and where resource may properly be allocated. In Maldives, restoration is at very early stage (Edwards, 2010), with very few reports in literatures and the majority of the information coming from website, technical reports or symposia, calling for a more in depth research and better understanding of the effectivness of restoration efforts.

1.1.8 The aims of the study

Several anthropogenic and natural stressors are degrading coral reef in the Maldives (Pisapia et al., 2016, 2019; Saliu et al., 2019; Stevens et al., 2019). Among those, predation of corals is rapidly emerging as one of the main issue causing high loss of alive coral, primarily in conjunction with other disturbances such as temperature-induced coral mortality (MEE, 2017; Stevens et al., 2019). However, few information are available in the Maldives on the ecology of corallivores calling for a need of studies to clarify interactions and impacts with coral reef dynamics and anthropogenic disturbances. Furthermore, beside the global increasing in surface water temperature caused by the over production of carbon dioxide by human activities (Al-Ghussain, 2019), the production of waste represent one of the biggest environmental problem of the last decades (Filatov et al., 2018). Therefore, anthropogenic marine debris (AMD) may represent a further threats for coral reef by

enhancing the stress on corals in addition to the predation pressure by corallivores. Thus, knowing patterns of accumulation in various marine compartments and interaction with marine organisms it may be possible to better constitute management strategies and aware users for a more sustainable practices. In the Republic of Maldives, the management of AMD is of primary importance due to the dispersive and isolated structure of the country and the presence of tourists which increase the production of waste. In particular there is a need for a standardized monitoring methodology, effort-effective and easily applicable on large-scale. Generally, impacts from synergistic effect of predatory pressure and anthropogenic source of disturbances such as increasing of temperature or AMD may increase the reduction of the alive coral cover. In this context, restoration techniques are widely developing in order to recover resilience of damaged reef. In the Maldives, research on coral restoration is still at very early stage, although resorts and local islands are more often starting such programs. Yet, the evaluation of effectiveness is often reduced at few ecological factors, such as coral survival and growth, but social attributes may be crucial in driving funding allocation as suggested by recent studies (Hein et al., 2017). Thus, the present work aims at:

- 1. addressing the ecology of the major corallivores in the Republic of Maldives, specifically of *Acanthaster planci*, *Culcita* spp. and *Drupella* spp. (Chapter 2,3 and 4);
- setting and improving the methodology in detecting and quantifying AMD on the Maldivian beaches (Chapter 5);
- evaluating the perception of users of coral reef on the conservation projects used in resorts, in particular coral restoration, as tools to drive financial support (Chapter 6).

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- CHAPTER 2 -

2.1 Monitoring and assessing a two-year outbreak of the corallivorous seastar Acanthaster

planci in Ari Atoll, Republic of Maldives.

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Outbreak.

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2.2 Abstract

Outbreaks of the corallivorous crown-of-thorns seastars have received increasing attention due to their negative impacts on coral reefs in the Indo-Pacific Ocean. However, outbreaks in remote and dislocated islands are still poorly understood. This study aims to begin filling informational gaps regarding outbreaks of *Acanthaster planci* in the remote islands of the central Ari Atoll, Republic of Maldives. The population of *A. planci* was monitored during three periods over two years (2015-2016) to evaluate variations in abundance and to characterise size structure and feeding behaviour. The outbreak appeared to be severe and active throughout the entire study period. The size structure analysis revealed a multimodal distribution dominated by individuals between 20 and 30 cm, suggesting that the outbreak may have resulted from a few nearby mass spawning events. Additionally, the most abundant live coral was *Porites*, which was also the most consumed genus; however, the electivity index showed a preference for corals of the genera *Favites* and *Pavona*. Finally, we also highlighted the need for more geographically extended surveys to better understand local patterns regarding outbreaks of *A. planci* in the Republic of Maldives.

2.3 Introduction

Outbreaks of the crown-of-thorns seastars (CoTS), *Acanthaster* spp. (excluding *A. brevispinus*), are a major threat to tropical reefs in the Indo-Pacific Ocean (e.g., Pratchett et al. 2017). CoTS are specialised coral predators whose populations display transitions between relatively long periods of low densities, with little effect on corals and episodes of high densities known as outbreaks (Pratchett et al. 2014). During outbreaks, their abundance can exceed 1000 CoTS ha⁻¹ (e.g., Kayal et al. 2012), resulting in severe impacts on coral reefs characterised by coral mortality as high as 80% (e.g., Pratchett 2010; Baird et al. 2013).

The first well-documented outbreak was reported in southern Japan in the late 1950s (see Yamaguchi (1986)). Since then, outbreaks have been reported throughout the Indo-Pacific Ocean (see Pratchett et al. (2014)). However, the determinants and mechanisms regulating outbreaks remain elusive. Several hypotheses have been proposed regarding the factors that may trigger outbreaks; these hypotheses aim to identify the main determinants in terms of both natural and anthropogenic sources (e.g., food limitation, typhoons, adult aggregation, nutrient enrichment, overfishing of CoTS predators) that may increase the survival rates of the larvae and juveniles (e.g., Fabricius et al. 2010; Babcock et al. 2016; Pratchett et al. 2017). However, there is still lack of consensus in the understanding of CoTS outbreaks, which calls for further research (Cowan et al. 2017; Pratchett et al. 2017).

Various authors have observed that such outbreaks can adopt two distinct forms, which may result in marked differences in population structure (e.g., Moran 1986; Johnson 1992; Pratchett 2005; Pratchett et al. 2009, 2014). A "primary outbreak" is characterised by multiple, successive recruitment events, which lead to a sustained build-up of individuals of various ages and sizes (see Pratchett et al. (2014)). A "secondary outbreak" is triggered by a single massive influx of recruits resulting from a single mass spawning, and this leads to a large population of CoTS of similar ages and sizes (see Pratchett et al. (2014)).

An obvious key aspect driving the effects of CoTS outbreaks on reef communities is related to their preferred diet (Ormond et al. 1976; De'ath and Moran 1998b; Pratchett 2007, 2010). Specifically, CoTS exhibit a strong preference for corals of the family Acroporidae, such as the genera *Acropora*

and *Montipora*, as well as corals of the family Pocilloporidae, such as the genera *Pocillopora* and *Stylophora*; in contrast, CoTS tend to avoid corals of the family Poritidae (De'ath and Moran 1998b; Pratchett 2007, Scott et al. 2017). However, those preferences reflect the order in which corals are consumed based on their availability, but these preferences are by no means strict; in fact, during severe outbreaks, once all other corals have been consumed, CoTS easily turn to 'sub-optimal' prey (Pratchett 2005, 2007). The factors influencing the prey preferences of CoTS include coral nutritional content, growth forms, means of defences (both by the coral itself and/or by associated organisms, such as trapeziid crabs found in Pocilloporidae corals). The combination of these factors and coral species availability, i.e., relative abundance, determine the diet of CoTS (Moran 1986; Pratchett 2001, 2007).

Although outbreaks of CoTS have been described throughout their entire geographical range, there is little information about outbreaks that occur on small and dislocated reefs, such as those in the Philippines (Bos 2010), in the South China Sea (Lane 2012), in the Chagos Archipelago (Roche et al., 2015) or in the Maldives (see Pratchett et al. (2014)). Moreover, little is known about species-specific biology, ecology and occurrence of outbreaks since most of the current knowledge was generated from research in the western Pacific Ocean on the species *Acanthaster* cf. *solaris* (Pratchett et al. 2017). Notably, recent genetic studies have highlighted the presence of at least four different species, of which *Acanthaster planci* is restricted to the northern Indian Ocean (Vogler et al. 2012; Haszprunar et al. 2017); thus, here we will refer to *Acanthaster planci* based on this up-to-date information.

The Maldivian coral reef is the seventh largest coral reef system, with a total surface of 8920 km², accounting for 5% of the world's reef area (Spalding et al. 2001). Maldivian reefs had one of the highest coral cover values in the western Indian Ocean until a major coral bleaching event, which occurred in 1998 (Goreau et al. 2000; McClanahan and Muthiga 2014), eradicated almost 90% of the living coral (Zahir 2000; Morri et al. 2015). In the last twenty years, Maldivian reefs have shown a high degree of recovery (Pisapia et al. 2016). However, further bleaching events that were reported in 2010 (Tkachenko 2012, 2015) and 2016 (Bruckner et al. 2017; Ibrahim et al. 2017; Perry and Morgan 2017) are complicating the process. In general, corals are under multiple stressors, including diseases (see Montano et al. (2012, 2015a, b, 2016); Seveso et al. 2012), increase in land

reclamation (Fallati et al. 2017), anthropogenic pressure (Brown et al. 2017), and predation from various corallivores (e.g., Hobbs 2013; Bruckner et al. 2017;). In this context, outbreaks of *Acanthaster planci* (Fig. 2.1) could play a crucial role in determining the fate of Maldivian reefs, as has been demonstrated for CoTS in other Indo-Pacific localities (e.g., Pratchett et al. 2014).

The first published documentation of an *A. planci* outbreak in the Maldives dates back to the 1990s (Ciarapica and Passeri 1993; Sluka and Miller 1999); additionally, a few later reports from the Maldivian government highlighted the presence of non-outbreak populations in various atolls between 2006 and 2010 (Zahir et al. 2010), and the widespread presence of *A. planci* in the Archipelago was also confirmed by a recent study (Ibrahim et al. 2017). However, most of this information is qualitative, which means the current knowledge on the population structure and ecology of *A. planci* in the Maldives is in a very early stage. In 2014, a large and apparently stable outbreak was reported in the Ari Atoll (Saponari et al. 2014), and this outbreak offered a unique opportunity to fill the knowledge vacuum; as a result, we conducted a two-year field study to describe the population abundance, size structure, and feeding behaviour of *A. planci* in the study area.

2.4 Materials and methods

2.4.1 Study Sites

Surveys were conducted intermittently from March 2015 to December 2016 in the central Ari Atoll, where 19 locations were randomly selected based on accessibility (Fig. 2.2).

Surveyed reefs ranged from 0.04 km (Emboodhoo Thila) to 3.75 km (Thudufushi) in diameter and consisted mainly of low-energy reefs with gentle slopes on all sides. Due to logistical issues, sampling efforts differed among different study sites and periods (see Online Resource 2.1 for details). A localised effort to control the outbreak occurred at one site; methods included removing individuals or killing them by injecting individuals with acid solution (Buck et al. 2016). Here, data were collected prior to each individual removal or killing effort to reduce anthropogenic influences.

The abundance (i.e., population density) of *A. planci* was documented during three different periods: from March to October 2015 (2015), from June to August 2016 (2016_A) and from October to December 2016 (2016_B). Surveys were conducted during daylight in three different reef zones (flat, crest and slope) by snorkelling at shallow depths (0-5 m) and by scuba diving at greater depths (5-30 m). We quantified the density of *A. planci* in a total of 108 belt transects; each belt transect was 100×5 m² (500 m² total area), spaced 5-10 m apart, and sampling was conducted at the same depth. The transects were laid parallel to the reef crest and started at the randomly selected points within the target reef zone, depth range and location.

One-way analyses of variance (ANOVA) were performed on the data set to test for differences in *A. planci* density among the three time periods and among the coral reef zones; transects were used as replicates. *A. planci* density was log-transformed to meet the assumptions of normality and homoscedasticity. When ANOVA indicated significant differences, we performed Tukey's HSD multiple comparison post hoc tests to assess the significance between individual factors. Statistical analyses were performed using SPSS ver. 24 (IBM, New York). All data are presented as the mean \pm standard error (SE) unless otherwise stated.

We also characterised the population size distribution of *A. planci*. The surveys were conducted by applying the roving diver technique. This technique consists of a 1-h dive as a sampling unit, and the diver progressively swims from the maximum depth (5-30 m) to the shallower depth (0-5 m), noting the data along the transect (Hoeksema and Koh 2009; Montano et al. 2017). Surveys were conducted from June to December 2016 by measuring the body diameter (from the tips of opposite arms) to the nearest centimetre (cm) of each individual encountered. Data were then pooled into 5-cm size classes (<10; 10-15; 16-20 cm, etc.). We used those measures as a proxy of seastar age; additionally, we assumed individuals smaller than 10 cm in diameter were younger than 1 year in age, those of 10-15 cm were between 1- and 2-years old, those of 16-25 cm were between 2- and 3-years old, those of 26-40 cm were between 4- and 5-years old, and those larger than 40 cm were greater than 5-years old (see Engelhardt et al. (1999); Pratchett 2005). Additionally, size distribution was tested for unimodality using the Hartigan's dip test (Hartigan and Hartigan 1985) which has

been used to study size distribution of land organisms (e.g., Kelt and Meyer 2009) and for marine and freshwater fish (e.g., Olden et al. 2007). The null hypothesis considered the distribution as unimodal; consequently, a rejected p-value (<0.05) suggested that the distribution was multimodal or at least bimodal. The statistical analysis was performed using RStudio (R Core Team 2017) and a specific dip.test package (Maechler 2016).

2.4.3 Feeding behaviour

We investigated the feeding behaviour of all recorded *A. planci* individuals from June to December 2016 (Online Resource 2.1) by applying the same roving diver technique described above (Hoeksema and Koh 2009; Montano et al. 2017). For this, we annotated every organism (coral or not) showing signs of *A. planci* predation within a radius of 2 m around the target individual. Specifically, we considered (1) the presence of a seastar with the stomach everted on a coral or on any other potential prey and (2) the presence of fresh scars on coral tissue as clear signs of predation. To recognise fresh scars, in situ evaluations were made by checking the presence/absence of tissue, and corals with scars and missing tissue were considered to have been preyed upon. We took notes in situ of the genus and size (to the nearest centimetre (cm) as the maximum diameter) of each preyed coral individual. The preyed corals were arranged into 10-cm size-classes, starting from corals <5 cm, which are considered recruits (Colgan 1987), up to corals >200 cm. In addition, the morphology of each colony was recorded according to the following categories: branching, digitate, encrusting, free-living, massive, and tabular.

To have a 'frame of reference' that could be used in comparison with the diet of *A. planci*, we used photoquadrats to determine the composition and structure of the benthic community. Photoquadrats were obtained from 28 random belt transects from June to December 2016 (Online Resource 2.1). The locations were chosen among those accessible considering the logistical limitations of boat accessibility and availability. Thus, to optimise the survey effort, three locations were chosen to represent the coral coverage and abundance of the central area of Ari Atoll. We used the same approach as Tkachenko (2015), who used three locations in Ari Atoll (of which two are in the same area of the present study) to assess the abundance of the main coral taxa following the 2010 thermal

anomaly in the Indian Ocean. Moreover, the belt transects were laid parallel to the reef crest in correspondence with the surveys described previously. Photographs were taken with a Sony rx100 iv camera in a Fantasea housing using 1×1 -m PVC frame to demarcate the photoquadrats. Data were collected and analysed using the following benthic categories: broken but alive coral colony, corals, coral diseases, coral rubble, coralline algae, dead corals, gorgonians, macro algae, pavement, sand, soft corals, sponges, Tunicata, turf algae, zoantharians, and unknown; in addition, a genus level was added within the benthic category 'corals'. The relative coverage of the different benthic categories was estimated for each quadrat from 100 randomly distributed points using Coral Point Count with Excel Extension software 4.0 (Kohler and Gill 2006).

The proportional consumption of corals was calculated accounting for the size of corals and the taxa being preyed upon, while the relative availability of the different coral genera were extrapolated a posteriori by counting each colony within the area marked by the photoquadrats using the pictures taken in the field. To explore the food preference of *A. planci*, the Ivlev's electivity index (Ivlev 1961) (E) was used following Lechowicz (1982). The electivity index E is commonly used in the literature to determine the feeding preferences of CoTS and other seastars (e.g., Kayal et al. 2011; Farias et al. 2012). The index E was calculated for group i (i.e., a genus or family of corals) as follows:

$$Ei = \frac{(ri - pi)}{(ri + pi)}$$

where pi is the prey relative abundance in the environment, and ri is the relative abundance in the predator's diet. We calculated ri as the frequency of predatory action on group i in relation to all the preyed colonies. Successively, we obtained pi as the mean relative abundance of each single genus or family. Values of Ei ranged from 1 and -1, indicating random feeding when Ei = 0, maximum preference when Ei = 1, and maximum avoidance when Ei = -1.

2.5 Results

2.5.1 Abundance and population size structure

During the monitoring period, we performed 108 transects and covered a total area of 54×10^3 m². We counted 1986 *A. planci* individuals, with an overall mean density of 18.1 ± 2.3 individuals/transect, which equated to 361.5 individuals/ha. The highest densities of *A. planci* were found in 2015, with 32.6 ± 9.8 individuals/transect; in contrast, densities were 18.9 ± 2.7 in 2016_A and 9.4 ± 2.5 in 2016_B (see also Online Resource 2.2 for more details). The differences in *A. planci* densities between the different periods were identified as significant by ANOVA ($F_{2.92} = 4.352$ p = 0.016; Fig. 2.3a). Tukey's HSD post hoc tests showed that *A. planci* density was significantly higher in 2015 than it was in 2016_B at the 0.05 level of significance. All other comparisons were not significant. Similarly, we found significant differences in *A. planci* densities among the coral reef zones ($F_{2.92} = 6.996$ p = 0.001), with the reef slope exhibiting the highest density of seastars (i.e., 25.3 ± 5.3 individuals/transect) compared to the reef flat and crest (7.5 ± 1.8 and 18.0 ± 3.2 individuals/transect, respectively; Fig. 2.3b). Tukey's HSD post hoc tests showed that *A. planci* density was significantly higher in the reef slope than in the reef flat at the 0.05 level of significance. Again, all other comparisons were not significant.

Body sizes were measured for 1521 individuals, yielding a mean value of 25.96 ± 0.12 cm. The range of diameter measurements ranged from 7 to 50 cm. The resulting population structure was not unimodal, as determined by the dip test (p-value $< 2.2^{-16}$). The size classes 21-25 cm (i.e., 2-3 years old) and 26-30 cm (i.e., 3-4 years old) accounted for 32.6 and 44%, respectively, of the sampled individuals. Conversely, young individuals (diameter <15 cm, age <2 years) and very old individuals (diameter >40 cm, age >5 years) accounted only for 1.4 and 0.3% of the sample size, respectively (Fig. 2.4).

The total percentage of individuals showing predatory activity was 51% (i.e., 775 out of 1521 total individuals), and more than half of these individuals (58.5%) had their stomach everted in the act of consuming their prey, and 41.5% of them were found close to a coral with a fresh scar. Most (774) of the seastars showing predatory activity were consuming scleractinian corals, with a single exception of one individual that was apparently feeding on a conspecific. The coral colonies were identified at the genus level (733 out of 774); however, 5.3% of the colonies were too small or damaged, so those colonies were not included in the analysis.

The list of documented prey included 23 genera of coral (Online Resource 2.3). *A. planci* exhibited a high proportion of consumption of corals of the genus *Porites* (53.5%), followed by the genera *Pocillopora* (9.6%) and *Acropora* (8.8%); in contrast, seven genera appeared to be less palatable (with only 1 to 4.7% of the sampled individuals having been preyed upon). The sampled seastars consumed less than 1% of the remaining 13 genera (Online Resource 2.3). The size of the preyed upon coral colony varied from 2 cm to 240 cm. The size class consumed most was 11-20 cm (23.5%), followed by 5-10 cm and 31-40 cm (11.1 and 13.9%, respectively). Notably, only 3% of the seastars were found consuming recruits, and only 7% of seastars were found consuming coral colonies larger than 100 cm. *A. planci* individuals were also found preying mainly on massive (53.6%) and branching (31.4%) corals (Online Resource 2.4).

The benthic coverage was dominated by dead corals ($56.7\% \pm 4.5$), followed by coral rubble ($11.5\% \pm 1.6$); in contrast, the coverage of live corals was very low, accounting for only $4.2\% \pm 0.9$ of the total coverage (Online Resource 2.5). *Porites* was the dominant genus among live corals, with a relative mean coverage of $79.5\% \pm 0.9$, followed by *Pocillopora* and *Acropora* ($7.9\% \pm 0.09$ and $5.1\% \pm 0.1$, respectively), and the remaining genera together accounted for less than 2.4% of the living coral coverage (Online Resource 2.6).

Regarding prey preferences, the Ivlev's electivity index calculated a positive value for the genera *Dipsastraea* and *Favites* (0.54 and 0.49, respectively); however, the results were negative for the remaining genera and families, with the exception of *Psammocora*, which had a value of zero, indicating random feeding (Fig. 2.5). Specifically, negative values close to -1 (i.e., maximum

avoidance) were found for the genera *Porites*, *Acropora* and the family Fungiidae (Fig. 2.5). The genera found to be <0.01 in abundance were not considered since the index may suffer from bias with very rare genera (Lechowicz 1982).

2.6 Discussion

During the survey, an overall density of 361.5 individuals/ha was observed, which was in accordance with the outbreak densities of CoTS reported elsewhere (e.g., Moran and De'ath 1992; Pratchett et al. 2014). These findings are similar to the localised outbreaks of *A. planci* reported in the Maldives in 2014, when Saponari et al. (2014) observed and quantified the density of seastars during an isolated outbreak in Mama Ghiri, a small reef located in Ari Atoll. Since it was only a few kilometres away from the survey area of the present study, we cannot exclude the possibility that Mama Ghiri may have been a source area for the outbreak described in the present study. The propagation of adult CoTS may occur between nearby reefs as observed in Moorea, French Polynesia by Kayal et al. (2012), where an 8-year monitoring programme revealed consecutive outbreaks due to the movement of adult CoTS.

Densities of *A. planci* were consistently high throughout the study period, but they tended to decrease through time, from 32.6 ± 9.8 individuals/transect in 2015 to 9.4 ± 2.5 individuals/transect in 2016_B. Such a negative trend could be due to the progressive reduction in prey availability due to the outbreak itself and/or to the recent bleaching event that occurred in 2016. The effect of a reduction in prey availability could be twofold. On the one hand, a reef with less coral may promote the migration of individuals to new coral-rich reefs (Keesing and Lucas 1992; Kayal et al. 2012; Sigl and Laforsch 2016). On the other hand, the lack of food could cause a natural decline in the *A. planci* population and increase their exposure to starvation and diseases (Moran and De'ath 1992; Pratchett 1999, 2005). In addition, even though the effort of removing or killing individuals in the study area was localised in only one reef, we cannot exclude the possibility that this effort may have partially contributed to the observed decline. We also found significantly high densities on the reef slope, which may be preferred to the reef flat and crest due to lower water movement and light exposure or because these habitats have more abundant palatable coral prey. Many authors have

highlighted the role of these factors in influencing the distribution of CoTS (e.g., De'ath and Moran 1998a, b).

The population of A. planci in Ari Atoll displayed a great range of size/age classes, spanning from individuals younger than 2 years old to individuals greater than 5 years old. The presence of seastars smaller than 10 cm, which was observed in 2016, was quite rare (0.1%), suggesting weak recruitment during the previous years. The low number of juveniles smaller than 10 cm in diameter may also be due to their cryptic and nocturnal behaviour (De'ath and Moran 1998a). Therefore, the differences in the detectability of early juveniles should be researched with dedicated surveys. In general, the individual size structure did not follow a unimodal distribution, suggesting that more than one cohort may dominate the size structure. Indeed, more than half of the individuals (76.6%) were between 21-25 cm and 26-30 cm, and hence, they represented individuals that were approximately 3-5-years old. However, these results are consistent with what has been observed in most secondary outbreaks, with few cohorts being very abundant (see Pratchett et al. (2014)). On the other hand, the origin of the outbreak cannot be clearly assessed in the current study area due to the limitations of our survey and the lack of previous information; furthermore, studies on A. planci in the Maldives are very few and are fragmented in terms of time and information provided (Adam 1989; Van der Knapp 1989; Ciarapica and Passeri 1993; Sluka and Miller 1999; Zahir et al. 2010; Saponari et al. 2014; Ibrahim et al. 2017). Thus, additional studies are needed to clarify the origin, timing and severity of A. planci outbreaks in the Maldives.

The feeding behaviour of *A. planci* has been poorly studied since most of the research has been concentrated on *A. cf. solaris* (Pratchett et al. 2017). In this study, *A. planci* mostly consumed corals of the genus *Porites*, which was the most abundant genus in the study area. However, *A. planci* appeared to prefer corals of the genera *Dipsastraea* and *Favites*, which were less abundant than *Porites* but were likely more palatable. However, *Acropora*, which is generally known to be a favourite prey (De'ath and Moran 1998b), appeared to be avoided, likely due to its rarity and difficult detectability. Similar preferences have been obtained from studies that explored the feeding preferences of a non-outbreak population of CoTS in Sulawesi, Indonesia by Tokeshi and Doud (2011). However, our results refer to a specific situation in which the 2016 coral bleaching increased coral mortality in combination with the predatory activity of *A. planci* (Ibrahim 2017). In

fact, the coral bleaching event in 2016 may have restricted the diet of *A. planci*, posing strong constraints on the food preferences. Nevertheless, CoTS have been reported to switch their diet in the absence of their favourite prey (De'ath and Moran 1998b; Pratchett 2007, 2010). In addition, similar behaviour has been observed in other corallivores, such as filefish (Hobbs 2013) and *Drupella* snails, especially following a major bleaching event (Hoeksema et al. 2013). These observations are consistent with previous studies that showed CoTS are capable of dynamically adapting their diets to coral availability, consuming even coral genera that they commonly avoid (Pratchett et al. 2010).

The outbreak documented in Ari Atoll may have contributed to the coral mortality caused by the bleaching event in 2016 (Perry and Morgan 2017). The damage may be have been even worse due to the 2-year continuous outbreak, which could last longer, as has been observed elsewhere (e.g., Kayal et al. 2012), and due to the capability of *A. planci* to consume the last-surviving coral genera. Further investigations on population dynamics on larger scales, larval dispersion, juvenile ecology and oceanographic modelling will clarify the processes driving the Maldivian outbreak. Nevertheless, comparative research is needed among species of the genus *Acanthaster* to identify geographical differences. In addition, the potential impact on the Maldivian coral reef may be enhanced by other corallivorous species, such as *Culcita* spp. or *Drupella* spp. (Bruckner et al. 2017). However, it may still be possible for the coral community to recover or shift in composition, and similar situations have been reported in other reefs that were previously damaged by multiple disturbances, including outbreaks of CoTS and coral bleaching (e.g., Kayanne et al. 2002). Rather, it would be of great interest to document the aftermath of these disturbances on the Maldivian coral reef over a long-term temporal scale.

2.7 Conclusions

The purpose of this study was to provide the first official report of the latest outbreak of *A. planci* in Ari Atoll, Republic of Maldives. The outbreak possibly arose as a secondary outbreak even though more information is needed to clearly assess its origin. However, it remained active throughout the 2-year monitoring period during which *A. planci* revealed a high capability of adapting its diet to

coral availability and therefore enhancing the damage on coral community in the study area. Yet,

the dynamics of A. planci outbreaks remain unclear, despite being one of the most significant

disturbances on coral reefs in the Indian Ocean. Thus, further studies on outbreaks of different

species of CoTS in different areas are fundamentally necessary. Finally, our results provide useful

insights to begin filling informational gaps regarding outbreaks of A. planci in remote areas such as

the Maldives.

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Compliance with ethical standards

Conflict of Interest: The authors declare that they have no conflict of interest.

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2.9 References

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2.10 Figures and Online Resource

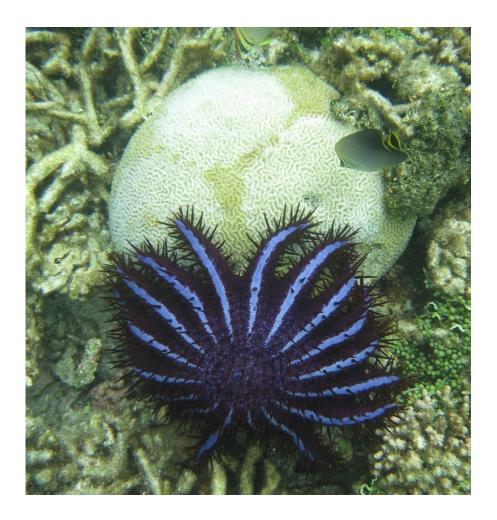


Fig. 2.1 The color morph of *Acanthaster planci* according to Haszprunar et al. (2017). Photo taken during a survey in 2016, Athuruga, Ari Atoll, Maldives

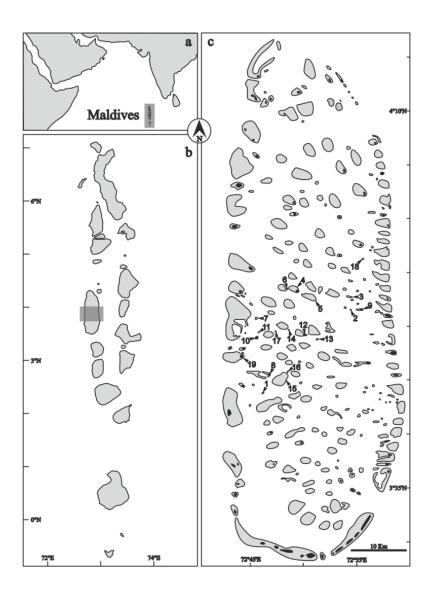


Fig. 2.2 a, b The map of the Republic of Maldives. **c** The map of the study area in the Ari Atoll with locations codes (see Online Resource 2.1), in black the islands and in grey the reefs.

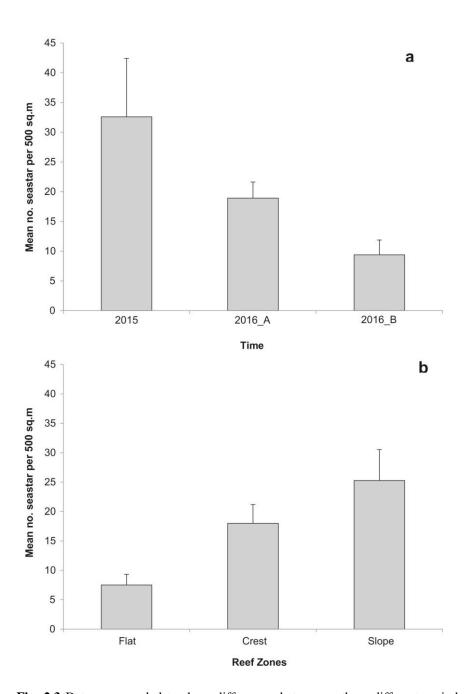


Fig. 2.3 Data were pooled to show differences between **a** three different periods: from March to October 2015 (2015), from June to August 2016 (2016_A) and from October to December 2016 (2016_B). **b** Three different reef zones (Flat, Crest and Slope) (bars indicates SE)

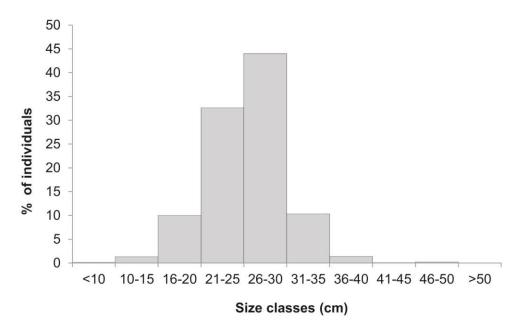


Fig. 2.4 Overall size structure for the population of *Acanthaster planci* monitored during the study period in central Ari Atoll. The histograms indicate the percentage of individuals belonging to the size classes considered.

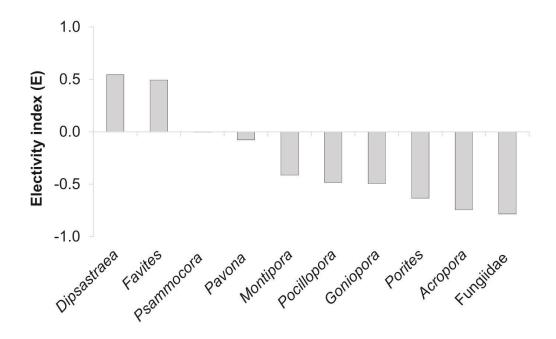
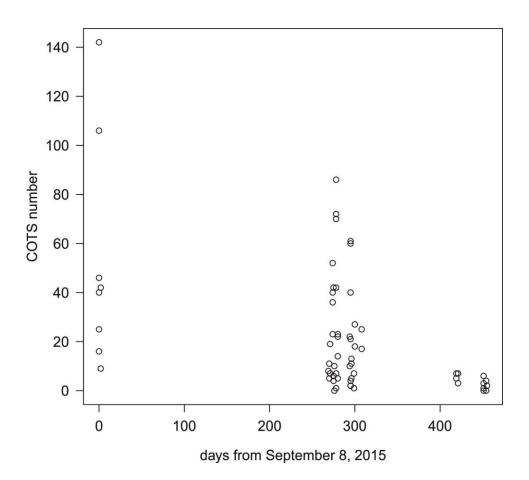


Fig. 2.5 Feeding preferences of *A. planci* according to the Ivlev's electivity index (1961) (E) for each genera or family of corals considered (*i*) in the study area

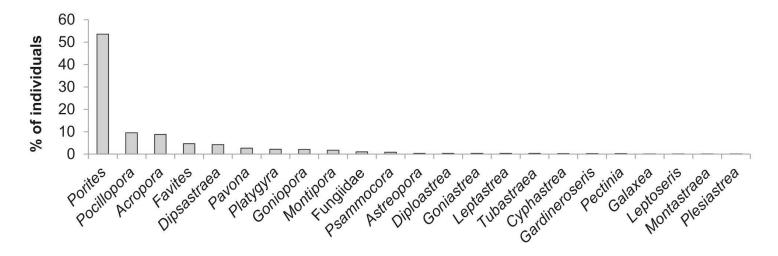
Online Resource 2.1 The data collected (abundance of *Acanthaster planci*, the size, feeding behaviour and coral cover) are listed for each location where the survey have been done (x). The code refers to the reefs surveyed, and reported on the map in Fig. 1. Abundance was monitored from March 2015 to December 2016. Size, feeding behaviour and coral cover were surveyed from June to December 2016. Due to logistic in-field limitations the surveys were separated.

Code	Lat	Long	Location	Abundance	Size	Feeding Behaviour	Coral Cover
1	3.729	72.760	Ali Thila	X	X	x	
2	3.859	72.899	Ali Thila (Athu)	X			
3	3.890	72.890	Alicoi	X			
4	3.887	72.816	Athuruga	X	X	X	X
5	3.885	72.822	Athuruga Ghiri	X	X	x	
6	3.890	72.800	Athuruga Thila		X	x	
7	3.845	72.752	Dega Ghiri	X			
8	3.758	72.771	Diggha	X			
9	3.860	72.915	Digghiri	X	X	x	
10	3.815	72.753	Emoodhoo	X			
11	3.819	72.756	Emboodhoo Thila		X	x	
12	3.819	72.829	Hennfaru	X			
13	3.812	72.846	Kuda Myaru Thila	X	X	x	
14	3.828	72.800	Mas Thila	X	X	x	
15	3.759	72.798	Myaru Gali	X			
16	3.761	72.799	Myaru Gali Thila		X	x	
17	3.825	72.781	Olo Ghiri	X	X	x	X
18	3.933	72.917	Shark Thila		X	x	
19	3.786	72.731	Thudufushi	X			x

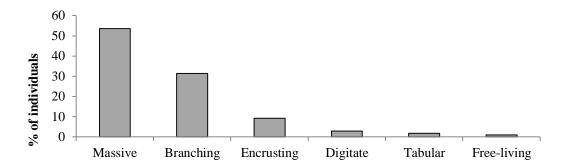
Online Resource 2.2 The temporal sequence of changes in abundance is represented by plotting the actual number of crown-of-thorns seastars (CoTS) per transect (circlets) against the specific date of sampling, which offers visual evidence for the decline in CoTS abundance



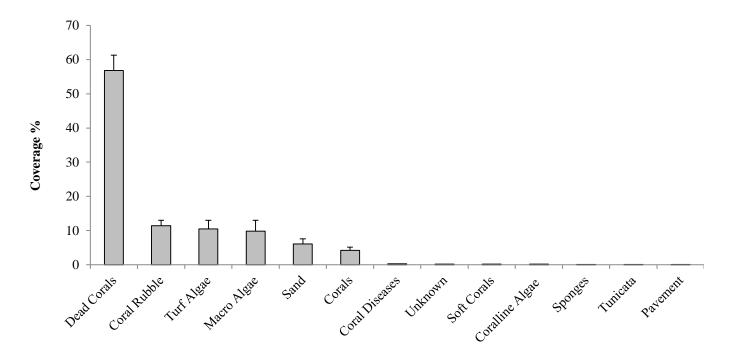
Online Resource 2.3 Overall percentage of individuals of Acanthaster planci for each genus of coral found eaten, comprising the family Fungidae

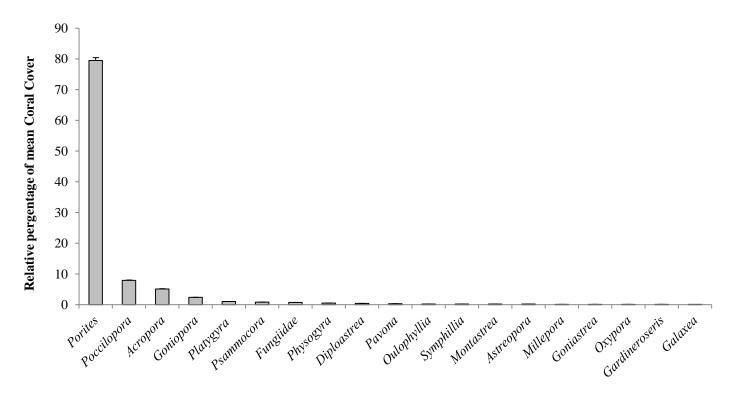


Online Resource 2.4 Overall percentage of individuals of *Acanthaster planci* for the main growth forms of corals found eaten by the starfish



Online Resource 2.5 Overall mean coverage (±SE) of the main benthic categories





- CHAPTER 3 -

3.1 New insights into the ecology and corallivory of *Culcita* sp. (Echinodermata: Asteroidea) in

the Republic of Maldives

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3.1.1 Abstract

Although corallivory is recognized as a threat affecting the structure and integrity of coral reef habitats, ecological data on most species of coral consumers remains limited, slowing down the development of conservation and restoration strategies of the reef ecosystems. In this study, the population distribution and corallivorous behaviour of the cushion sea star *Culcita* sp. were investigated in the south region of Faafu Atoll, Maldives. Most sea stars were found on reef slopes within 0-10 m depth and in areas characterized by low live coral cover. Several coral genera were preyed on by the sea star. Although most of the consumed corals belong to the genus *Acropora*, a feeding preference for the genera *Pocillopora* and *Pavona* and a consistent avoidance of the genus *Porites* were observed. Furthermore, the majority of the prey corals were small colonies (< 10 cm diameter), even though *Culcita* sp. appeared to be capable of partially consuming larger colonies. Dietary preferences for specific coral colonies or genera have the potential to generate local shifts in coral community composition and structure and may affect reef recovery following natural and anthropogenic disturbance in an already impacted environment such as the Maldivian reefs.

3.1.2 Introduction

The role of corallivorous sea stars in coral reef ecology and their impact on coral health have become increasingly important and investigated, due to the devastating effects from outbreaks of the crown-of-thorns sea star (COTS) (*Acanthaster* spp.) (Sweatman & Syms, 2011; De'ath et al., 2012; Pratchett et al., 2017). Under these circumstances, other species of coral-eating sea stars have been neglected, likely due to their apparent inability to cause population outbreaks or to represent an immediate and macroscopic risk to reef integrity. Among these less-recognized corallivorous organisms are the cushion sea stars of the genus *Culcita*.

Currently, only three species are described; C. coriacea (Müller & Troschel, 1842), C. schmideliana (Bruzelius, 1805) and C. novaeguineae (Müller & Troschel, 1842). In the Indo-Pacific area, the predominant species are C. schmideliana and C. novaeguineae, with a large abundance of the former in the western Indian Ocean (Yasuda et al., 2012), whereas C. coriacea is considered endemic to the Red Sea (Clark, 1976). Like Acanthaster spp., Culcita spp. are also coral predators, which feed on corals by everting their stomachs and removing only the coral tissue without affecting the carbonate skeleton. Ultimately, the tissue is digested in situ, and this predation leaves scars that appear as large white spots (Goreau et al., 1972; Glynn & Krupp, 1986). However, both sea stars include a variety of other organisms in their diet (Thomassin, 1976; De'ath & Moran, 1998). The two sea stars also share similar spawning periods and larval ecology in many geographic areas (Yamaguchi, 1977; Yasuda et al., 2010; Otha et al., 2011).

Nevertheless, differences in the feeding behaviour of *Acanthaster* spp. and *Culcita* spp. have been reported, with cushion sea stars consuming comparatively less coral and at slower rates. For example, while a *Culcita* sea star can remove up to 1 m² yr ⁻¹ of *Pocillopora damicornis* (Linnaeus, 1758) or up to 0.9 m² yr ⁻¹ of mixed coral prey (Glynn & Krupp, 1986), individual COTS have been estimated to consume an average of 5 to 6 m² yr ⁻¹ of coral surface, reaching values of 12 to 13 m² yr ⁻¹ in some locations (Birkeland & Lucas, 1990).

The main difference between these two sea stars is that *Acanthaster* spp. have a higher fecundity and cause large population outbreaks with disastrous consequences for coral reefs (Birkeland &

Lucas, 1990; Kayal et al., 2012; Scott et al., 2017), whereas similar bursts of cushion sea star populations have not yet been reported. Nevertheless, average densities of *Culcita* spp. are typically higher than the number of individuals observed in non-outbreak populations of *Acanthaster* spp. (Goreau et al., 1972; Glynn & Krupp, 1986). This suggests that the cushion sea star, due to its continual removal of living coral, may represent a persistent force in affecting coral cover abundance and composition (Pratchett et al., 2011), and therefore, similar to COTS, may influence broader ecological processes and interactions in coral reefs.

Although our knowledge of *Acantahster* spp. is continuously increasing (Pratchett et al., 2017), little information about the ecology and corallivorous behaviour of *Culcita* spp. in natural environments is currently available. To date, these often limited and preliminary field observations indicate that dietary preferences are highly variable, with *Culcita* spp. feeding on different coral species depending on location (Goreau et al., 1972; Thomassin, 1976; Glynn & Krupp, 1986; Quinn & Kojis, 2003). However, these studies often report a general preference for small coral colonies and/ or recruits, potentially as the size and shape of *Culcita* individuals limits their ability to climb and feed on larger colonies (Glynn & Krupp, 1986; Quinn & Kojis, 2003).

In this study, the dietary ecology and corallivorous behaviour of the cushion sea star were investigated for the first time in a geographic area where this organism is poorly investigated: the Republic of Maldives. In the Maldives, the presence of the genus *Culcita*, and in particular, the species *C. schmideliana*, has been reported (Clark & Rowe, 1971; Jangoux & Aziz, 1984; Moosleitner, 1997; James, 2004); however, the Maldivian morpho-type shares common morphological features both with *C. schmideliana* and *C. novaeguineae* and, without a precise taxonomic identification, should therefore be referred to with the generic name *Culcita* sp.

The Maldivian coral reef is the seventh largest reef system on earth, with 8920 km² of total surface area (Spalding et al., 2001). Recently, its health status has been affected by multiple stressors, such as coral bleaching events (Tkachenko, 2015; Ibrahim et al., 2017; Perry & Morgan, 2017), disease outbreaks (Montano et al., 2015, 2016; Seveso et al., 2015, 2017), land reclamation and anthropogenic pressure (Brown et al., 2017; Fallati et al., 2017), algal overgrowth (Montano et al., 2012a; Seveso et al., 2012) and COTS outbreaks (Saponari et al., 2014, 2018). Hence, understanding the ecology of coral-consuming animals is of critical importance, in order to identify

their role in mediating coral community structure and better predict their ecological impact under changing conditions. To this end, we examined a population of cushion sea stars within a Maldivian coral reef system to determine (a) whether the distribution and size of individuals are related to depth, habitat composition, structural complexity and reef zonation, and (b) what the diet of these sea stars consists of and if they exhibit preferences for specific coral genera, growth forms, or colony sizes.

3.1.3 Materials and Methods

3.1.3.1 Study area and sampling design

Underwater surveys were performed on SCUBA diving during a number of field campaigns between February 2015 and April 2016 in the southern region of the Faafu Atoll, Republic of Maldives (Fig. 3.1.1). Our logistic base was the Marine Research and High Education Center (MaRHE) on Magoodhoo Island (3°4'49.08"N, 72°57'57.19"E, WGS84), an inhabited island that measures 900 x 450 m and is located on the south-eastern region of the atoll rim, approximately 140 km south of the capital Malè. Magoodhoo reef is approximately 3 km long and 1.55 km wide (Montano et al., 2012b).

Around the island, 17 different sites showing heterogeneous characteristics in terms of reef morphology and exposure were randomly selected among those accessible (Fig. 3.1.1). Some sites exhibited typical low-energy reef features with ample growth of coral and gentle slopes, whereas others were characterized by shallow patchy lagoon reefs or steep reef walls. Moreover, some sites were located inside the atoll, either as lagoon-patch reefs or lagoon-facing sides of the atoll rim (inner reefs), whereas others were positioned on the external, ocean-facing side of the atoll rim (outer reefs), which are often subject to more intense hydrodynamic conditions. The presence of *Acanthaster* spp., whose signs of predation can be confused with those of *Culcita* spp., was not observed on any of the selected sites.

3.1.3.2 Determination of coral and substrate coverage in the study area

To determine the composition and structure of the benthic community in the study area as well as the diversity and the abundance of the scleractinian corals, a photo quadrat method was used. For each sampling site, a minimum of 8 PVC quadrats of 1 x 1 m were randomly placed at different depths and spaced 10 to 20 m apart. Photographs were taken using a Canon G11 camera in an underwater housing (Canon WP-DC34) and the photos were analysed using Coral Point Count with Excel extension software 4.0 (CPCe 4.0, Kohler & Gill, 2006). Substrate coverage was estimated through the identification of organisms below 100 randomly distributed points per quadrat. Data relative to each site were collected and analysed together as study areas, using the following benthic categories: corals (scleractinians), rock, sand, coral rubble, dead corals, coralline algae, algae, soft corals, sponges and other sessile invertebrates (tunicates, zoantharians and unknown). In addition, the genus level was added within the benthic category "corals" (identification according to Veron, 2000).

3.1.3.3 Field observations of *Culcita* sp.

At each study site, the presence of *Culcita* sp. was recorded by applying the roving SCUBA diving technique, which consists of a 1-h dive sampling unit, where the diver moves progressively from the maximum depth (approximately 30 m) to shallower water, swimming freely throughout the dive locality (Hoeksema & Koh, 2009; Montano et al., 2017a). To avoid resampling the same individuals, the divers swam in the same direction without turning back.

3.1.3.4 Distribution and size

For every cushion sea star encountered, the depth at which it was observed (< 10 m, 10-20 m and > 20 m) and its location in terms of reef zones (reef flat, reef crest, reef slope) was recorded. To determine the coral cover of the area where each individual was found, the living coral cover percentage was evaluated visually within a 2 m radius calculated through a tape measure around

each sea star. A coral covering score from 1 to 4 was assigned, where "1" indicated a coral coverage between 0 and 10%, "2" a coral coverage between 10 and 30%, "3" a coral coverage between 30 and 50% and "4" a coral coverage > 50%.

Furthermore, the size of each *Culcita* sp. encountered was noted by measuring with a ruler the maximum diameter of the specimen to the nearest centimetre (cm).

3.1.3.5 Feeding activity and coral prey preferences

To define the behaviour of the organism, 3 main activities of the cushion sea star were considered: "moving", when tube feet were visible and the motion of the sea stars was easily detectable; "preying", when sea stars with everted stomachs were feeding on corals or other organic matter; and "stationary," when specimens were not moving or preying.

Within the investigated area (2 m radius around the sea star), we also counted the number of corals consumed by the sea star. In particular, corals showing clear signs of external digestion and the absence of coral tissues were considered as preyed corals (Fig. 3.1.2). Importantly, the size of the investigated area (2 m radius) was selected considering the slow movement of the sea stars. This ensured that the coral colonies, showing fresh and bright white scars due to the total removal of living coral tissue as well as a visible thin layer of mucus, had been recently preyed upon by *Culcita* sp. (Fig. 3.1.2). The diameter of the targeted colonies was measured to the nearest centimetre (cm) considering their longest axis, and these corals were classified into 3 size-classes (< 5 cm, 5-10 cm and > 10 cm). Furthermore, the dimensions of scars on the coral surface were noted, and the coral was classified as partially or totally consumed. In addition, the genus of the targeted coral colonies was recorded as well as their growth morphology, according to the following categories: branching, encrusting, massive, foliaceous and mushroom.

3.1.3.6 Data analysis

Data are presented as the mean ± standard error (SE) unless otherwise stated. Data normality was verified using the Shapiro-Wilk test. Three separate one-way ANOVAs, followed by a Tukey's

HSD post hoc test, were used to evaluate significant differences in the abundance of sea stars at different depths, in different reef zones (reef flat, crest and slope) and in relation to different coral cover in the area around the sea star (2 m radius). Likewise, three separate one-way ANOVAs, followed by a Tukey's HSD post hoc test, were used to evaluate significant differences in the sizes of sea stars using the same three previous factors.

Spearman's rank correlation order test was used to examine if the size of the scars produced by *Culcita* sp. on the preyed coral colonies was correlated to the size of the colonies. All statistical analyses were performed using SPSS ver. 24 (IBM, New York) and R ver. 3.2.3 (R Development Core Team, 2015).

Sea stars' preferences for different groups of prey, here considered as coral genera, were calculated following Chesson (1983), as the standardized forage ratio (S_i) and defined for a group i as:

$$S_i = \frac{(\frac{ri}{p_i})}{\sum_{1}^{n}(\frac{ri}{p_i})}$$

where P_i is the prey relative abundance in the environment, r_i is the relative abundance of the prey in the predators' diet and n is defined as the number of groups in the system. The values of the standardized forage ratio ranged between 0 and 1, with $S_i = 0$ indicating avoidance and $S_i = 1$ indicating exclusiveness for a group of prey. We calculated P_i as the mean percentage cover for a coral genus in all the sites obtained from photo quadrats analysis and r_i as the percentage of predatory actions on a coral genus in relation to all preyed colonies.

The Ivley's electivity index, E_i (Ivley, 1961), was obtained by the following calculation:

$$E_i = (r_i - P_i) / (r_i + P_i)$$

where r_i and P_i are defined as above. Values of E_i ranged between -1 and 1, such that $E_i = -1$ represents total avoidance, $E_i = 0$ means non-selective feeding and $E_i = 1$ represents exclusive feeding on a given prey item. As reported by many authors, this index is not independent of prey density, and thus has a major shortcoming for comparative purposes versus the Standardized Forage Ratio (Jacobs, 1974; Chesson, 1983). Both indices have been used in feeding studies on Asteroidea and hence provide an efficient tool for the comparative analyses of sea star feeding behaviour (Farias et al., 2012; Saponari et al., 2018).

3.1.4 Results

3.1.4.1 Coral and substrate coverage in the study area

Considering the community structure and composition of the investigated area (Fig. 3.1.3a), benthic community composition was dominated by corals (29.9 \pm 3.6%), followed by rock (18.9 \pm 2.1%) and sand (13.5 \pm 1.6%). The coral genus *Acropora* was the most abundant (13.2 \pm 6.4%), followed by *Porites* (6.7 \pm 4%), *Pocillopora* (1.2 \pm 1%), *Favites* (0.9 \pm 0.7%), *Montipora* (0.8 \pm 1.3%), *Pavona* (0.8 \pm 0.5%) and other less represented genera (Fig. 3.1.3b).

3.1.4.2 Distribution and size of *Culcita* sp.

During the study period, 163 specimens of *Culcita* sp. were found in the area examined. The abundance of sea stars showed significant variations depending on the depth (one-way ANOVA, F $_{(2,50)} = 24.825$, p = 0.000), with the majority of organisms (approximately 70%) found between 0 to 10 m. Below 10 m, the number of observed sea stars decreased progressively with increasing depth, even if no significant difference was detected between the depth ranges 10-20 m and > 20 m (Fig. 3.1.4a). No significant difference was observed in the number of sea stars in the different reef zones (one-way ANOVA, F $_{(2,50)} = 0.268$, p = 0.766, Fig. 3.1.4b), whereas their abundance varied significantly depending on the coral cover percentage recorded in the 2 m radius area around the sea star (one-way ANOVA, F $_{(2,66)} = 6.219$, p = 0.001). In particular, significant differences were detected between areas characterized by a coral coverage between 10 and 30%, where the highest number of *Culcita* sp. was found (~ 41.5%) and those with a coral coverage > 50%, where the lowest abundance of specimens was observed (~ 9.5%), (Fig. 3.1.4c).

The average size of all sea stars sampled was 18.74 ± 0.15 cm, which did not show significant variation depending on the depth (one-way ANOVA, $F_{(2,153)} = 1.624$, p = 0.107, Fig. 3.1.4d). However, the average size of the individuals showed significant variation depending on the location of the organisms in the different reef zones (one-way ANOVA, $F_{(2,153)} = 3.217$, p = 0.0427, Fig.

3.1.4e) and the coral cover abundance in the 2 m radius area around each sea star (one-way ANOVA, $F_{(3,153)} = 5.933$, p = 0.000, Fig. 3.1.4f). In particular, significant differences in average sizes were detected between the reef crest and the reef slope (Tukey HSD post hoc, p < 0.05; Fig. 3.1.4e) and between areas where the coral coverage was more than 50% compared to areas where this percentage was less than 50% (Tukey HSD post hoc, p < 0.05; Fig. 3.1.4f).

3.1.4.3 Feeding activity and coral prey preferences of *Culcita* sp.

Only 14 sea stars (~ 9%) out of a total of 163 were observed to perform feeding activities during the sampling, and all were found to be preying on scleractinian corals. Most sea stars (~ 68%) were found stationary on the reef, whereas approximately 23% of the specimens were moving.

Scars typical of predation by *Culcita* sp. were found on 433 coral colonies belonging to 16 different coral genera (Table 3.1.1). Most of the preyed corals belonged to the genera *Acropora* (38.1%) and *Pocillopora* (~ 36.3%). A considerable number of colonies belonging to the genus *Pavona* were also found to be preyed upon by sea stars (~ 12.5%), whereas predation on other reef-building genera represented only a small percentage of the total predation (Table 3.1.1). Considering the growth morphology of the consumed colonies, *Culcita* sp. predation was highest on corals with a branching growth morphology, whereas the predation on massive forms was less intense, although it encompassed 9 different coral genera (Table 3.1.1).

Most of the sampled sea stars consumed small adult corals with a diameter between 5 and 10 cm (260 colonies out of 433, \sim 60%) followed by juvenile corals with a diameter < 5 cm (129 colonies out of 433, \sim 30%), whereas large adult colonies with a diameter > 10 cm (average size: 15.6 \pm 0.84 cm; maximum size: 38 cm) appeared to be the least predated (44 colonies out of 433, \sim 10%).

Furthermore, considering all the preyed colonies, both totally and partially, a significant positive correlation (Spearman's rho = 0.897, p < 0.001) was detected between the size of these colonies and the size of the scars created by the predatory activity of the sea star (Fig. 3.1.5). However, as the size of the predated corals increased, the number of partially consumed colonies also increased. In fact, partial predation by *Culcita* sp. occurred on only approximately 7% of all consumed juvenile colonies and on approximately 19.5% of small and 25% of the larger (> 10 cm) predated colonies

(Fig. 3.1.6a). As shown in Fig. 3.1.6b, the coral genus *Montipora* was the most partially consumed (3 colonies out of 7, ~ 42%) followed by *Pavona* (17 colonies out of 54, ~ 31%), *Porites, Acropora*, *Galaxea* and finally *Pocillopora* (only 12 colonies out of 157, ~ 8%).

The use of the Standardized Forage Ratio (S_i) and the Ivlev's Index (E_i) allowed us to compare the abundance of prey categories in the environment and in the predator's diet. The values obtained for both of these indices are shown in Table 3.1.2. Remarkably, the Standardized Forage Ratio produced values that indicate a preference for corals of the genus *Pocillopora* and *Pavona*, with values of 0.592 and 0.298, respectively. In contrast, the value generated for the genus *Porites* (0.005) was close to zero, indicating avoidance. The Ivlev's index showed a similar pattern, with values corresponding to preferences for *Pocillopora* and *Pavona* (0.761 and 0.576, respectively) and a negative value, indicating strong avoidance for *Porites* (-0.885), (Table 3.1.2).

3.1.5 Discussion

The direct consumption of live coral, or corallivory, represents an important biotic stressor for reefbuilding corals that can accelerate the rate of coral reef decline (Knowlton et al., 1990; Rotjan et al., 2006; Lenihan et al., 2011). However, although a wide variety of species from numerous taxa consume living coral (Carpenter, 1997; Cole et al., 2008; Rotjan & Lewis, 2008), to date, most studies have focused on a few specific organisms (e.g., COTS, butterflyfishes and *Drupella* spp.), indicating that the actual impact of corallivores on reefs may be underestimated. This study increases the knowledge of the little-investigated cushion sea star *Culcita* sp. in the Maldives, showing how this sea star is widely distributed over the reef, although exhibiting a preference for a specific depth range, reef zone and coral coverage of the habitat, and has a predilection for specific colony sizes and genera.

Regarding the distribution patterns of the cushion sea star, our results indicate that in the study area *Culcita* sp. can be found at a wide depth range (from 0 to at least 30 m) and in all reef zones (flat, crest, slope), even though a preference for reef slopes characterized by a shallow depth, within 10 m, was evident. In previous studies, *C. novaeguineae* was observed on reef slopes to 20 m depth, on reef flats, and on patch reefs in protected areas (Goreau et al., 1972; Yamaguchi, 1975;

Grosenbaugh, 1981; Glynn & Krupp, 1986). However, in line with our observations, C. novaeguineae always displayed a higher density on the reef slopes below 3 and 7 m (Goreau et al., 1972; Glynn & Krupp, 1986). In contrast, C. schmideliana mainly colonizes coral reef flats (Thomassin, 1976). In this study, the reef slope may have been preferred over the reef flats and crests due to lower water movement and light exposure, a higher presence of palatable coral prey, or an abundance of coralline algae that induce the recruitment of sea star larvae and provide juveniles with food and shelter, which has been proposed to explain similar patterns of distribution of COTS (Johnson et al., 1991; De'ath & Moran, 1998; Pratchett, 2005; Kayal et al., 2012). Variations in seawater temperature could also affect cushion sea star distribution. For example, C. novaeguineae was observed in areas characterized by a temperature of between 29°C and 31°C, with highest abundances recorded at approximately 29°C (Yokley, 2016), which corresponds to the average surface temperature recorded in our study area (Seveso et al., 2015, 2017). Our data also showed that most of the sea stars were located on substrates characterized by a low live coral coverage (10-30%), whereas fewer specimens were located in reef zones with a coral coverage > 50%. This observation suggests that *Culcita* sp. may prefer areas where in addition to corals, several types of prey are found, increasing the food resource diversity. In fact, Culcita spp. are not obligate corallivores because they feed on other organisms, such as the epilithic algal matrix growing on dead coral, sponges, soft corals, bryozoans, small fauna (nematodes, crustaceans) and algae (Thomassin, 1976; Glynn & Krupp, 1986).

Regarding the size of *Culcita* individuals in relation to the coverage of live coral, our results suggest as in areas characterized by elevated coral coverage, the average size of the *Culcita* sp. population appeared to be the lowest. Considering that corals represent the main framework builders of the Maldivian reef systems, areas characterized by a high coral abundance generally also show a high structural complexity. Small sea stars may prefer a complex three-dimensional habitat that allows them to reduce both the pressure of predation, through hiding and crypsis, and the effects of waves and currents that could easily transport them away from the corals into deeper parts of the reef. During the sampling activities conducted in daylight conditions, very few *Culcita* sp. specimens were actively feeding upon corals, in line with previous evidence suggesting that this animal is

preferentially a nocturnal feeder (Glynn & Krupp, 1986), and the majority of individuals were observed hidden and stationary inside ravines, crevices or under coral colonies.

In addition to habitat preferences, our study suggests that Culcita sea stars on Maldivian reefs exhibit strong patterns of prey preference and feeding behavior. Based on previous observations, the feeding preferences of Culcita spp. appear to vary depending on geographical area. C. novaeguineae in Guam fed mainly on small heads of Acropora, Pocillopora, various faviids, Porites lichen, and possibly Hydnopora and Montipora (Goreau et al., 1972). In Hawaii, the same sea star species mainly preved on Pocillopora meandrina (Dana, 1846), followed by Montipora verrucosa (Lamarck, 1816) and *Porites lobata* (Dana, 1846), showing the same food preferences when offered a single coral prey species in captivity (Glynn & Krupp, 1986). In Madagascar, C. schmideliana has been observed to more frequently eat coral colonies of small sizes and large polyps, such as Galaxea fascicularis (Linnaeus, 1767) or Goniopora stokesi (Milne Edwards & Haime, 1851), and less commonly small Acropora (Thomassin, 1976). Our results showed that in the Maldives a wide range of coral genera are preved upon by *Culcita* sp.; however, the majority of predation occurred on Acropora and Pocillopora corals, which are both characterized by a branching growth morphology. Interestingly, no predatory event on colonies of Acropora displaying a tabular morphology was observed. All other corals preyed on had either an encrusting (Pavona) or massive growth form; however, this accounted for only ~ 25% of total predation events. The preference for particular coral morphologies may be in part due to the surface complexity of the coral (De'ath & Moran, 1998). For example, Keesing (1990) found that branching forms of Acropora have the greatest complexity, thereby providing the sea star A. planci (Linnaeus, 1758) with greater surface area and hence more tissue per feeding. However, the availability in the investigated area of the different coral genera targeted as prey should be considered in order to detect real food preferences. While sea stars fed on a number of coral general, Si and Ei values suggest that sea stars have a preference for the genus Pocillopora followed by Pavona, which are both characterized by a low abundance in the area. Considering that *Culcita* sp. has a small stomach and low feeding rate (Glynn & Krupp, 1986; Birkeland, 1989), its preference for *Pocillopora* could be related to the high digestibility or nutritional quality of this coral genus. In fact, the tissues of Pocillopora are very thin and, because they are not intricately connected and deeply penetrating in the skeleton, they form a superficial layer that makes them easily available for the sea star (Glynn & Krupp, 1986; Loya et al., 2001). Moreover, this genus is characterized by a low level of protective mucus, absence of large nematocysts, and ineffective protection of the juvenile colonies by the *Trapezia* spp. symbiotic crabs, which often fail to prevent the predatory action of *C. novaeguineae* (Glynn & Krupp, 1986; Rouzé et al., 2014).

The genus *Pavona* showed a high index of preference but also a high percentage of partially eaten colonies. The genus *Acropora*, despite the large number of predatory acts, did not appear to be preferentially chosen by *Culcita* sp. and therefore its high rates of predation may be due to the large abundance of this genus in the study area. The genus *Porites*, although one of the most abundant in the area, was among the least eaten and all these few colonies targeted by *Culcita* showed a massive growth form. *Porites* could be less palatable due to its thick tissue, which penetrates deeply into the skeleton and is characterized by a low protein content, as well as the numerous and large nematocysts that can cause arm rearing, tube feet retraction and stomach withdrawal in sea stars (Brauer et al., 1970; Moore & Huxley, 1976; Glynn & Krupp, 1986; Keesing, 1990; Lough & Barnes, 2000; Loya et al., 2001). *Porites* is also known to host organisms that may prevent feeding (DeVantier et al., 1986; DeVantier & Endean, 1988). In addition, *Porites* is generally considered a large growing genus, and it is possible that *Culcita* sp. would have difficulties climbing it (Glynn & Krupp, 1986). Not surprisingly, the average size of the few colonies of *Porites* eaten by *Culcita* sp. was relatively small and the largest ones were only partially consumed.

In this context, small colonies (< 10 cm in diameter) have been found to be preferred by *Culcita* sp., which is in line with previous studies, suggesting that the lack of prehensile arms prevents *Culcita* spp. from climbing onto large or high growing corals (Goreau et al., 1972; Thomassin, 1976; Glynn & Krupp, 1986). However, *Culcita* sp. also preyed upon larger coral colonies, which were only partially eaten. In general, we observed that as the size of the colonies targeted by the sea stars increased, both the size of the scars on the colonies as well as the amount of partially consumed colonies increased. This result suggests that *Culcita* sp. is seldom able to kill an entire large colony at one time, perhaps preferring to change coral once a certain amount of its tissue has been assimilated, rather than consuming an entire large colony during multiple feeding attacks. We hypothesized that *Culcita* sp. might be more easily deterred by the developed defensive mechanisms

of large adult colonies, which could also be more effective in their defence than smaller colonies due to the higher number of host symbiotic organisms (Abele & Patton, 1976; Glynn, 1980). However, even the partial mortality of coral colonies can affect reef health because it reduces coral growth rate and reproduction, due to the expenditure of the energy stores used for tissue regeneration processes (Veghel & Bak, 1994; Henry & Hart, 2005). In addition, if tissue regeneration is incomplete, portions of the colony could become susceptible to colonization by spatial competitors such as algae, sponges, other colonial invertebrates or disease-causing agents (Bak & Steward-Van Es, 1980; Nugues & Bak, 2009).

3.1.6 Conclusion

This study provides an overview of the feeding behaviour of a corallivorous organism, *Culcita* sp., that has received scarce attention, but whose impact on coral reef composition and health should not be underestimated. Considering its preferential predation on recruits and juvenile coral colonies and its predilection for some specific coral genera, *Culcita* sp. may affect reef recovery and resilience following other stress events and generate local shifts in coral community composition and structure. In this regard, further ecological investigations on larger scales and quantitative analyses on population dynamics should be considered.

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3.1.9 Tables and Figures

Table 3.1.1 – Relative percentages of colonies belonging to each coral genus that have been eaten by Culcita sp. and their average size (\pm SE). The growth morphology (B: branching, E: encrusting, M: massive, MSH: mushroom, F: foliaceous) and the relative percentage of the eaten colonies by growth morphology are also reported. The data shown in the table were obtained analysing 433 coral colonies eaten by Culcita sp.

Coral genus	% eaten (genus)	Average size (±SE) eaten colonies (cm)	Growth Form	% eaten	
Acropora	38.11	8.91 (±0.62)	В	74.37	
Pocillopora	36.26	6.97 (±0.34)	Б	74.37	
Pavona	12.47	7.44 (±0.61)			
Montipora	1.62	8.43 (±2.22)	Е	15.01	
Leptoseris	0.92	8.25 (±0.85)			
Porites	1.62	11.71 (±3.18)			
Galaxea	1.62	8.57 (±0.92)			
Favia	1.62	5.71 (±8.5)			
Hydnopora	1.15	6.8 (±0.37)		9	
Platygyra	0.92	6.75 (±1.03)	M		
Coeloseris	0.69	7.33 (±1.2)			
Isopora	0.69	9 (±3.51)			
Favites	0.46	7 (±2)			
Gardinoseris	0.23	4			
Fungidae	1.39	8.5 (±2.22)	MSH	1.39	
Pachyseris	0.23	9	F	0.23	

 $\textbf{Table 3.1.2} - \text{Ivlev's Index } (E_i) \text{ and Standardized Forage Ratio } (S_i) \text{ referring to the six most abundant coral genera in the study area.}$

Index	Coral genus	Coral genus							
	Acropora	Porites	Pocillopora	Favites	Montipora	Pavona			
Ei	-0.173	-0.885	0.761	-0.779	-0.352	0.576			
Si	0.057	0.005	0.592	0.01	0.039	0.298			

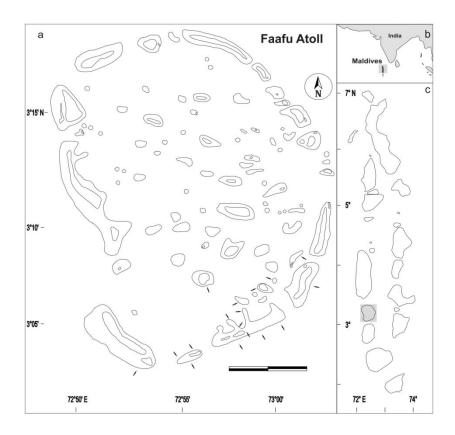


Fig. 3.1.1 – Map of the study area. Seventeen sites (indicated by arrows) were selected both on the inner and outer side of the Southern part of the Faafu Atoll (a), in the Republic of Maldives (b, c). Scale bar: ~ 10 km. Modified from Montano et al., 2017b.

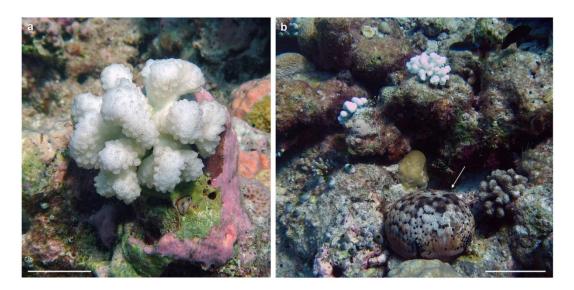


Fig. 3.1.2 –Small adult colony of *Pocillopora* spp. consumed by *Culcita* sp. (a). Note the absence of living tissue. Cushion sea star (indicated by the arrow) close to recently consumed coral colonies (b). Scale bars: ~ 4 cm in (a), and ~ 10 cm in (b).

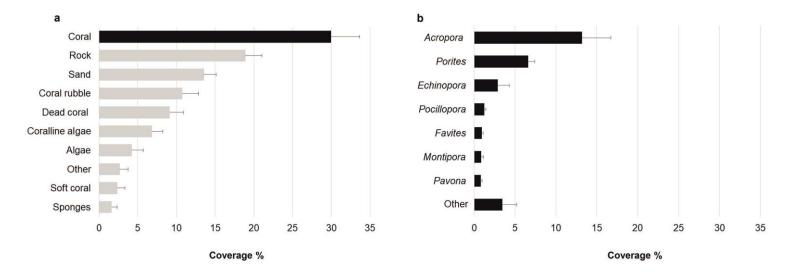


Fig. 3.1.3 – Overall coverage of the benthic categories (a) and coral genera (b) in the whole study area. Coral genera showing a coverage % lower than 0.5 were included in the category "Other" (represented by 30 coral genera). Data are expressed as the mean ± SEM.

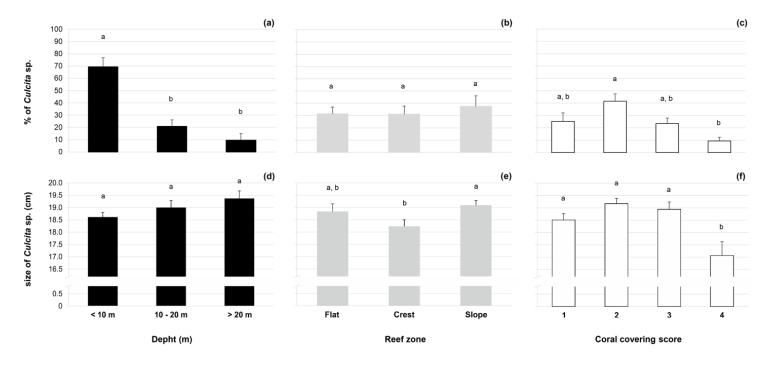


Fig. 3.1.4 – Mean percentage of abundance of *Culcita* sp. in three depth ranges (a), in three reef zones (b), and in 2 m radius areas around sea stars, showing 4 different coral cover percentages (1: coral coverage between 0 and 10%, 2: 10-30%, 3: 30-50%, 4: > 50%) (c). Data from 17 sites were analysed and percentages expressed as the mean \pm SEM (one-way ANOVA followed by Tukey's HSD multiple pair-wise comparisons). Average size of *Culcita* sp. (cm) in three depth ranges (d), in the three reef zones (e) and in areas showing different coral cover percentages (f). Data are expressed as the mean \pm SEM (one-way ANOVA followed by Tukey's HSD multiple pair-wise comparisons). In each graph, letters denote Tukey's significant difference among the different groups (p < 0.05), thus the same letter indicates no significant difference (p \geq 0.05).

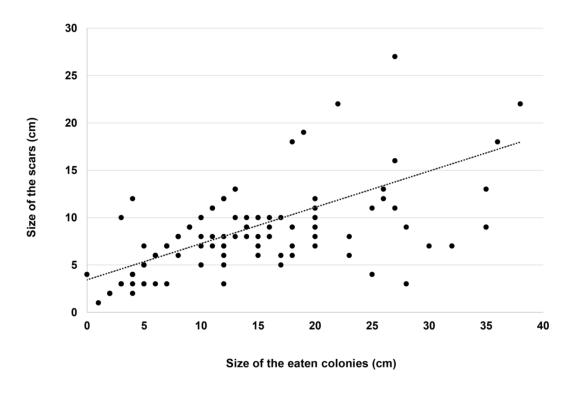


Fig. 3.1.5 – Relationship between the size of the scars produced by *Culcita* sp. on the preyed coral colonies and the size of the eaten colonies.

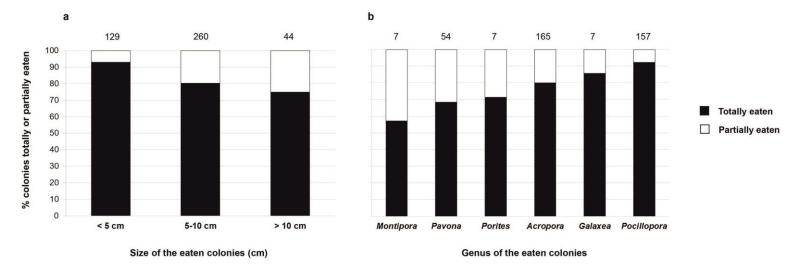


Fig. 3.1.6 – Relative abundance (%) of coral colonies eaten totally (in black) or partially (in white) by *Culcita* sp. based on their size range (diameter < 5 cm, between 5-10 cm and > 10 cm) (a). Numbers above each bar indicate the total number of eaten colonies (both totally and partially) per size range. Relative abundance (%) of coral colonies eaten totally or partially by *Culcita* sp. based on their genus (b). The coral genera reported in the graph are the only ones for which partially consumed colonies were observed. Numbers above each bar indicate the total number of eaten colonies (both totally and partially) per coral genus.

3.2 Another diner sits at the banquet: evidence of a possible population outbreak of Culcita sp.

(Agassiz, 1836) in Maldives

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3.2.1 Text

The cushion seastars *Culcita* spp. are facultative corallivores that can feed on different coral species. In Maldives, they

showed general preference for small coral colonies and recruits belonging to the genera *Pocillopora* and Pavona (Montalbetti et al. 2019). Contrary to what has been described for the most investigated coral predator Acanthaster spp., large population densities of Culcita spp. have never been recorded to date, since the cushion seastar abundances have been reported to be less than 0.5 individuals per 100 m2 on average, although these numbers can slightly vary from one place to another (Bruckner and Coward 2018). However, during different surveys performed from May to October 2018 in Faafu Atoll, Republic of Maldives, an unusual high density of cushion seastars was observed. Three belt-transects of 50 m x 4 m were performed on each reef in order to count the number of specimens within a unit area. In particular, a density of 42 seastars per 600 m2, thus meaning 1 specimen every 14.3 m2, was observed on the reef flat (~ 1 m depth) of Magoodhoo (Fig. 3.2.1A), an inhabited island located on the south-eastern region of the atoll rim whose reef is approximately 3 km long and 1.55 km wide. Other 37 seastars per 600 m2, meaning 1 seastar every 16.2 m2, were counted on the reef slope (~ 15 m depth) of Adhangau, an uninhabited island with a relatively smaller reef than the one of Magoodhoo, measuring 1.3 km in length and 250 m in width. These specimens were often found forming groups of several individuals close to small coral colonies presenting scars due to seastars predation (Fig. 3.2.1B). These colonies appeared white in color, without any living tissue, and often covered by a thick layer of mucus. These observations, along with other evidences previously reported from other reefs in Maldives (Bruckner and Coward 2018), meet the criteria of most of the definitions of Acanthaster spp. outbreak used to define these phenomena (Pratchett et al. 2014) and at the same time definitely exceed usual abundances of cushion seastars found on coral reefs (Bruckner and Coward 2018). For these reasons, we hypothesized that the large numbers of individuals of Culcita sp. described here may indicate the occurrence of a population outbreak.

Since the cushion seastar shows selective feeding, both from a dimensional and a qualitative point of view, preferring small colonies of few coral genera (Montalbetti et al. 2019), the impact of a possible population outbreak could severely affect the structure and the resilience of coral reefs. Thus, we suggest more detailed studies to better understand the population dynamics and clarify the possibility of an outbreak of the cushion seastar in Maldives.

3.2.2 References

Bruckner AW, Coward G (2018) Abnormal density of *Culcita schmideliana* delays recovery of a reef system in the Maldives following a catastrophic bleaching event. Mar Freshwater Res 70 (2): 292-301

Montalbetti E, Saponari L, Montano S, Maggioni D, Dehnert I, Galli P, Seveso D (2019) New insights into the ecology and corallivory of *Culcita* sp. (Echinodermata: Asteroidea) in the Republic of Maldives. Hydrobiologia 827: 353-365

Pratchett MS, Caballes CF, Rivera-Posada JA, Sweatman HPA (2014) Limits to understanding and managing outbreaks of crown-of-thorns starfish (*Acanthaster* spp.). Ocean Mar Bio 52: 133-200

3.2.3 Figures

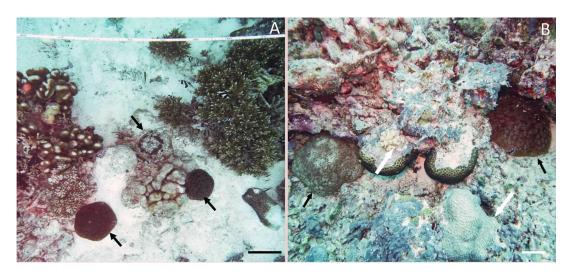


Fig. 3.2.1 (A) View of three specimens of *Culcita* sp. grouped together (indicated by black arrows) found on a reef flat in Magoodhoo island. Scale bar 15 cm. (B) Detail of two individuals found close to eaten coral colonies (indicated by white arrows). Scale bar 5 cm.

- CHAPTER 4 -

4.1 Assessing population collapse of *Drupella* spp. (Mollusca: Gastropoda) in the shallow reef after a catastrophic bleaching event in the Republic of Maldives.

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Keywords: Corallivory, Coral bleaching, Drupella, Population dynamics, Republic of Maldives

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4.2 Abstract

Corallivory, or predation of scleractinian corals, is responsible for changes in coral community structures. Among coral predators, Drupella spp. have been considered as potential delayers of coral recovery in the Republic of Maldives. However, very little information is available on the ecology of this gastropod. Population structure, distribution and feeding preferences of Drupella spp. was studied around 2 years after a mass coral bleaching event in 2016. We surveyed 6 shallow reefs in two atolls of the Republic of Maldives between October 2018 and March 2019. Drupella spp. resulted to be homogenously distributed in the study area, with significant differences only between localities which resulted to be significantly correlated with changes in coral cover rather than coral genera. In fact, feeding preferences confirmed the plasticity in changing coral genera in relation to the availability. Population size structure was dominated by adults suggesting low rate of recruitment in the last year and/or after the bleaching event in 2016. In general, our data showed an average collapse of the gastropod population density compared to the few previous data available in the Maldives. Thus, impact of *Drupella* spp. during the recovery of the reef appeared to be minimal with the population suffering from the disappearing of the primary resource of food and shelter caused by the temperature-induced coral bleaching in 2016. We also suggest to improve a monitoring program of this gastropod to better understand the dynamic of predation in relation of diverse origin of anthropogenic and natural impacts, and the concurrence effect of more corallivores in different reef health conditions.

4.3 Introduction

Coral reefs are among the most diverse and dynamic ecosystems on the planet, and critically important for providing ecological goods and services to human communities (Williams et al., 2019; Woohead et al., 2019). Yet reefs are continuously deteriorating due to anthropogenic and natural disturbances such as global warming, coral predators and extreme weather events (De'ath et al., 2012). Knowing the scale of these impacts is essential for driving well-informed management decisions on reef conservation (i.e. Hughes et al., 2017; Shiver et al., 2018). In particular, anthropogenic disturbances are reported to exacerbate the negative effects of coral predation (Rice et al., 2019). For example, the crown-of-thorns, Acanthaster spp., has been reported to cause high coral mortality during outbreaks, reducing up to 80% coral cover (Pratchett et al., 2014, 2017) with an increased effect when associated to temperature-induced coral bleaching (Saponari et al., 2018). Other corallivores have been reported as responsible of reduction in coral cover, among those, Drupella spp. feed exclusively on living coral tissue and it is known to cause large-scale disturbances to coral reef ecosystems (i.e. Bruckner et al., 2017; Koido et al., 2017). Drupella is a genus of marine gastropods composed by four species D. rugosa, D. cornus, D. fragum and D. eburnea (Claremont et al., 2011). These tropical gastropods occur on coral reefs of the Indo-Pacific Ocean (Claremont et al., 2011) at low densities (0-2 ind/m²; Cumming 1999, 2009a), although populations of Drupella spp. may display transitions between low density periods with outbreak and/or local aggregation events with up to 20 ind/m² or 250 ind/colony, respectively (Cummig et al., 2009a; Bruckner et al., 2017). Large aggregations of D. rugosa have been observed in the Indo-Pacific Ocean and well documented on the Great Barrier Reef (Cumming, 1999), while outbreaks of Drupella spp. were reported from a wide range of countries including Kenya (McClanahan, 1994), Western Australia (Ayling and Ayling, 1987), Hong Kong (Cumming, 1998; Morton and Blackmore, 2002), Red Sea (Antonius and Riegl, 1997), Thailand (Scott et al., 2017) and India (Marimuthu and Tripathy, 2018). These events appeared to be caused by overfishing of natural predators, high coral mortality, changes in temperature and salinity (Turner, 1994a,b; Cumming, 2009a, b), although the causes are complex and still not completely understood (McClanahan, 1994, 1997; Lam et al., 2007; Ratianingsih et al., 2017). When these events occur, the damage on coral reefs may be severe reducing coral cover by 35 to 70% (Cumming, 2009). Furthermore, impacts mediated by corallivores may synergically act with other coral stressors exacerbating coral mass mortality (Pisapia et al., 2016, 2019; Saponari et al., 2014, 2018) and delay reef recovery (Bruckner et al., 2018). Thus, both outbreak and aggregation events may affect coral reef communities by reducing reef resilience and recovery (Lam et al., 2007), causing population shifts and increasing disease incidences (Nicolet et al., 2013, 2018; Scott et al., 2017). However, most of the research on the ecology of *Drupella* spp. has focused mainly on these specific events with very few information regarding non-outbreaking or non-aggregating population (Turner, 1994a; Cumming, 1999).

Drupella spp. has been recently reported to aggregate in the Republic of Maldives concurrent to the coral bleaching event in 2016 by Bruckner et al. (2017), but very few information is available prior to this event (see Taylor 1978). The Republic of Maldives has been affected by a coral bleaching event in 2016 resulting in a loss of 30 up to 95% of corals (Perry and Morgan, 2017; Pisapia et al., 2019). In addition, the Maldivian coral reef is threatened by increasing tourism and coastal development (Brown et al., 2017), land reclamation (Fallati et al., 2017) and natural stressors such as diseases (see Montano et al., 2012, 2015a-b, 2016; Seveso et al., 2012). The impact of corallivores in Maldives has recently started to attract scientific attention focusing on outbreaks of Acanthaster planci (Saponari et al., 2014, 2018), the ecology of Culcita spp. (Bruckner et al., 2018; Montalbetti et al., 2019a, b) and the population dynamic of Drupella spp. straight after a bleaching event (Bruckner et al., 2017). However, generally the population dynamics of corallivores are still poorly described in the Maldives calling for more in depth research. Here, we aim at describing the ecological traits of Drupella spp., in particular, we surveyed a population around two years after a mass coral bleaching event to define population structure, feeding preferences and variation in spatial density in the shallow coral reef of the Republic of Maldives.

4.4 Materials and Methods

Variations in density, population structure and feeding preferences were assessed for the corallivorous gastropod *Drupella* spp.. Although both the species *D. rugosa* and *D. cornus* are reported from the Maldives (Taylor, 1978) the in-field identification was difficult since the shells of

the specimens were often heavily encrusted with coralline red algae and different epifauna. Thus, to avoid misidentification, gastropod snails were identified to genus level and considered as *Drupella* spp. (Fig. 4.1).

Surveys were performed in a total of 6 sites in two different Atolls (3 in Ari Atoll and 3 in Faafu Atoll) in the Republic of Maldives (Fig. 4.2) between November 2018 and March 2019. Sites were randomly selected according to accessibility and surveyed by snorkelling between 0–5 m, in accordance with other studies who found *Drupella* spp. mainly in the shallower reef (Taylor, 1978; Turner, 1994a; Cumming, 2009; Hoeksema et al., 2013; Bruckner et al., 2017; Koido et al., 2017; Scott et al., 2017; Hamman, 2018; Marimuthu et al., 2018)

To evaluate density variation within and among sites belt transects of 200 m^2 (50 x 4 m each) were conducted both on the reef flat (0-2 m) and along the reef crest (2-5 m). The belt transects were randomly placed parallel to the shoreline and spaced 10-30 m apart. Each individual encountered in each belt transect was counted and removed from the position to avoid recount bias. In addition, size data was collected by measuring the long axis of each shell, from the apex to the tip of the siphonal canal, to the nearest cm. The data was pooled into three size classes comprising recruits (< 1cm), juveniles (1-2cm) and adults (> 2cm), following Turner (1994). The Hartigan's dip test (Hartigan and Hartigan, 1985) was used to test the size distribution for unimodality. The distribution is considered unimodal in the null hypothesis, thus, a rejected p value (< 0.05) suggests a multimodal, or at least bimodal, distribution. The statistical analysis was performed using RStudio (R Core Team 2017) with the dip.test package (Maechler, 2016).

During the survey, it was also noted whether an individual showed preying, stationary or moving behaviour. When a fresh feeding scar was found on the coral the individual over or in the proximity of the scar was considered 'preying', while if no scar or old scars were found or the individuals were hidden at the base of the branches they were considered 'stationary'. The individual was considered 'moving' when found on a different substratum than live coral, since they need to move from one colony of coral to the other to find shelter and food (Cumming, 1999; Morton et al., 2002). In addition, the substratum was noted specifying the genus of corals, when the individuals were found on a coral colony, or other substrata, such as coral rubble, dead coral and sand. Size, morphology and genus of the coral preyed upon by *Drupella* spp., with old or fresh scars, were

noted. A further general qualitative impact assessment was performed and the maximum diameter of the scar area and the coral colony were measured. The scar size was then divided in two categories, specifically >50% or <50% of the size of the coral colony.

Furthermore, we investigated the feeding preferences of *Drupella* spp. following Saponari et al. (2018). The Ivlev's (1961) electivity index (*Ei*) was used with the formula:

$$Ei = (ri - pi) \div (ri + pi)$$

For this equation *ri* represent the proportion of prey in the diet and *pi* represent the proportion of prey in the environment. Values of *Ei* range between 1 (maximum preference) and -1 (maximum avoidance), whereby 0 indicates random feeding. We calculated *ri* as the frequency of snails on a specific *i* genus or family of corals in relation to all the preyed colonies. After, we obtained *pi* as the mean relative abundance of each single genus or family of corals in the environment. Data for the abundance of corals in the environment were collected by using the Line Intercept Transect (LIT) method with each transect of 10 m long and spaced 10 m apart, on the same belt transect used for abundance and size structure. Benthic categories were selected following Saponari et al. (2018), specifically: corals, coralline algae, coral rubble, dead coral with algae, gorgonians, macro algae, sand or pavement, soft coral, sponge, Tunicata, turf of algae, zoantharian and unknown. The genus level was added within the category 'corals'.

Statistical comparisons of the spatial variation, number of individuals per aggregation within genera, morphology, locations, differences in shell size between locations and differences in coral cover between locations were analysed using the Mann-Whitney U-test and the Kruskal-Wallis test with Tukey's post-hoc test because data failed in meeting the normality assumption (Zar, 1999).

Comparisons within coral genera occupied by *Drupella* spp., the number of individuals per aggregations and occurrence of aggregations on different coral morphologies, genera and locations were analysed with Chi-square goodness-of-fit tests. Spearman's rank correlation was used to examine whether density of *Drupella* spp. was related to benthic categories and to the number of genus on each location in order to verify which variables among benthic coral or occurrence of

genera drives *Drupella* spp. density. Statistical analysis was performed using SPSS ver. 24 (IBM, New York). All data is represented as arithmetic means ± standard deviation.

4.6 Results

During the study period, the monitoring survey for the population of the snail *Drupella* spp. was performed with a total of 72 transects covering an area of 1.44 ha. We counted 651 individuals of Drupella spp. with an overall mean density of 9.04±19.72 ind/200m². The distribution of the snail resulted to be homogeneous at large spatial scale, as highlighted by the U-test, which didn't show significant differences in densities between Ari and Faafu Atoll (U=741.5, z=1.103, p=0.270). Similarly, no significant differences were found comparing the density of individuals on the flat and the crest reef area (U=762, z=1.351, p=0.177) and the comparison within flat and crest area in each single location (U-test, p>0.05 for all comparisons). Contrarily, at location scale, the distribution of individuals varied significantly (Fig. 4.3). Thudufushi, Magoodhoo and Adhanga presented the highest density with 22.8±26.9, 22.3±33.3 and 5.25±5.6 ind/200m² respectively, while Drupella spp. occurrence was lowest in Athuruga with 0.25±0.9 ind/200m² (K-W, p<0.001). The data on coral cover, from 216 LIT pooled, showed that benthic coverage was mostly dominated by dead corals (50.23%±29.90) and coral rubble (35.01%±30.44) with alive coral coverage accounting for 8.58%±13.24. Further, coverage of alive coral resulted to be significantly different between locations (K-W, p<0.001; Fig. 4.4) with Magoodhoo, Thudufushi and Adhanga showing the highest values (14.49%±18.11, 13.85%±9.37, 13.02%±9.2, respectively) and Maaga showing the lowest (0.74%±1.25). The number of genera in each location didn't correlate significantly with the density of Drupella spp. (Spearman's rho $\rho = 0.771$, p = 0.72). While, a significant positive correlation was found between density of *Drupella* spp. and alive coral coverage (Spearman's rho ρ =0.546, p<0.000), with the snail density negatively correlated with the presence of macro algae (Spearman's rho ρ =-0.330, p=0.005) and dead coral (Spearman's rho ρ =-0.266, p=0.024). Prey preferences changed in different locations following the availability of coral prey, *Pocillopora* had a positive index value for Bathala, Thudufushi, Adhanga and Maaga, while Acropora for Bathala and Thudufushi and *Porites* for Athuruga and Magoodhoo. The genus *Payona* had a positive index only for Bathala and the genus *Hydnophora* only in Adhanga. All the other cases for all the genera considered were negative values indicating avoidance (Tab. 4.1). Looking at the pooled data, the electivity index (Ei) showed a positive value for the genus *Pocillopora* and *Acropora*, 0.35 and 0.17 respectively, while random choice resulted for *Porites* with 0.002. Negative value resulted for *Pavona* and *Hydnophora*, -0.75 and -0.79 respectively (Fig. 4.5). In addition, the qualitative assessment of the impact of the predation was not found to be lethal at the moment of the survey, since 82% of the occupied colonies were damaged by less than 50% of the colony size and only 1 colony was completely wiped out.

The size of the preyed upon coral colony varied from 8 to 500 cm in maximum diameter. The size class most represented was 25-35cm (17.9%), followed by 15-25 and 35-45cm (15.38% and 10.86%, respectively). Notably, no individuals of *Drupella* spp. were found preying upon coral recruits <5cm in maximum diameter, despite their occurrence in every location.

Individuals of *Drupella* spp. were mostly found on live coral colonies (85.6%) although few individuals were found on dead corals (13.2%) and coral rubble (1.2%). The snails were mostly preying, or have recently preyed, on the corals (62.5%) leaving a fresh scar with visible damage once removed from the colony. 31.2% were found hidden between the branches at the base of live coral colonies, while the remaining 6.3% were found on different substrata while moving, likely searching for other coral colonies. The snails were found on 232 colonies of corals, and considering the whole dataset pooled, the genus *Porites* was the most frequently occupied (43.7%) followed by *Pocillopora* (33.4%), *Acropora* (22.4%), *Pavona* (1%) and only 1 individual was found on a single colony of the genus *Hydnophora* ($\chi^2_{(4)}$ =440.708, p<0.000).

Drupella spp. was found aggregating on 44.8% of the colonies, with a minimum of 2 and a maximum of 62 individuals (Fig. 4.6), which were found on a single branching colony of the genus *Acropora*. Aggregations were mostly constituted of a few individuals, with an average of 5.03 ± 6.4 ind per aggregation and the majority in between 2 and 5 individuals ($\chi^2_{(13)}$ =148.808, p<0.000). The data didn't show significant differences in number of individuals per aggregation within different coral genera (K-W, p=0.525), morphology (K-W, p=0.658) nor for locations (K-W, p=0.796). Thus, the number of individuals was randomly distributed among 4 genera of corals (*Porites* spp., *Acropora* spp., *Pocillopora* spp. and *Pavona* spp.) and there was no preference in morphology nor

in locations. Furthermore, *Drupella* spp. was aggregating more frequently on branching corals (75%) than massive (10.5%), digitate (12.5%) or encrusting (1.9%) ($\chi^2_{(3)}$ =141.308, p<0.000). The aggregations occurred more frequently on *Porites* (50%) than *Pocillopora* (32.7%), *Acropora* (15.4%) and *Pavona* (1.9%) ($\chi^2_{(3)}$ =54.462, p<0.000) and more frequently in Magoodhoo ($\chi^2_{(4)}$ =88.212, p<0.000) than other locations.

Shell size was measured for all the individuals, ranging from 0.2 to 4.8 cm, and yielding a mean value of 2.62 ± 0.53 cm. The resulting population structure was not unimodal as determined by the dip test ($p<2.2x10^{-16}$). The snail population was constituted mainly by adults (>2 cm) with 90.3% of the individuals, while only the 9.1% were juveniles (1-2 cm) and 0.6% were recruits (<1 cm) (Fig. 4.7). Size was significantly larger in Faafu than Ari atoll (U=79998, z=2383, p<0.001) as well as on the crest area compared to the flat area considering the data pooled (U=51246, z=2231, p=0.027). The analysis revealed a statistical significance between locations (K-W, p<0.000), the post-hoc test suggested Magoodhoo as location with bigger shell sizes compared with Bathala, Thudufushi and Adhanga, as well as Adhanga compared to Thudufushi (K-W, p<0.05).

4.6 Discussion

The population of *Drupella* spp. was monitored and documented around 2-years after the 2016 large scale coral bleaching event. In this study, the total mean density of the snail resulted to be one of the lowest ever recorded compared to other works (e.g. Ayling and Ayling, 1987; Turner, 1994; Al-Moghrabi, 1997; Cumming, 1999, 2009a,b; Bruckner et al., 2017). Notably, it was around 70-fold less than the density reported by Bruckner et al. (2017), in South Malé Atoll, in 2016 (0.045 ind/m² in this study, against 3.4 ind/m² from Bruckner et al., 2017). This result suggests a possible collapse of *Drupella* spp. population in the aftermath of the bleaching event. Furthermore, no outbreak was reported as the mean density never exceeded the outbreak threshold of >2 ind/m² defined by Cumming (2009) and it was also lower than the level of 0.62 ind/m² suggested by Bessey et al. (2018) as the density beyond which consumption of prey is faster than its growth rate (specifically for *Acropora spicifera*). So far, no outbreak of *Drupella* spp. has been reported in the Maldives, in contrast to other countries of the Indo-Pacific Ocean (Ayling and Ayling, 1987; Antonius and Riegl,

1997; McClanahan, 1994; Cumming, 1998; Morton and Blackmore, 2009; Scott et al., 2017; Marimuthu and Tripathy, 2018).

Although not in outbreak, the population showed the presence of aggregations, the majority were small and constituted of 2 to 5 individuals, with a maximum of 62 ind and an average of 5.03 ± 6.4 ind per coral colony. The majority of the aggregations were found in locations (Magoodhoo and Thudufushi) with highest coral cover, those were also the largest found during the study, in accordance with the theory of optimal foraging, which predicts that predators will aggregate most likely in areas with high prey density (see Cumming, 2009a). Generally, the number of aggregations and the individuals per aggregation were lower compared to other studies (Cumming, 2009a,b) and especially compared to the findings of Bruckner et al. (2017) who reported aggregations of up to 250 individuals on a single coral colony with an average of 16 ± 13.3 individuals.

In general, it is possible that the population of *Drupella* spp. has decreased compared to the findings of Bruckner et al. (2017). The reduction in live corals caused by the bleaching event in 2016 may have influenced population dynamics of the snail which uses coral colonies as food and shelter (Hamman, 2018). *Drupella* spp. may have even moved to deeper areas of the reef looking for alive corals, since the shallow reef is also the more exposed to the bleaching event and thus with the highest coral mortality (Pisapia et al., 2018, 2019) although more research is needed to clarify this aspect. Additionally, the snail may be more exposed to predators (Hamman, 2018) and to population decline as reported also for other obligate corallivores such as the coral-dwelling crab *Trapezia cymodoce* (Stella et al., 2011), butterflyfish (Graham et al., 2009), the filefish *Oxymonacanthus longirostris* (Hobbs et al., 2010) and other coral-dependent fish (Booth et al., 2002).

Drupella spp. was found feeding only on scleractinian corals which is in accordance with its ecology as obligate corallivore (Morton et al., 2002; Rotjan and Lewis, 2008). Few individuals were found on dead corals and coral rubble. Morton et al. (2002) reported that the snails were strongly attracted by corals with fresh scars and the presence of conspecifics. Thus, the snails found on other substrata were probably moving in search for corals following scars or conspecific cues.

The snails were aggregating mostly on branching corals, particularly of the genus *Porites*, followed by *Pocillopora* and *Acropora*, which reflects also the general prey preferences in the study area, apart from the genus *Porties* which resulted a random choice. It is known that acroporids are the

favourite prey of *Drupella* spp. (Moyer et al., 1982; Fujioka and Yamazato, 1983; Turner, 1994; Cumming, 1999; Morton et al., 2002; Al-Horani et al., 2011), however, prey preferences may change according to the availability of corals. In Thailand *Drupella* spp. was reported to shift from acroporids to fungiid corals following a temperature-induced coral mortality event (Hoeksema et al., 2013; Moerland et al., 2016). Also, in the Maldives, the snail was found extending the prey range from *Acropora* and *Pocillopora* to more than 10 genera, including *Porites*, in the aftermath of the 2016 bleaching event (Bruckner et al., 2017). However, we found the snail occupying only 5 genera of corals, likely due to the highest mortality of other genera which may have different susceptibility to heat waves (Pisapia et al., 2017, 2019) and the slower recovery from the mortality event (Pisapia et al., 2017).

In this study, looking at location scale, *Drupella* spp. feeding preferences changed following coral cover, with preference for *Porites* in locations with low coral cover such as Athuruga and Maaga, while *Acropora* and *Pocillopora* were preferred in locations with higher coral cover such as Thudufushi and Magoodhoo.

Size structure of the population surveyed revealed a dominant proportion of adults (90.3%) compared to juveniles (9.1%) and recruits (0.6%), with the presence of more than one generation as suggested by the dip test. Such results are in accordance with Bruckner et al. (2017), who found a majority of adults in the population of *Drupella* spp. in South Malé Atoll straight after the bleaching event, but with a percentage of juveniles of 38% which is higher than the juveniles percentage (9.1%) in this study. In a scenario of limited resource, such as a decreasing coral occurrence as food and shelter, aggregations may lead to intraspecific competition where size-dependent density tends to consist of the better competitors with large consequence on population dynamics (De Roos et al., 2003; De Roos and Persson, 2013). In this case, adults may be considered as better competitors dominating the resource and hence causing juvenile mortality (Nicholson, 1954; May et al., 1974; Furness and Birkhead, 1984). An additional explanation may be related to a lower rate of reproduction, the recruits were uncommon (0.6%) similarly to the findings (0.99%) of Bruckner et al. (2017). Such limitations in reproduction may be caused by the reduction of substrate for eggs capsule attachment. *Drupella* spp. lays egg capsules on the bare skeleton of corals (Sam et al., 2017;

Scott et al., 2017), but the reduction of prey presence and the increased competition with algae may lead to a reduction of space for the eggs and hence reduction in reproduction success.

The snail resulted to be homogeneously distributed along the surveyed area at atolls (Ari and Faafu) and reef scale. Contrarily, differences were found when comparing localities, likely because coral cover was differently affected by coral bleaching in 2016 in different localities (Pisapia et al., 2017, 2019). Generally, the density of *Drupella* spp. was higher in locations with higher coral cover, such as in Magoodhoo and Thudufushi, independently from the number of genera, as suggested by the Spearman's correlation probably due to the plasticity in shifting feeding preferences according to the availability of coral genera.

Drupella spp. is likely to reduce resilience of the reef when acting synergically with other factors of stress for the corals, including temperature-induced mortality, diseases, pollution or other corallivores (e.g. Nicolet et al., 2013, 2018; Scott et al., 2017; Bruckner et al., 2017). In this study, we showed that 2-year after the coral mortality event, Drupella spp. impact may be considered lower, possibly due to the collapse of the population. Further, the majority of the corals preyed upon were only partially damaged (<50%) at the moment of the surveys, indicating a lower effect on coral cover. In this condition, the presence of coral guards such as Trapezia cymodoce (Samsuri et al., 2018) or the presence of deterring hydrozoans (Montano et al., 2017) may also reduce the predatory pressure, although further dedicated research are needed to be verified. In addition, no coral recruits were preyed upon, despite their presence, indicating that reef recovery is on-going, unless other factors will threat coral survival, and that recruits seems to be safe from Drupella spp. predation.

In conclusion, *Drupella* spp. showed a large-scale homogeneity in density and only at reef scale a variation dependent from coral cover. The population collapsed and showed less aggregations, around 2-year after the coral bleaching event in the study area, compared to previous reports in the Maldives (Bruckner et al., 2017). Thus, the snail may represent a less impacting corallivore in a post bleaching condition, compared for example with the seastar *Culcita* spp., which is known to feed on coral recruits and delay reef recovery (Bruckner et al., 2018; Montalbetti et al., 2019a,b). Further research should focus on larger geographical areas, including other atolls both in the north and in the south of Maldives and expand to deeper areas of the reef to have a better overview of the snail

distribution. In addition, snail behaviour should be monitored including reefs with pre-bleaching coral cover to clarify the predatory activity and quantify the impacts with a focus on the co-occurrence with other corallivores in the Maldives.

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4.8 References

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4.9 Figures and Tables



Fig. 4.1 a) The picture was taken during the survey in Bathala. The specimens of *Drupella* spp. (highlighted by an arrow) were feeding on a colony of the genus *Pocillopora*. b) Upper and lower view of *Drupella* spp.. Bars in a) and b) indicate 1 cm.

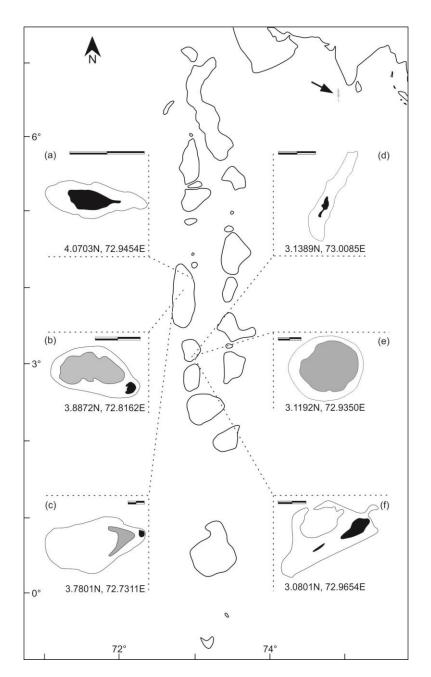


Fig. 4.2 Map of the study area located in Ari and Faafu atolls in the Republic of Maldives (black arrow). (a) Bathala, (b) Athuruga, (c) Thudufushi (Ari atoll), (d) Adhanga, (e) Maaga and (f) Magoodhoo (Faafu atoll). Solid lines represent reef limits, in black the islands and in grey the closed lagoon. Total scale bars are 0.5 Km

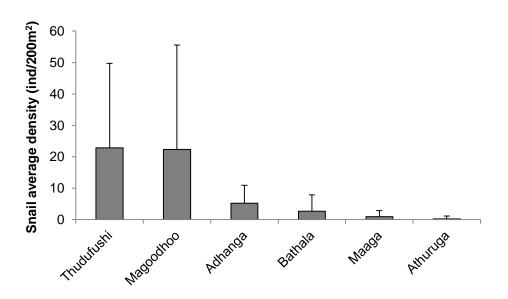


Fig. 4.3 Data showing the differences in snail density for each location. Bars indicate Standard Deviation.

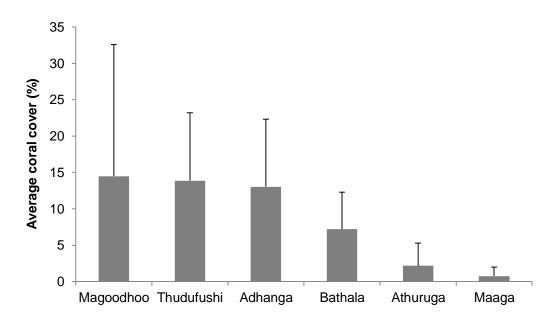


Fig. 4.4 Average coral cover in percentage for each locations. Bars indicate Standard deviation.

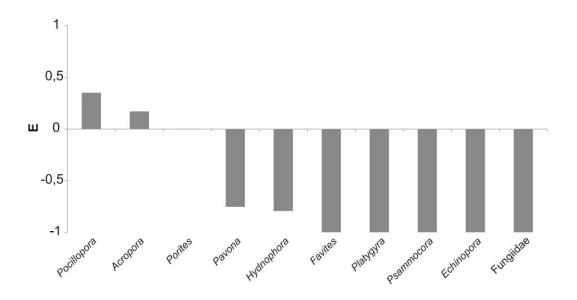


Fig. 4.5 Feeding preference of *Drupella* spp. according to the Ivlev's Electivity Index (E) for each genera or family pooled in the study area

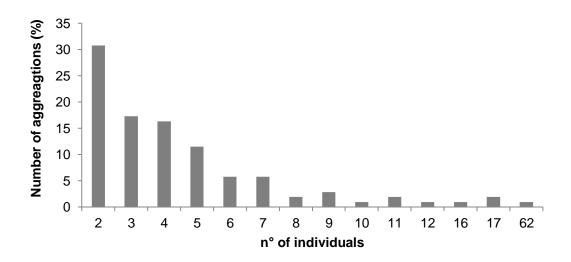


Fig. 4.6 Distribution of the percentage of aggregations following the number of individuals per aggregations.

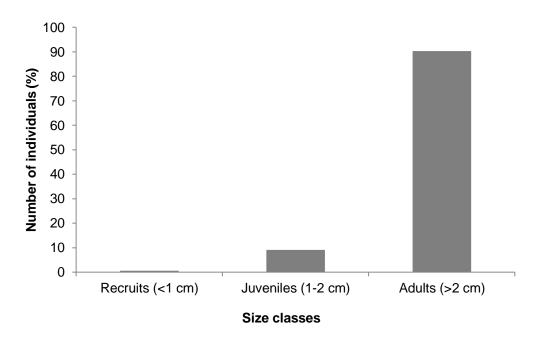


Fig. 4.7 Distribution of *Drupella* spp. divided into size classes following Turner (1994).

Tab. 4.1 Feeding preferences of *Drupella* spp. according to the Ivlev's Electivity Index (E) considering the genera preyed upon at each location. The genera *Pavona* and *Hydnophora* were absent in Maaga, thus no data (n.d.) were reported.

	Porites	Acropora	Pocillopora	Pavona	Hydnophora
Bathala	-0.77	1.00	0.75	0.45	-1.00
Athuruga	0.79	-1.00	-1.00	-1.00	-1.00
Thudufushi	-0.80	0.26	0.44	-0.39	-1.00
Adhanga	-0.24	-0.02	0.59	-0.80	0.24
Maaga	-1.00	-1.00	0.38	n.d.	n.d.
Magoodhoo	0.17	-0.16	-0.64	-1.00	-1.00

- CHAPTER 5 -

5.1 Anthropogenic Marine Debris assessment with Unmanned Aerial Vehicle imagery and Deep Learning: a case study along the beaches of the Republic of Maldives

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5.2 Abstract

Anthropogenic Marine Debris (AMD) is one of the major environmental issues of our planet to date, and plastic accounts for 80% of total AMD. Beaches represent one of the main marine compartment where AMD accumulates, but few and scattered regional assessments are available from literature reporting quantitative estimation of AMD distributed on the shorelines. However, accessing information on the AMD accumulation rate on beaches, and the associated spatiotemporal oscillations, would be crucial to refining global estimation on the dispersal mechanisms.

In our work, we address this issue by proposing an ad-hoc methodology for monitoring and automatically quantifying AMD, based on the combined use of a commercial Unmanned Aerial Vehicle (UAV) (equipped with an RGB high-resolution camera) and a deep-learning based software (i.e.: PlasticFinder). Remote areas were monitored by UAV and were inspected by operators on the ground to check and to categorise all AMD dispersed on the beach. The high-resolution images obtained from UAV allowed to visually detect a percentage of the objects on the shores higher than 87.8%, thus providing suitable images to populate training and testing datasets, as well as gold standards to evaluate the software performance. PlasticFinder reached a Sensitivity of 67%, with a Positive Predictive Value of 94%, in the automatic detection of AMD, but a limitation was found, due to reduced sunlight conditions, thus restricting to the use of the software in its present version. We, therefore, confirmed the efficiency of commercial UAVs as tools for AMD monitoring and demonstrated - for the first time - the potential of deep learning for the automatic detection and quantification of AMD.

5.3 Introduction

Environmental contamination generated by Anthropogenic Marine-Debris (AMD) represents one of the most ubiquitous and long-lasting environmental change of our planet (Laist, 1987; Ryan, 2015). AMD is responsible of several ecological, ecotoxicological, economic and social impacts. However, the extent to which it is harming wildlife and plants, endangering human health and reducing the availability of ecosystem good and services (Laist, 1987; Rochman et al., 2013; Hengstmann et al., 2017) is still to be properly understood and quantified (Eriksen et al., 2014; Thompson et al., 2009). It has been estimated that from 5 to 13 million tonnes of litter enter the oceans each year (Jambeck et al., 2015; Geyer et al., 2017) and that plastic accounts for over 80% of the total AMD (UNEP 2005; Laist, 2011; Thiel et al., 2013; Penca, 2018).

Plastic is persistent and for the most part (roughly 60%) less dense than seawater (Andrady, 2011; Ryan et al., 2009). Once introduced into the marine environment from multiple sources (both seaand land-based), buoyant plastic can be transported by surface currents and winds (Kako et al., 2010), recaptured by shorelines (Kako et al., 2014) or degraded into microplastic (Barnes et al., 2009; Cinner et al., 2018). Distribution and accumulation of plastic into the marine environment are indeed controlled by circulation patterns and prevailing winds, coastal and seafloor geomorphology (Barnes et al., 2009; Galgani et al., 2000; Savini et al., 2014) and anthropogenic activities (Ramirez-Llodra et al., 2013). Well known hotspots of accumulation include the sea surface, where aggregations of a large amount of persistent and light plastic take place at ocean gyres, creating giant "garbage-patches" (Eriksen et al., 2014; Law et al., 2010, 2014), but also submarine canyons, where litter originating from land accumulates in large quantities (Pierdomenico et al., 2019) and the shores, particularly beaches (Corcoran et al., 2009). Although data documenting the occurrence of plastic everywhere in the oceans (from the surface to the deep seafloor - Thompson et al., 2004 and Van Cauwenberghe at al., 2013) are quite exhaustive, a consistent quantification of the total amount accumulated within the diverse marine compartments, has not been accurately outlined. While reliable estimations have been provided for the giant surface garbage-patches (Lebreton et al., 2018; Eriksen et al., 2014), scarce information is available from the deep and poorly unexplored seafloor, but nonetheless for the shorelines, where only a few and scattered regional assessments

were provided (Martin et al., 2018; Vlachogianni et al., 2018; Andrades et al., 2016; Ebbesmeyer et al., 2012). Plastic accumulation on beaches may represent the terminal phase of oceanic transport or a transient stage with a successive washed to the sea following storms or tides movements (e.g. Shimizu et al., 2008). Knowing the accumulation rate on beaches and associated spatiotemporal oscillations would be a crucial information to refine global estimation on the dispersal mechanisms of plastic in the marine environment and its amount in each compartment. Most of our knowledge on the quantity of plastic accumulated on beaches, at different temporal scales, is based on sparse and regional monitoring activities, performed following different protocols and without standardized procedures, making difficult data integration and comparisons among regions (e.g. Galgani et al., 2015; Watts et al., 2017). Beach litter estimation, at places performed within the framework of dedicated monitoring activities (among others the Marine Strategy Framework Directive - Directive 2008/56/EC - Galgani et al., 2014), is also commonly subjective and timelabor consuming, since it relies on visual census where items are recorded along transects (Lavers et al., 2016; Layers and Bond, 2017). Only recently the use of aerial imagery has been proved to be an appropriate and efficient method to monitor beach litter (Kako et al., 2012; Kataoka et al., 2018; Sha et al., 2018; Deidun et al., 2018). In particular, the use of Unmanned Aerial Vehicles (UAVs) equipped with RGB cameras, beside the advantage of the low-cost, allows the collection of high resolution imagery data (i.e.: at centimetre level - Casella et al., 2016; Flynn and Chapra, 2014) over quite large areas (e.g. hundreds of hectares), also not easily accessible, with great flexibility in terms of time and frequency of data collection (i.e. decades of hectares per day), and under conditions where satellites would be of limited use (i.e.: high cloud cover, limited image resolution). Nevertheless, estimation of beach litter from RGB imagery of various sources (UAVs included), over large and even remote areas, still requires standardization of sampling techniques and data processing. Also, objective identification of plastic items on aerial imagery, based on automatic image classification is a novel field of investigation. To the best of our knowledge, only one work has been recently published on the use of UAV Remote Sensing combined to Artificial Intelligence (AI) for beach-litter monitoring by Martin et al. (2018). They proved the ability of machine learning (ML) in performing less time-labour consuming (40 times faster than humans) and subjective

methodologies to detect AMD, but the best sensitivity reported in Martin et al. (2018) for AMD automatic quantification was low (i.e.: 44%).

In our study, we therefore focused on the improvement of the sensitivity of the AI algorithm and the associated positive predicted values, which account for the false positive AMD. For this purpose, we provided a deep learning, rather than a random-forest, machine-learning approach, as previously implemented by Martin et al. (2018), being deep learning more beneficial for object detection (LeCun at al., 2015; Chollet 2017; Guest at al., 2018). An essential output of our work is, in addition, the formulation of a combination of protocols to automatically detect and quantify beach litter along the shores of selected remote islands in the Republic of Maldives, defined by the 2010 UNPD's Assessment of Development Results a vulnerable "Small Island Developing State (SIDS)". The protection of the environment from pollution is indeed extremely important for SIDS as, aside from other reasons that are common to all countries, two important industries (tourism and fisheries) depend on a pristine environment (UNEP, 1999).

Our study proposes an ad-hoc combination of protocols to: 1) collect UAV-images suitable for the training of a deep-learning algorithm, 2) provide smart gold standards to estimate the algorithm performances, 3) train and test the deep-learning algorithm in near real-time conditions. We believe that our work could be useful to propose new best-practices for applying deep learning to automate the procedure of litter detection and quantification by UAV systems on beaches, which in turn could offer an instrumental tool for sustainable solid waste management.

5.4 Materials and Methods

5.4.1 Study Area

The case-study area consists of different islands of the Republic of Maldives (Fig. 5.1a), an archipelago composed of 1192 atoll islands stretched for 860 km, in North-South direction, located in the middle of the Indian Ocean. The islands are grouped in 20 administrative atolls and divided under three distinct categories: inhabited, uninhabited and resort islands (Fallati et al., 2017). The archipelago, with its peculiar geographical location and its 644 km of coastline, represents the

perfect place for the deposition of plastic debris that are drifted from the surface currents of the Indian Ocean (Barnes, 2004). In addition, local sources of littering are represented by the waste production on the inhabited islands and those discharged into the sea from the numerous boats that daily cross the atolls. In the Maldives, the high dispersion of land mass and population, both of them spread over a distance of 860 km, creates a negative effect on solid waste management issue. With the exception of resort islands, which represent the 6% of the total archipelago surface and where beach clean-up is a daily routine operated by resort employees, most part of the coastline of each Maldivian island, is indeed covered, to an undefined extent, by AMD coming from different sources (Fig. 5.2).

As testing regions for our study, we selected three different coastal areas. Two of these islands (Adangau and Jinnathuga) are in Faafu Atoll, one (En'Boodhoo) is in Alif Dhaalu Atoll (Fig. 5.1b, c). The three islands were chosen as representatives of small-size uninhabited islands of the archipelago, where beach-cleaning cannot be guaranteed by resort employees or government personnel. En'Boodhoo is an island of 1.8 ha, located in the western lagoon of Alif Dhaalu Atoll. The island is desert, and the human presence is mainly related to the safari boats that stop nearby, and to touristic picnic and barbeques on the beach. Adangau (1.1 ha) and Jinnathuga (1.9 ha) are two islands of Faafu Atoll located in the Atoll's eastern lagoon. These two are similarly used as picnic island from the inhabitants of the Atoll. The primary sources of litter on these islands are both the direct release of waste and oceanic transport. The target testing area within the islands was selected as a portion of the beach with direct access to the sea, different exposition to the winds and currents, with the presence of psammophytes plants and natural debris (leaves, roots and twigs) as well as litter.

5.4.2 Aerial Surveys

5.4.2.1 UAV

In order to achieve large-scale reproducibility of a protocol to collect UAV images, we propose to use a consumer-grade UAV, equipped with a high-resolution RGB camera, to survey the study area.

For this purpose, we used the DJI Phantom 4 drone, a quadcopter with high sensing qualities, equipped with a 1/2.3" CMOS camera sensor (12.4 MP) that can collect images with a resolution (R) of 4000x3000 pixels and an integrated GPS/GLONASS system. Compared to fixed-wing UAVs, that can cover with a single flight a larger area and can handle a higher quality camera, Phantom 4 is lightweight, easy to carry, and can smoothly fly at low altitude to obtain good ground-resolution images. Moreover, easy take off and landing procedures make this drone an outstanding, cost-effective solution for low altitude and short-range studies. All the metadata are recorded in an EXIF (Exchangeable Image File Format) file, which includes information on the pictures such as shutter speed, apertures, ISO and GPS coordinates (latitude, longitude and altitude). Flight time with a single battery is roughly 25 min.

5.4.2.2 UAV Survey Protocols

Three different altitudes, namely 10, 15 and 35m were considered to define the optimal protocol in terms of image quality and number of images required to cover the area of interest (AOI):

1) ground sample distance (GSD) being defined as:

$$GSD \ mm/pix = \frac{SW \times FH}{FL \times IW}$$

where SW is the sensor width, FH is the flight high, FL is the focal length of the camera, and IW is the image width (Ventura et al., 2018), and

2) number of images of interest (IOI) being defined as:

$$IOI = \frac{AOI [m^2]}{D [m^2]} = \frac{AOI [m^2]}{R [pixels^2] GSD^2 [m^2/pixels^2]}$$

where D is the dimension of the area covered by a single image at a specific GSD, and R is the resolution in terms of pixel of the images, as defined in paragraph 2.2.1.

The surveys were planned using DJI GS PRO (www.dji.com/it/ground-station-pro) a free Ipad application released by DJI. This app allows designing all the aspects of the drone mission: generate optimal flight paths, set camera parameters and directly monitor data acquisition on the Ipad screen. For all the surveys we set a fix flight altitude with a frontal and lateral overlap of 80 % and 70 %, respectively, a -90° gimbal angle (nadir orientation), a shooting interval of 2 seconds (equal time interval mode) and a constant velocity of 1.3 m/s. Before starting the UAV overflight weather condition (wind speed, cloud coverage) and the presence of obstacles along the path was checked. A metric tape was laid on the beach (e.g. for several meters), in order to check the spatial accuracy of the orthomosaic during the postprocessing. Once all the parameters were set, the UAV automatically took off and completed the mission (e.g. trajectory in Fig. 5.3 a, b).

5.4.2.3 Reconstruction of the AOI

The images were processed by Agisoft PhotoScan (www.agisoft.com), a commercial Structure from Motion (SfM) software, widely used by the scientific community for its user-friendly interface, spontaneous workflow and the excellent quality of the point cloud output (Burns at al., 2017; Cook, 2017; Bonali et al., 2019). The process is dived in three main steps (Fig. 5.3c): drone photos alignment using high accuracy setting; high-quality dense 3D point cloud generation; creation of a Digital Terrain Model (DTM) from the dense could. As final outputs we obtained, from the DTM, orthomosaics with a GSD of 4.4 mm/px, 8.2 mm/pix and 14 mm/pix respectively for the three flights altitude (10, 15 and 35 m). The models are geo-referenced thanks to the coordinates stored into the EXIF files of each image. For more extensive information on the process, see Verhoeven (2011) and Ventura et al., (2016). The orthomosaics generated from images collected at 10 m altitude, were considered as our AOI and used for data assessment.

5.4.3 Gold Standards

5.4.3.1 In-Situ Ground Assesment

We performed an in-situ ground assessment (GA) of the AOI, aimed at quantifying the AMD on the studied shorelines in order to calculate the efficiency of the UAV survey-protocols. This quantity represents our first Gold Standard (GA-GS). As a first step, a recognition of the investigated shorelines was conducted to detect AMD. The items found during the inspection were counted and classified by the operators into different subtypes (Table 5.1). The category named "other" refers to objects and fragments that were smaller than 5 cm, which is the minimum size of the target objects that we decided when defining the optimal protocol described in terms of the image quality. Then, these objects were re-arranged in the specific AOI to be monitored by UAV overflights.

5.4.3.2 Image Screening

In order to evaluate the quality of the images, a comparison was made between the number and type of items counted by the operators during the in-situ GA on the beach, and the number and type of items counted by an operator during an image screening (IS) of the orthomosaic on a PC. The AMD recognised via IS represents our second GS (IS-GS), and a smart best-estimate of the actual GA-GS. Indeed, proving the feasibility of identifying and estimating the AMD by screening images collected by UAVs, rather than by operators on the ground, is crucial to choose the optimal UAV survey-protocol for the collection of images suitable for deep learning, and to establish procedures that allow avoiding the time-consuming GA of the operators.

5.4.4 The Deep-Learning Algorithm

In order to allow easy access to AI non-expert users, we used a commercial software – PlasticFinder (Italian software license 012677 D011755, DeepTrace Technologies, www.deeptracetech.com/) – to detect and quantify AMD. The core algorithm of the software is a deep-learning convolutional neural network (CNN). CNNs are a class of multilayer architecture suitable for processing RGB images for classification and object detection tasks, where the stack of convolutional layers allows for translation invariance – i.e. the net is trained to recognize an object independently of its position within the image.

The adoption of a deep learning approach has one main motivation. In order to provide a tool that could favour a scalable approach, i.e. adaptable to different scenarios, a large image-database was needed to provide a general training set, i.e. a set of images to let the algorithm learn the classes of interest. The main advantage of deep learning is that it automates the most critical part of a ML workflow: the feature extraction. In contrast to conventional ML methods (e.g. Random Forest, Support Vector Machine, Gradient Boosting Machines), that require hand-design features as input, a neural network is made of trainable multilayers that learn automatically the features through geometric transformations and gradual adjustments of learning weights with respect to a feedback signal, thus being more suitable than conventional ML for large dataset training (LeCun 2015, Chollet 2017). The PlasticFinder CNN has been tailored for 5 classes of images, namely: "vegetation", "sea", "sand", "AMD" and "other" (i.e. sand with small pieces of wood, stones, algae). UAV images obtained from the survey of Jinnathuga island were used for the collection of the training set (Fig. 5.4).

The island was chosen on the basis of the fact that all the classes of interest were present. Therefore, we selected training images, within the AOI, representing the classes of interest. For each class, a balanced number – of the order of thousands – of different samples was collected, in order to tailor the algorithm on the specific experimental settings. A subsample of UAV images (N=3) collected on the other two islands, Adangau and En'Boodhoo, were used for the testing set. The surveys of the testing-set islands were finalised at different experimental conditions (Table 5.1) which allowed to investigate the influence of climate, light and shadow on the efficacy of the algorithm. When a tested image is input in the software, it returns pixel-wise classification heatmaps, representing a pixel probability-map for each class, and a bounding-boxes map with the detected AMD.

The performance of the automatic detection, classification and quantification were measured by comparing the results with the two GS. The metric is expressed in terms of true positive (TP), false negative (FN) and the false positive (FP) items, rather than in terms of pixels, for an easier interpretation. The statistical measure of the performances is expressed through the *Sensitivity = TP* / (TP+FN), the Positive Predictive Value PPV = TP/(TP+FP), and the harmonized mean of Sensitivity and PPV, given by the F-score = 2TP/(2TP+FP+FN).

5.5 Results

5.5.1 Optimization of UAV Survey Protocols

The optimal protocol for the UAV survey, as a compromise between image resolution and number of IOI to cover the AOI, was found at a UAV altitude of 10 m, corresponding to a GSD of 4.4 mm/pixels. The know dimension of the objects (metric tape) in the orthomosaics strongly matches the true dimensions measured on the beaches with an average accuracy of ≈1mm. Table 5.1 reports the different AOI covered following the optimal protocol for three selected islands, namely Jinnathuga, Adangau and En'Boodhoo islands, and the corresponding number of minimum IOI.

5.5.2 Gold Standards

Table 5.1 reports the experimental results of the in-situ GA and of the IS of the AOI. The matching scores express the ratio of the AMD found via IS to the AMD found during the GA. This score accounts for the estimated error in the use of the GS produced via IS.

5.5.3 The Deep-Learning Algorithm

5.5.3.1 Training, testing and performance

The training of the tailored CNN, performed on images from Jinnantuga-island (Fig. 5.4), achieved a validation accuracy higher than 95%. Adangau and En'Boodhoo-islands images (Fig. 5.5-a. – Fig 5.6-a.) were used to test the algorithm. A pixel-wise probability heat-map of each input image has been obtained by the software, as well as a bounding-boxes map for the detected AMD (Fig. 5.5-a. – 5.6-a.). In particular, for each pixel, a probability is given to be classified as AMD, thus allowing a visual understanding of the specific areas that might be subjected – with a different probability of risk exposure – to the presence of plastic debris.

Figure 5.5-c. and 5.6-c., shows bounding-boxes maps with all AMD detected. Table 5.2 reports the numerical results obtained by comparing the software output and the IS-GS for each image. The results highlight the average software-performance for En'Boodhoo drops of a factor of about 3 with respect to the Adangau case.

Therefore, these results give the evidence that the collection of UAV- images suitable for the training and testing of the deep-learning algorithm, should rely on specific recommendations regarding the optimization of the UAV survey, the collection of the GS, and the development of the algorithm itself.

5.6 Discussion

5.6.1 The UAV survey and the AMD detection

The low cost, the high resolution and the high flexibility of UAVs quickly turned out to make them extremely versatile and useful tools for the investigation and analysis of a number of environmental issues. Small UAVs are used indeed with increasing frequency, in many research activities with applications in different fields: structural geology (Bonali et al., 2019), forestry sciences (Baron et al., 2018; Mlambo et al., 2017), mapping of sensitive marine habitats (Ventura et al., 2018), marine megafauna surveys (Colefax et al., 2018; Kiszka et al., 2016), coral bleaching detection (Levy et al., 2018). These platforms, especially the commercial drones, are proving to be useful tools for high-resolution remote sensing data collection, especially because of their small size, the increased lifetime of the batteries and the possibility to plan autonomous flights with user-friendly ground station software. Moreover, SfM algorithms allow obtaining accurate Digital Terrain Models (DTMs) and orthomosaics over large areas.

AMD was monitored worldwide through aerial surveys, along the beaches, since 2012 (Kako et al., 2012; Deidun et al., 2018; Kataoka et al., 2018; Martin et al., 2018; Sha et al., 2018) but explored locations are still limited (Fig. 5.7). Besides, more significant, it is the absence of a standardized protocol for data acquisition and elaboration. Previous studies, performed using a balloon equipped with a digital camera (Kako et al., 2012) and aerial photographs (Kataoka et al., 2018), faced

problems related to the orthorectification and to the pixel-size of the images: a GSD of 10 cm/pix allowed identifying only groups of debris and not the single objects. The recent adoption of UAVs for AMD monitoring overcome a number of limitations mainly related to the flight altitude and to the GSD, to the orthorectification of the images, and to the repeatability of the surveys in a short time. However, the data-processing procedures are not uniform, ranging from visual interpretation of the images (Deidun et al., 2018) and spectral profile analysis of litter (Sha et al., 2018), to the use of machine learning methods (Martin et al., 2018). The use of AI classifiers (Martin et al., 2018) is more complex for different reasons, among which the lack of publicly available large databases (providing adequate images to train algorithms) is notable. The difficulty of developing scalable approaches, i.e. procedures that do not depend on local environmental constrains, is also a major issue for the use of AI classifiers.

The advantages in using UAVs, in terms both of resolution and monitoring repeatability, match perfectly with the need of understanding the pattern of aggregation in a remote area such as the Republic of Maldives. Here, a considerable amount of marine litter has been reported, despite the remoteness of the location (Imhof et al., 2017). However, Imhof et al. (2017) highlighted the need for a robust protocol, allowing extensive sampling in space and time to collect scientifically sound data (Imhof et al., 2017). Remote-sensing studies, related to the accumulation and transportation of AMD, were not conducted before this work in the Republic of Maldives (Fig. 5.7). The lack of such monitoring studies for this area is significant, considering that plastic debris from the rivers of South Asia contributes to 67% of the global annual input (Lebreton et al., 2017) and that countries on the Indian Ocean are among the principal producer of mismanaged plastic waste (Jambeck et al., 2015). Besides, it is not clear where all this plastic, that should accumulate in the Indian Pacific, gyre is going (Mheen et al., 2019). Thus, the proposed methodology will improve and standardise the data collection of marine-litter accumulation on beaches and shorelines, gathering valuable and comparable data, even in remote and isolated areas.

The results of our study confirm most of the advantages of using a consumer-grade drone to carry out environmental monitoring. In particular, the use of a DJI Phantom 4 drone allowed to speed up considerably the standard walking beach survey and to access remote areas such as Maldives.

On the surveyed islands, anthropogenic debris were found everywhere: on the water's edge, just left there from waves and tides; on the upper part of the beaches and in the bushy coastal vegetation, likely carried there from storm tides and winds, or left by local tourists. The selected GSD allowed the identification and categorization of debris for each single detected item, making our remote observations comparable to the ones performed by operators on the ground. In fact, the matching between the ground-assessment and the visual screening of the images is higher than 87% (Tab. 5.1). In addition, the results of our survey protocol shows that the majority of the detected objects (Tab. 5.1) were plastics bottles and aluminium cans. Particularly abundant were also flip flops on En'Boodhoo and Adangau. These three categories of debris were observed with different degradation level: from brand new, with labels and the colours still intact, to partially disrupted. This can indicate the heterogeneity of the sources: some of them can be just washed up on the shore from the closest inhabited island or discharged from boats that passed nearby; others may have float in the ocean for thousands of kilometres before reaching the shore. Instead, the higher presence of plastic bags (foods wraps and plastic bags) on Adangau island is most probably due to the use of the island as a picnic and barbeque location from the inhabitants of the atoll.

The graphical outputs and the numerical results for Adangau Island show good performances (Figure 5.5, Table 5.2) and, in particular, in the face of an average sensitivity of 67%, the average PPV reaches 94%. This means that the deep-learning algorithm performance is affected by a non-negligible number of FN items – impacting on the sensitivity – but also that, on the other hand, the software is highly specific in the ability to recognize AMD with respect to FP items. In addition, it should be noted that, for Adangau island, the software performance is quite stable, even when the number of real AMD-items on the beach increases (Table 5.2). The pixel-wise classification heatmaps for En'Boodhoo-island, shown in Figure 5.6-b, qualitatively confirm the good ability of deep learning algorithm, in recognizing the presence or absence of elements belonging to the "sea" class. Also, the software correctly reports on a low-probability with respect to the presence of vegetation (below 30%). On the contrary, the heatmaps for the other classes draw attention to issues that become evident when looking at the zoomed bounding-boxes maps in Figure 5.8, and to the quantitative results shown in Table 5.5.

In this case, the average software-performance for En'Boodhoo drops of a factor of about 3 with respect to the Adangau case. This limitation can be explained by considering the different lighting conditions. In particular, Adangau-island images were collected at 12 am of a sunny day, therefore with similar sunlight-conditions of the testing set of Jinnathuga island. On the contrary, En'Boodhoo images were collected at 5 pm, and the shadows in the proximity of footprints (Fig. 5.8) or of real AMD, represent pitfalls for the algorithm, as clarified by the high number of FP items in Table 5.2. As a matter of facts, the software was not trained to recognize footprints or shadows, and therefore such a limitation restricts the use of PlasticFinder, in its present version, to specific sunlight-conditions. For these reasons, we suggest conducting the survey with the sun high on the horizon, in order to avoid excessive shadows on the surveyed areas. However, to date, and to the best of our knowledge, there is only another algorithm presented to the scientific community, that has been developed for the specific purpose of automatically detect and quantify AMD along the shores by using a combination of UAV images and AI. In their pioneering work, Martin et al. (2018), focused their efforts in the Saudi-Arabian shorelines. They faced the highly-challenging task of both detecting and classifying the AMD typology with a series of multi-class random-forest classifiers, based on the extraction of HoG features. The authors validated the feasibility of using AI for AMD detection, but pointed out that the use of deep learning would have been more beneficial with respect to their approach that achieved a maximum sensitivity of only 44%. Therefore, our work represents the first implementation to automatically detect and accurately quantify AMD, based on a deep-learning approach. Results in Table 5.3 point out that PlasticFinder performances give better results, with respect to all the metrics, especially if used in the appropriate sunlight conditions. In particular, PlasticFinder PPV is much higher than the one obtained by Martin et al. (2018), allowing for a more specific tool to alarm on and quantify the presence of AMD. In fact, it is important to highlight that, in order to monitor the presence of AMD and to know which are the areas that require an urgent intervention (i.e. those where AMD accumulate the most), it is essential to have a tool that is able to detect only AMD, without mistaken false positives. To this extent, reaching a high PPV is more crucial than a high Sensitivity. Also, the fact that the reached Sensitivity is constant, despite the different loads of litter on the beach, is also a good result because it shows that the more is the AMD, the higher is the litter detected by PlasticFinder, i.e. this technology is able to detect accumulation zones.

These results reflect the major advantage of deep learning, with respect to conventional ML methods, which is the fact that it is not necessary to pre-transform data (e.g. an image) into selected features to feed models, but data can be input into neural-network models to let them automatically identify the best representations that allows tasks such as detection or classification (LeCun et al., 2015).

5.6.2 Best-practices optimization and future improvements

In order to optimize and enhance best practices for AMD remote-sensing monitoring, further improvements should be applied to the adopted protocols and methods.

In terms of the UAV survey, we reckon that once all the flight parameters have been set, the monitoring can be carried out from a small boat, in the proximity of the shores, without the need of reaching the beach, often inaccessible for the presence of coral reefs all around the islands. Therefore, this methodology can be particularly useful in geographical sites, such as the Maldives, where the presence of many small remote uninhabited islands, and the need to optimise the AMD beach-monitoring, represents a pressing matter.

In terms of the ground assessment protocol, such assessment is used to validate the reliability of the methodology, but, in implementing the protocol, it is not supposed to happen every time (otherwise the protocol would lose its time-efficiency). For this reason it has been not included in the Protocol recommendations (Table 5.4). In general, the beach should be left untouched before the UAV survey to avoid footprints or other environment manipulations that could affect the methodology performance, especially in remote places.

Another important remark is that, for a relevant fraction of the AMD, the deposition on the beach is only a transitory phase before being taken up by the currents to resume the floating travel in the ocean. Instead, other AMD can be trapped on the upper part of the beach, where environmental factors and the erosive action of the sand can accelerate the plastic degradation processes. The microplastics particles (< 5mm) produced by the degradation of the AMD trapped on the upper part

of the shore can enter in the sediments or can be released as contaminants in the water of the lagoon (Saliu et al., 2018; Saliu et al., 2019). Therefore, fast and efficient data collection and image analysis of the distribution of AMD on the shore, as well as specific AI tools for its automatic and objective assessment are necessary, but not sufficient, since microplastic is lost from this detection and quantification. However, the distribution and quantitation of AMD, as obtained from our protocol, could be used to understand which are the most impacted areas, and the AMD depositional seasonal trends connected to the Indian Ocean currents patterns (Mheen et al., 2019). The creation of an integrated model could allow stakeholders (e.g. governments, NGO) using this information in order to promote mitigation actions, such as specific citizenship awareness initiatives, beach—clean up events, but also addressing — with a data-driven approach - the interception of the floating AMD, before reaching the shorelines.

Finally, we would suggest some improvement for PlasticFinder, for example, by a more in-depth training, with the aim of avoiding sunlight-conditions dependence for its use, which represents, to date, one of its major limitations. Also, optimization should be implemented to scale up the algorithm speed and ability to process full orthomosaic images, overcoming time-scale limitations due to processing of a large amount of data. In Table 5.4, we summarize and suggest an optimal protocol, with key recommendations.

5.7 Conclusion

Our work was aimed at proposing an efficient and reliable monitoring protocol, to address a pressing worldwide environmental issue such as AMD deposition along the shores. Low altitude remote-sensing data are essential for obtaining a synoptic overview of extended areas, and UAVs are powerful tools to acquire them. Our study, confirmed the use of a commercial drone for AMD monitoring as a fast and reliable surveys methodology. The use of UAV is instrumental to survey remote areas and the spatial resolution achieved in the collected images allowed to detect a percentage of the objects on the shores higher than 87.8%. A deep-learning based software, PlasticFinder, has been used for the automatic detection and quantification of AMD, providing analysis of the UAV collected images. In the Maldivian case study, the overall performances were good, reaching a PPV of 94% with the better sunlight conditions, much greater than the only state-of-the-art AI algorithm so far published in literature. The only critical limitations, observed in our study, are determined by environmental circumstances encountered during the survey, and especially sunlight conditions and the associated terrain shading effects: restrictions are given for the images that can be analysed with the deep-learning algorithm in its present version, where the PPV is reduced to 54%.

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5.9 Bibliography

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5.10 Tables and Figures

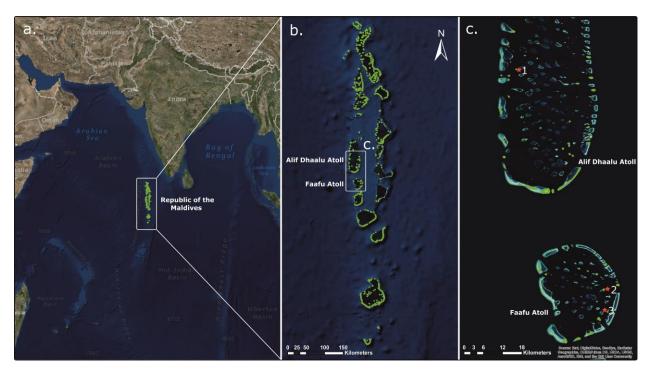


Figure 5.1. Geographic location of the study area, Republic of Maldives (a.), Alif Dhaalu and Faafu Atolls (b.), and islands selected as testing sites (c.): 1 En'Boodhoo, 2 Jinnathuga, 3 Adanga



Figure 5.2. Debris accumulation in the Maldives: (a) one of the ferries port in Malè (capital city) full of plastic bottles release in the ocean from the boats or from the streets of the city; (b) debris accumulated near the shore of Thilafushi, the only landfill island of the archipelago; (c, d) plastic waste deposited by the high tide on the beach of Adangau; (e, f) plastic waste deposited in the bushes and on the shores of En'Boodhoo.

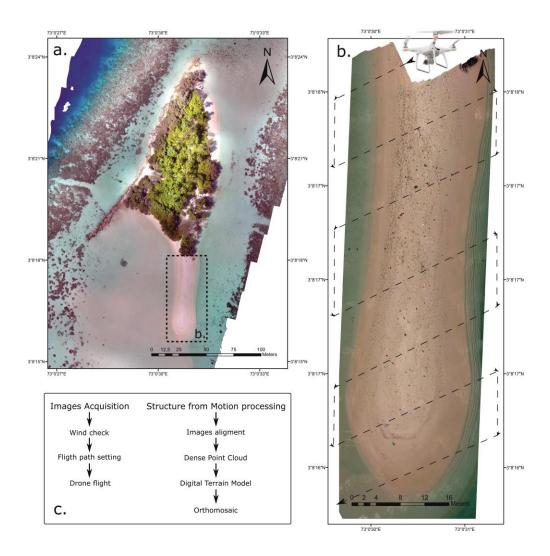


Figure 5.3. Example of drone survey over Adangau (a.) sandy long beach. The path followed by the drone (b.) on the high-resolution orthomosaic of the monitored area produced from SfM workflow (c.).



Figure 5.4: Examples of Jinnathuga island images (a., b. and c.), used for the training set for the deep-learning algorithm. Note the presence of the different classes in the images, in particular "AMD" in (a), "sand" in (a), (b) and (c), "sea" in (b), "vegetation" and "other" in images (a) and (c).

Table 5.1. AOI, number of minimum IOI, climate, light and weather conditions of each investigated Maldivian island during the UAV survey and the in-situ ground assessment (GA). The number of items collected during the GA on each beach is listed per each class, and the items identified via image screening (IS) via PC are also reported.

	Jinnathu	ıga	Adangau		En'Boodhoo		
AOI (m ²)	216	216		1056		225	
IOI	1		5	5		1	
Climate (Month)	April		Novembe	November		October	
Light (Time)	12 pm		12 pm	12 pm			
Weather (Conditions)	Sunny		Sunny		Cloudy		
Use	Training	Set	Testing S	et	Testing S	et	
AMD Class	GA	IS	GA	IS	GA	IS	
Lighter	4	1	1	1	4	4	
Bottle	21	21	50	54	47	43	
Straw	1	0	1	0	0	0	
Net	11	8	3	3	2	3	
Plastic Bag	7	7	43	50	3	2	
Aluminum Can	8	6	11	13	21	14	
Plastic containers	3	3	1	1	12	7	
Plastic utensils	0	0	20	8	1	0	
Flip Flop	1	1	13	8	32	29	
Other	26	26 25		3	13	19	
TOTAL	82 72		147	141	135	120	
Matching Score (%)	87.8	87.8		95.9		88.8	

Table 5.2. Results for the Adangau and En'Boodhoo testing-set images A1, A2, A3 and E1, E2, E3, respectively. Average scores (AVG) are also given for each set. AMD accounts for the total real items in each image, as identified by the gold standard. True positive (TP), false negative (FN) and false positive (FP) items are combined to express the software performance in terms of sensitivity, PPV and F-score.

IMG	AMD	TP	FN	FP	Sensitivity (%)	<i>PPV</i> (%)	F-score (%)
Adangau	1					1	
A1	37	25	12	0	0.68	1.00	0.81
A2	58	40	18	4	0.69	0.91	0.78
A3	89	56	33	6	0.66	0.90	0.74
AVG	61.3	40.3	21	3.3	0.67	0.94	0.78
En'Bood	hoo					1	
E1	71	23	48	66	0.32	0.26	0.29
E2	43	5	38	6	0.12	0.45	0.19
E3	98	25	73	65	0.26	0.28	0.27
AVG	70.6	17.6	53	45.6	0.23	0.25	0.33

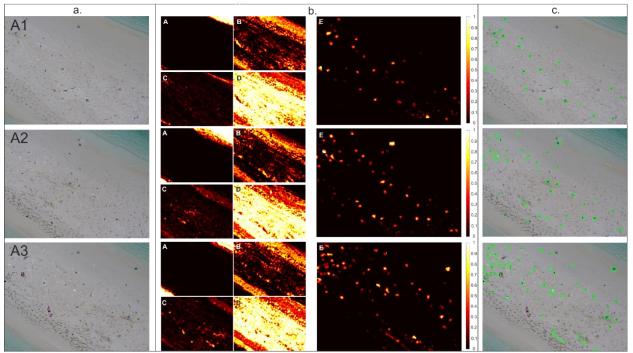


Figure 5.5. Canvas on the left (a.), from top to bottom: testing-set images A1, A2 and A3 for Adangau-island. Central canvas (b.), from top to bottom: PlasticFinder pixel-wise classification heatmaps for Adangau-island images A1, A2 and A3, respectively, representing the classes sea (A), sand (B), vegetation (C) other/AMD (D), and AMD (E) with the probability scale ranging from 0 to 1. Canvas on the right (c.), from top to bottom: PlasticFinder bounding-boxes maps for the Adangau-island images A1, A2 and A3, respectively. Each green bounding box is identified as an AMD by the software.

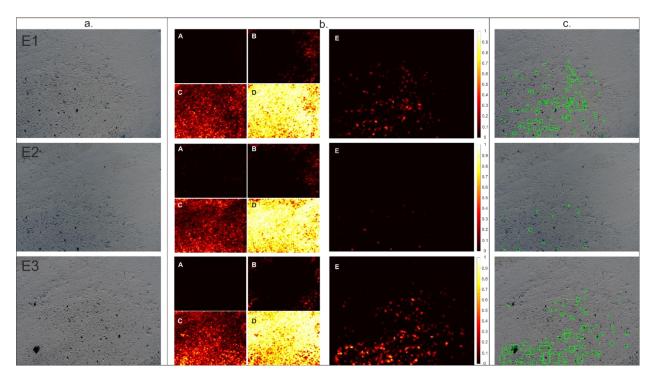


Figure 5.6. Canvas on the left (a.), from top to bottom: testing-set images E1, E2, E3 for En'Boodhoo island. Central canvas (b.), from top to bottom: PlasticFinder pixel-wise classification heatmaps for En'Boodhoo-island images E1, E2, E3, respectively, representing the classes sea (A), sand (B), vegetation (C), other/AMD (D), and AMD (E) with the probability scale ranging from 0 to 1. Canvas on the right (c.), from top to bottom: PlasticFinder bounding-boxes maps for the En'Boodhoo-island images E1, E2, E3, respectively. Each green bounding box is identified as an AMD by the software.

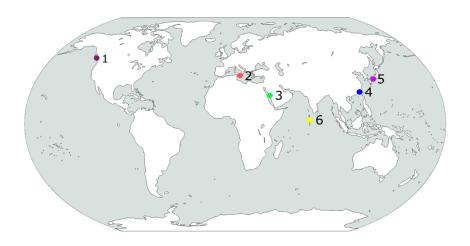


Fig. 5.7 Geographic distribution of studies that used remote sensing techniques to monitor and detect beach debris: 1 Vancouver Island, Canada (Kataoka et al., 2018); 2 Malta (Deidun et al., 2018); 3 Saudi Arabia (Martin et al., 2018); 4 Fuzhou, Fujian, China (Sha et al., 2018); 5 Seto Inland Sea, Japan (Kako et al., 2012); 6 Republic of the Maldives, present study area.

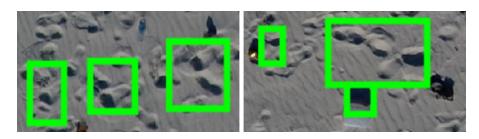


Figure 5.8. Examples of shadows in the proximity of footprints (left image) or of AMD (right image), that are mistaken as AMD by the software, representing pitfalls for the algorithm in its present version.

Table 5.3. Comparison between results from Martin et al. (2018) (average on the overall results), PlasticFinder results for Adangau island and averaged results for Adangau and En'Boodhoo island

Algorithm	TOT	TP	FN	FP	Sens (%)	PPV (%)	F-score (%)
Martin et al.	415	164	251	1941	0.40	0.08	0.13
PlasticFinder	61.3	40.3	21	3.3	0.67	0.94	0.78
(Adangau)							
PlasticFinder	131.9	57.9	74	48.9	0.44	0.54	0.49
(AVG A/E)							

Table 5.4. Optimal protocol and key recommendations for the optimization of the UAV survey, the collection of gold standards and of UAV images suitable for the training and testing of a deep-learning algorithm.

UAV Survey	
Flight Altitude	10 m
GSD	4.36 mm/pix
Camer Gimbal Orientation	-90° (nadir orientation)
Images acquisition along fixed	
paths	00% Holitai Overlap
pauls	700/ lateral everlan
	70% lateral overlap
	2 seconds of shooting interval
	1,3 m/s costant velocity
Gold Standard	
Ground Assessment	AMD inspection and subtype classification (to be
	limited to few representative areas for the
	validation of methodology)
	AMD Size > 5 cm
Image Screening	AMD counting and subtype classification
	Matching Score > 80%
Deep Learning	
Training	# of images per class ~ 10 ³
	Validation accuracy for tailored CNN > 95%
Testing	# of images ~ # IOI
	Use IS-GS to test performances with metrics
	Use GA-GS to estimate error on the IS-quantified
	performance
	r · · · · · · ·

- CHAPTER 6 -

6.1 Users' satisfaction on coral restoration projects: the case of the Maldives

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Authors contribution: FF, SM conceived and designed the research; SL, FS performed the experiment; FF, PG, analysed the data; FF, FS, SM wrote and edited the manuscript.

Keywords: Coral Reef, Rehabilitation, Reef users' satisfaction, IPA analysis.

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6.2 Abstract

The worldwide degradation of coral reefs due to local and global threats has, in the past decade, sparked the diffusion of active coral restoration programs which support the natural process of recovery. Yet, most coral restoration projects limit monitoring to few ecological factors. The ability of measuring the effectiveness of these projects, including their socio-economic dimensions, could increase their credibility and determine their further expansion through ease access to financial resources. Here, we measured the satisfaction and the relative importance of reef attributes for visitors and residents of resorts in the Maldives through Importance-Performance Analysis (IPA). Results of the fieldwork show that reef users in two resorts were not satisfied about the color, the quantity and the variety of the coral present in the locations. On the same items, importance ratings were very high thus indicating that a gap between satisfaction and importance and possibly highlighting a need for intervention. Some results were also gender-specific: women were much less satisfied than man but more willing to participate to a local coral restoration project.

We recommend that evaluation of coral restoration programs include reef users' satisfaction to provide a measure of effectiveness and potentially to ensure initial and longer-term support to the initiative.

6.3 Implications

- Currently the socioeconomical perspective of coral restoration projects is very rarely taken into account with assessment of projects focusing on ecological aspects.
- Evaluation of users' satisfaction of reefs may allow management decisions for the allocation of funds and resources towards coral restoration activities rather than other initiatives.
- Our data revealed that reef users are not satisfied about the color, the quantity and the
 variety of the coral present in the local barrier reef. Yet, these attributes are considered
 rated as very important.

•	Some results were also gender-specific with women being less satisfied than man on the
	health of the reef and more willing to participate to a local coral restoration project.

6.4 Introduction

Coral reefs are among the most fragile and delicate ecosystems in the world threatened by numerous stressors of anthropogenic origin and by natural disturbances connected to environmental changes (Hoegh-Guldberg & Bruno 2010). Thermal stress on corals, ocean acidification (Stat et al. 2006) and ecosystem alterations can lead to the disease or even death of the coral (Rosenberg & Ben-Haim 2002; Gates & Ainsworth 2011).

With current rapid decline of coral reefs, their diminished capacity to absorb disturbances and remain resilient (Carpenter 2008), active efforts have been put in place worldwide, in the past few years, to enhance the recovery of reefs (Grimsditch & Salm 2006, Young et al. 2012). Yet, while restoration on land is a widely used discipline (Putz et al. 2001, Wortley et al. 2013), restoration of coral is still in development with a lot of research currently being done to innovate on the techniques used and to find low-cost solutions that can be applied to the large-scale (Bayraktarov et al. 2016). As many practices in their infancy, the real long-term benefits of coral restoration have been challenged by the lack of scientific assessment of the outcomes as effectiveness and impact have been linked to transplant growth and transplant survival (Hein et al. 2017). In their work, Hein et al. (2017) analysed the indicators used in 83 coral restoration programs deployed worldwide. Their review revealed that growth and survival of coral fragments were the most widely used indicators of restoration success (88% of studies), rarely in combination with other ecological factors. Hein et al. (2017)'s results highlighted that coral restoration has so far focused on shortterm rather longer-term results. With the rapid increase of projects, better planning and more managerial judgment will be required to allocated resources. In this scenario, the introduction of sociocultural, economic, and governance indicators such as stewardship or reef user satisfaction (Okubo & Onuma 2015) will definitively be required. Reef user satisfaction is directly linked to increased prosperity of stakeholders and sources of satisfaction may vary among stakeholder groups (Hein et al. 2017 Appendix S1, Millennium Ecosystem Assessment 2005, Okubo & Onuma 2015). A recent study on four geographically different coral restoration projects (Hein et al. 2019) suggested that perceptions on coral restoration benefits vary by location thus being context-specific and by stakeholders' groups. Satisfaction is also subjective; for example, satisfaction of managers of restoration projects may be linked to positive ecological indicators such as survival and growth of fragments. Local community satisfaction may be linked to increased revenues through alternative income opportunities while tourists' satisfaction a result of increased recreational activities (Hein et al. 2017, Okubo & Onuma 2015). Yet, managers of coral restoration initiatives, could use reef users' satisfaction as a measure of effectiveness of their efforts to ensure initial and longer-term support to their projects.

In the Maldivian region, the global decline in coral reef health (Montano et al. 2015, 2016; Saponari et al. 2018) has coupled with direct impacts deriving from the heavy use of reefs and the fast development of resorts and local islands through dredging and construction of overwater structures (Edwards 2010). Coral restoration projects have become widely present thanks to the initiative of private resorts that consider them a way to fund conservation and engage tourists in environmentally conscious activities. The purpose of this work was to measure satisfaction of reef users in order to potentially indicate to reef practitioners and managers the need for an effective intervention and allocation of funding for restoration programmes on Maldivian coral reef.

6.5 Materials and Methods

6.5.1 Study Area

The surveys were conducted in the two selected resorts between the end of the spring and mid-summer 2018. The two study areas are located in different Atolls in the Maldives and were selected both for the presence of the interest of the resident marine biologists to test indicators on their coral restoration project and the support of the management to run the survey on reef users' satisfaction: visitors and residents. In particular the surveys have been conducted in a 5-star resort called Diamonds Thudufushi Beach and Water Villas (3.786268N; 72.731228E), part of the Planhotel Hospitality Group, located in South Ari Atoll, and a 5-star resort located in the Baa Atoll (5.201210N; 73.011111E), a UNESCO Biosphere Reserve.

The Importance-Performance Analysis (IPA), already successfully employed to analyse importance and satisfaction on natural features in natural areas (Tonge & Moore 2007), has been used to measure the satisfaction of reef users of leisure destinations in the Maldives. In particular, we used the standard IPA three-step process by selecting of a set of attributes; rating their importance and satisfaction to users; and displaying the results on a two-dimensional matrix (Lai & Hitchock 2015). In addition, because reef user satisfaction is directly linked to increased wellbeing of stakeholders, we considered residents of the resort islands as the first users of the health and biodiversity of local marine ecosystem, by activities such as snorkelling and diving, and we included them in our survey (Boley et al. 2017). Thus, the analysis were performed considering the respondents as clients, the paying visitors, and residents, the employee of the resorts.

A multiple choice questionnaire has been provided to measure importance and performance. Respondents were asked to assign a value using a five-point unidirectional scale. The final questionnaire for this fieldwork included seven coral reef related attributes (Supporting information S6 & S7). Attributes 'Fish abundance' and 'Fish variety' aimed at verifying users' perception on the quantity of fish and on the different types of fish present on the reef. The attributes "Information on house reef", "Briefing Excursion(s)" and "Reef accessibility" were specifically used for helping the resort in improving management decision, thus they were not included in the IPA analysis. The 'Coral cover' attribute is an indicator of the general reef health and it was explained as the percentage of reef covered with corals. All the other attributes were reef specific in terms of size, variety of corals, color of corals and accessibility of the reef from the island.

The distribution of importance ratings which presented a major ceiling effect was re-estimated through regression-based indirect measure of predicted importance ratings. The resulting measures were subsequently used for the final gap analysis, testing for I-S (Importance – Satisfaction) differences statistical significance of the null hypothesis I=S with a *p*-value= 0.05 (Lai & Hitchcock 2015). Correlations significance was tested with t-values and only those successfully validated represented in the map. To give a stronger visual representation and prioritise among attributes, we preferred to use the data-centred quadrants approach (DCQA) and drew Satisfaction-Importance as x-y axes with their arithmetic mean values as cross-points (Bacon 2003).

Given the richness of data available, we performed an overall correlation analysis to identify which items used for the questionnaire showed significant interrelationships. The overall correlation analysis was performed using personal data, purpose of the visit to the Maldives, number of visits to the country, and importance and satisfaction ratings expressed on the reef attributes. The results of the correlation analysis were represented in a Pearson's matrix and graphically shown through a heat map where the color red indicated the maximum positive correlations (+1) and the color blue indicates the maximum negative correlations (-1) while 0 indicates no correlation. Anaconda Python 3.6 was used to perform the analyses.

Finally, we collected answers on the 'Willingness to participate to local coral project' and combined them to verify the presence of gender, age or nationality commonalities across the two different samples.

6.6 Results

6.6.1 Clients

A total of 61 clients participated to our survey. Most respondents were males (55%), 35% were females, while 10% had decided not to declare their gender. The average age of the sample was 36.2 years with a range from 10 to 71 years and a standard deviation 13.1 years. In terms of Nationality, the sample was mainly composed by Italians (48%), British (16%), Swiss (15%), German (8%). Most respondents (47%) were repeaters with a 1.8 visit average number to the Maldives. For most clients, the main purpose of the visit was snorkelling or diving (48%). A vast majority (51) declared interest in marine life with a preference for Fish, (55%) combined Fish and Coral (37%) and an 8% minority on Coral. Some respondents (28) expressed their willingness to participate to the local coral project.

In terms of Importance, the least important attribute for clients was 'Reef size' while 'Information on house reef' and 'Briefing on excursion(s)' collected the highest average importance (Table S6.2). After testing data, 'Reef accessibility' was not used for the IPA mapping as the null hypothesis where importance equals satisfaction could not be validated. For satisfaction, clients indicated very

clearly their lowest satisfaction on 'Reef color' and 'Coral cover' and 'Reef Variety' (Table S6.3) while they were very satisfied with 'Information on house reef' and 'Briefing on excursion(s)'. Except for 'Reef color', all attributes totalled more than three in average satisfaction (Tab. 6.1).

The Figure 6.1 shows all the attributes in the quadrants of the IPA with 'Reef Color' and 'Coral Cover' being placed in Quadrant I high importance, low performance and 'Reef Variety' at the border between Quadrant I and II.

In addition, correlation analysis regarding information to participate to coral initiatives revealed that Gender has a statistical relationship with items relative to importance: importance of fish abundance (-0.17), importance of fish variety (-0.15), importance of coral cover (-0.12), importance of reef variety (-0.09) are shown in light blue color (Fig. 6.2). On average, female clients gave higher scores than males to these attributes. The attribute 'Visits to the Maldives' correlated positively (0.40) with 'Purpose of the visit'.

6.6.2 Residents

A total of 48 residents participated to our survey, males were 54% of the sample and 46% females, with a general average age of 29.5 years with a range from 17 to 45 years and a standard deviation of 6.9 years. Maldivian nationals represented most of the sample (65%) with an average of 6 years of work experience in the tourism industry. Non-Maldivian nationals (35%) had an average of 7 years of work experience in the Maldives. Only 17% of the residents had a marine occupation and overall 94% could swim. A large representation of the sample could also dive (48%). In this sample, 90% of the respondents declared to be willing to participate to the local coral project (43 respondents).

While the single means were different showing higher importance and lower satisfaction ratings than clients, the final ranking of priorities expressed by residents is the same: 'Reef Color', 'Coral Cover' and 'Reef Variety' are the attributes that require attention and intervention to increase their satisfaction (Table 6.2; Fig. 6.3; Table S6.4 – S6.5).

Also in this case, Gender Female shows a statistical relationship with items relative to importance attributes as indicated by the light blue color: importance of fish abundance (-0.05), importance of

fish variety (-0.12), importance of coral cover (-0.10), importance of reef variety (-0.12), importance of reef color (-0.10) (Fig. 6.4). On average, females gave higher scores than males to these attributes. The variable age shows negative correlation with satisfaction variables 'Fish variance' (-0.09) and 'Coral cover' (-0.20) meaning that younger residents are less satisfied than older residents on these items. The satisfaction on 'Reef variety' shows positive correlation with 'Coral cover' (0.71) and 'Reef variance' (0.71), with 'Reef color' (0.66) and 'Fish abundance' (0.49).

6.6.3 Combined analysis and personal comments

By combining the two samples, we identified twelve different nationality groups with Maldivian and Italian being the most represented nationalities. All other nationalities were grouped in a bucket category called Other. Gender and Age were used to sub-divide the nationality groups, and set a threshold to 32 years of age, corresponding to the age value of the 50th percentiles, that was then used to create cohorts of respondents aggregated by age. The answers for a total sample of 102 respondents show an overall average willingness of 62% with differences across the different segments. Overall, Maldivians declared to be the most willing to participate across different genders and ages. In general, women of all nationalities were more prone to participate than men (Table 6.3).

On top of rating the reef's attributes, 13 residents (27% of respondents) left a personal comment. All these contributions were written by young respondents below 35 years, with majority under 25 year (70%) and mostly women (70%). Their comments were mainly on two main topics: awareness on the coral emergency, conservation and restoration initiatives.

6.7 Discussion

Despite the differences between the two analysed samples, respondents expressed their lowest satisfaction on the same attributes, in the same order: 'Reef color', 'Coral cover' and 'Reef variety'.

This first conclusion tells us that overall respondents – clients and residents – clearly rate reef related attributes as a priority while expressing their relative unhappiness about the 'coral experience' while

snorkelling or diving. This result is evident from the individual satisfaction analysis here presented which suggest that users can perceive the destructive impact of the 2016 El Niño mass bleaching event, responsible for killing in the Maldives about 66% of the corals at depth between 0 and 7 m (Ibrahim et al. 2017). In our IPA matrices, the average overall satisfaction across all items, which represents the cross-point, is very close for the two samples (3.4 vs 3.5) while on importance items the cross-point for the residents is 4.6 versus 4.2 indicating either higher sense of urgency for resident employees than clients or alternatively a greater 'ceiling effect' in expressing votes on attributes importance. Though with different cross-point, the graphical evaluations of the IPA matrices showed both present attributes 'Fish abundance' and 'Fish variance' with high importance, high performance in Quadrant II, defined as the quadrant 'Keep Up the Good Work' (Martilla & James 1977). The attribute 'Reef size' for both group of respondents and 'Reef accessibility' and 'Information of reef' for residents were evaluated above average in terms of satisfaction and do not require actions. Unfortunately, 'Reef color' and 'Coral cover' fall in Quadrant I, defined as 'Concentrate Here' quadrant, suggesting that there is a need for intervention on the color and the cover of coral. This result can support the management in deciding to invest in a coral conservation project: at the time of the survey, the resort in Baa was only testing a single nursery with 1500 fragments of coral with unclear plans for the future. The results of the IPA potentially blaze the trail for moving to a fullyfledged project where more nurseries are established to support the growth of new coral colonies and be later transplanted where coral died in 2016.

It is more difficult to interpret the result of the IPA in the case of Thudufushi where a coral project has been on-going for the past three years and where, about 100 frames are already underwater. Yet, iron structures/frames core to this project are artificial reefs (AR) and cannot be considered as real reef restoration initiative where new coral colonies replace dead ones thus accelerating natural recovery. Respondents might perceive this difference and might be more satisfied with projects bringing biological restoration. There are three possible facts that might support this hypothesis. Firstly, a high percentage of respondents are returnee clients and they might remember the beauty and colors of the reef before the bleaching event of 2016 and which, unfortunately, the coral project was not able to prevent. This hypothesis is supported by the negative correlation on all items between the number of visits and the satisfaction of clients. Secondly, the presentation during the

Biology Night, at the end of which the survey took place, might have increased clients' awareness on the status of coral bleaching in South Ari Atoll. Thirdly, participants to our field work might simply dislike this type of ARs and female participants might not like ARs in general. On the third point, a survey conducted along the northern shore of Eilat, Israel, investigated divers 'preferences toward artificial reefs (Shani et al. 2012): 222 divers, mostly men (about 75%) between 25-44 years (average 33.9) with diving as favourite recreational activity, were surveyed between October 2009 and February 2010. The results demonstrated that 90% of the participants were very supportive of ARs but declared that tyres, concrete blocks, and pipes, which can be assimilated to the frames of Thudufushi, were their least favouritetype of ARs (average score of 1.54, 1.96 and 2.06 when rated on 5-point Likert scale). A clear difference pointed out by this study was the attitude based on divers' gender: male divers expressed an overall higher level of support than female divers for establishing ARs. Our analyses support even further the identified gender differences: answers to the question about the willingness to participate to coral projects confirmed that females have a higher desire to do something, and only slight differences around nationality and age appear.

The correlation analyses highlight statistical relationship between feminine gender and importance ratings on all attributes. The personal comments at the end of the questionnaire were left mostly by women (8 out of 13 contributions), almost all below 30 years (seven out of eight contributions). In addition, some of their comments present clear signs of accountability and commitment.

Another study confirms our finding that women have higher value orientation toward coral reef and could therefore be more motivated than men to actively participate to coral restoration projects (Needham, 2010). The study concluded that females have a stronger protectionist orientation to reefs than males and managers of leisure destinations should leverage on this value orientation to identify target groups for information and education campaigns aimed at minimizing impacts on natural resources such as educating on the consequences of touching or standing on coral.

Also, at Diamonds Thudufushi, on the same line indicated by Needham (2010), the management of the resort confirmed that female clients are usually more motivated to participate to the Biology Night and usually ask more questions relative to status of marine life. Furthermore, 47% of the clients at Thudufushi are returnees and it could be possible to keep them involved thus nurturing a culture of respect and conservation.

In the second resort, our results suggest the management and the resident marine biologist should initiate a coral restoration project and in doing so they could focus on training female residents as they might have a more direct and indirect positive impact on a coral restoration project. They could change their attitudes and behaviours toward coral reef as value orientations can predict higher-order cognitions (Fulton et al. 1996; Vaske & Donnelly 1999) and they would be more willing to use their time to volunteer for activities related to a project aimed at conserving the local house reef.

Again, based on the analyses, the local Maldivian community should be the second most important target for training and active involvement in a future coral restoration project together with the employees currently involved in a marine activity. The Table 6.3 on the 'Willingness to participate' shows that Maldivians declared a much higher willingness to participate to the coral projects than other nationalities. In addition, the heat map shows a statistical relationship with the variable Maldivian Nationality on all satisfaction and importance attributes but 'Fish abundance' which correlates more with other Nationalities. Also, insights from personal comments support this finding as most comments where written as voluntary contributions from Maldivian residents with clear reference to what they could do first-hand:

'We can start growing corals and need to look after corals well' (BA08MM).

Our results also show the variable Marine Occupation in correlation with all items on satisfaction supporting the finding that the residents who work at the marine sport centre or at diving shop would be more eager than others in participating to training activities and possibly contributing to a local coral initiative sponsored by the resort.

In conclusion, coral reef restoration initiatives will increase in response to the need for conservation to address global decline of coral. So far, they have focused primarily on evaluating short-term biological measures of success. The ambition of this work was to contribute to the evaluation of coral restoration effectiveness with the adoption for the first time of the IPA approach on attributes of coral reef in two Atolls of the Maldives. Our data supported us in assessing the low satisfaction of users on reef attributes and in identifying priorities with regard of coral restoration activities. The consistency of the results across the two user groups, reinforced our assumption that this approach could be used in the Maldives as well as in other destinations to allocate resources on coral initiatives andmonitoring clients 'satisfaction over time.

6.8 Acknowledgments

The authors would like to express their gratitude for all logistic support provided by Stefano Malatesta, Phanor Montoya-Maya, Inga Dehnert and Davide Seveso. The authors would like also to thanks the Planhotel Hospitality Group and the anonymus resort in Baa atoll for the willingness in being involved in the study.

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6.10 Tables and Figures

Table 6.1. Overall Results for importance and satisfaction of reef attributes of clients; Three attributes could not be statically validated (null hypothesis I=S) and were indicated in the table as not significant (NS) and they were not represented in the IPA matrix. All other gaps presented statistical significance with p-value= 0.05.

Attribute	Satisfaction mean	Predictive	Gap Value	p-value	Rank
	·	Importance mean	•	•	
Fish abundance (FA)	3.827	4.230	-0.403	0.004	4
Fish variety (FV)	3.915	4.288	-0.373	0.003	5
Coral cover (CC)	3.086	4.226	-1.140	5.19854E-09	2
Reef size (RS)	3.694	3.858	-0.164	0.191	8
Reef variety (RV)	3.293	4.182	-0.889	2.98365E-08	3
Reef colors (RC)	2.610	4.384	-1.774	1.194E-10	1
Reef accessibility (NS)	4.034	4.235	-0.200	NS	7
Information on house reef (NS)	4.232	4.448	-0.218	NS	6
Briefing on excursion(s) (NS)	4.372	4.511	-0.139	NS	9

Table 6.2. Overall Results for importance and satisfaction of reef attributes of residents. All the items tested were statistically significant and were represented in the IPA matrix.

Attribute	Satisfaction mean	Predictive Importance mean	Gap Value	p-value	Rank
Fish abundance (FA)	3.617	4.744	-1.128	8.45E-11	4
Fish variety (FV)	3.958	4.893	-0.936	1.73E-08	6
Coral cover (CC)	3.170	4.652	-1.478	5.05E-11	2
Reef size (RS)	3.625	4.446	-0.809	0.000244	7
Reef variety (RV)	3.417	4.666	-1.250	6.96E-09	3
Reef colors (RC)	2.938	4.687	-1.750	5.67E-12	1
Reef accessibility (RA)	3.894	4.489	-0.596	0.000935	8
Information on house re	eef3.625	4.625	-1.000	1.18E-05	5
(IR)					

Table 6.3. Willingness to participate to a coral project - Overall sample.

Group	Nationality	Gender	Age	Willingness	N
1	Italian	Male	=<32 years	30%	10
2	Italian	Male	>32 years	58%	12
3	Italian	Female	=<32 years	66%	3
4	Italian	Female	>32 years	83%	6
5	Maldivian	Male	=<32 years	76%	13
6	Maldivian	Male	>32 years	85%	7
7	Maldivian	Female	=<32 years	72%	11
8	Maldivian	Female	>32 years	100%	1
9	Other	Male	=<32 years	57%	11
10	Other	Male	>32 years	54%	11
11	Other	Female	=<32 years	76%	13
12	Other	Female	>32 years	35%	8

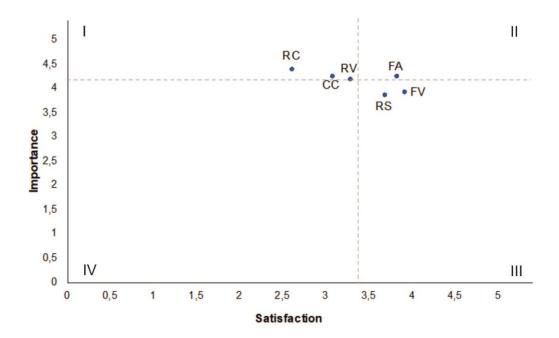


Figure 6.1. IPA Matrix for clients. Attributes acronyms: Fish abundance (FA), Fish variety (FV), Coral cover (CC), Reef size (RS), Reef variety (RV), Reef colors (RC). Attributes which were not statically validated (null hypothesis I=S) were not represented. Roman numbers indicating the quadrants.

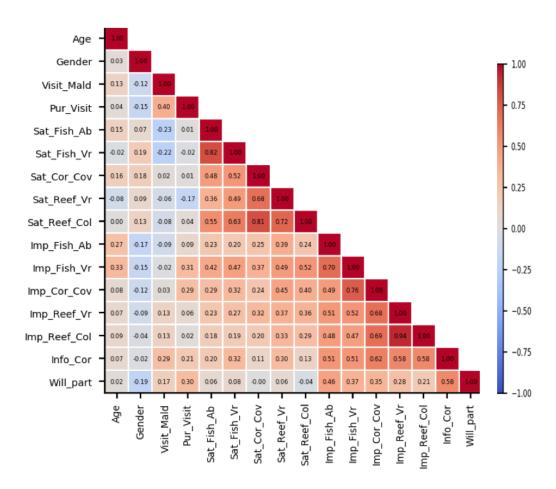


Figure 6.2. Pearson's Matrix for clients. The heat map shows the strong positive correlations between all satisfaction and all importance ratings which appear graphically distributed in a homogenous fashion below the diagonal line.

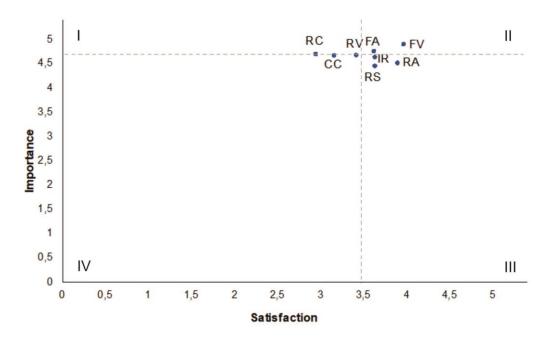


Figure 6.3. IPA Matrix for residents. Attributes acronyms: Fish abundance (FA), Fish variety (FV), Coral cover (CC), Reef size (RS), Reef variety (RV), Reef colors (RC), Reef accessibility (RA), Information on house reef (IR). Roman numbers indicating the quadrants.

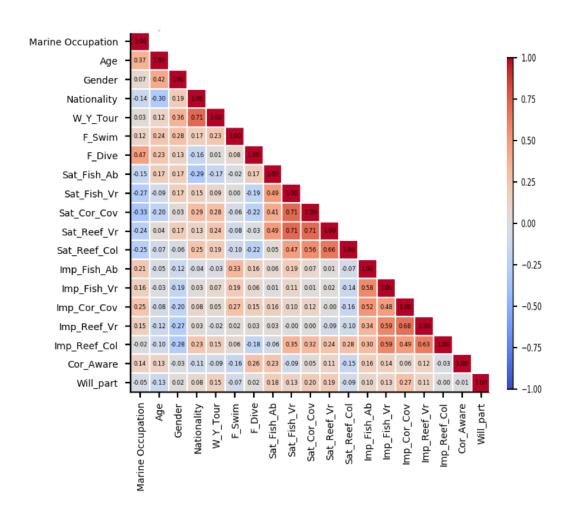


Figure 6.4. Pearson's Correlation Matrix for residents. The heat map shows the strong positive correlations between all satisfaction and all importance ratings which appear graphically distributed in a homogenous fashion below the diagonal line.

6.9 Supporting Information

Table S6.1. Data manipulation for the correlation analysis through Pearson coefficient.

Gender = Male 1; Female 0	Nationality = Italian 1; Other nationalities 0 (Thudufushi)
Nationality = Maldivian 1; Other nationalities 0 (Baa Atoll)	Age = Number
Visit_Mald = Number of visits 1, 2, >2 = 3	Pur_Visit = Snorkeling & Diving 1; Others 0
Sat_Fish_Ab = Satisfaction on Fish Abundance; scale 1 – 5	Imp_Fish_Ab = Satisfaction on Fish Abundance; scale 1 – 5
Sat_Fish_Vr = Satisfaction on Fish Variety; scale 1 – 5	Imp_Fish_Vr = Satisfaction on Fish Variety; scale 1–5
Sat_Cor_Cov = Satisfaction on Coral Cover; scale 1 – 5	Imp_Cor_Cov = Satisfaction on Coral Cover; scale 1 – 5
Sat_Reef_Vr = Satisfaction on Reef Variety; scale 1 – 5	Imp_Reef_Vr = Satisfaction on Reef Variety; scale 1 – 5
Sat_Reef_Col = Satisfaction on Reef Color; scale 1 – 5	Imp_Reef_Col = Satisfaction on Reef Color; scale 1 – 5
Brief_Cor = Briefing on excurision(s); scale 1 - 5	Info_Cor = Interest to receive information on local coral Project; Yes 1, No 0
Marine Occupation = Yes 1; No 0	Will_part = Willingness to partecipate; Yes 1, No 0
Work_Y_Mald = Number of years of work in the Maldives	Work_Y_Tour = Number of years of work in the tourism industry (Maldivians only)
F_Swim = Yes 1; No 0	F_Dive = Yes 1; No 0
Cor_Aware = Awareness of the local coral project; Yes 1, No 0	Will_part = Willingness to partecipate; Yes 1, No 0

 Table S6.2. Importance on reef attributes - Clients.

Not Important = 1, Slightly Important = 2, Moderately Important = 3, Very Important = 4, Very Important = 5.

^{*: &#}x27;False'= not normal distribution

Attribute/Importance	(1)	(2)	(3)	(4)	(5)	N	Mean	SD	Skewness	Kurtosis	Normality*
Fish abundance	0	0	6	28	18	52	4.230	0.645	-0.253	-0.620	False
Fish variety	0	0	7	23	22	52	4.288	0.695	-0.460	-0.820	False
Coral cover	0	2	10	15	26	53	4.226	0.891	-0.809	-0.403	False
Reef size	1	2	16	18	16	53	3.858	0.952	-0.652	-0.671	False
Reef variety	0	2	11	15	25	53	4.179	0.904	0.683	-0.668	False
Reef colors	2	0	6	12	32	52	4.384	0.973	-1.917	3.928	False
Reef accessibility	0	2	8	18	24	52	4.230	0.854	-0.863	-0.025	False
Information on house reef	0	0	7	13	31	51	4.470	0.730	-1.014	-0.357	False
Briefing on excursion(s)	0	1	5	11	34	51	4.529	0.757	-1.538	1.655	False

Table S6.3. Satisfaction on reef attributes - Clients.

Not Satisfied = 1, Slightly Satisfied = 2, Moderately Satisfied = 3, Very Satisfied = 4, Very Satisfied = 5.

*: 'False'= not normal distribution

Attribute/Satisfaction	(1)	(2)	(3)	(4)	(5)	N	Mean	SD	Skewness	Kurtosis	Normality*
Fish abundance	0	3	18	23	14	58	3.827	0.861	-0.166	-0.741	True
Fish variety	0	2	17	24	16	59	3.915	0.836	-0.202	-0.778	True
Coral cover	1	14	26	13	4	58	3.086	0.903	0.268	-0.210	True
Reef size	0	3	20	28	8	59	3.694	0.771	-0.106	-0.305	True
Reef variety	0	11	25	16	6	58	3.293	0.898	0.277	-0.596	True
Reef colors	13	18	14	7	7	59	2.610	1.286	0.475	-0757	True
Reef accessibility	0	4	11	22	21	58	4.034	0.917	-0.635	-0.425	True
Information on house reef	0	1	10	20	25	56	4.232	0.808	-0.668	-0.488	True
Briefing on excursion(s)	0	0	6	20	25	51	4.372	0.691	-0.652	-0.671	True

Table S6.4. Importance on reef attributes – Residents.

Not Important = 1, Slightly Important = 2, Moderately Important = 3, Very Important = 4, Very Important = 5.

*: 'False'= not normal distribution

Attribute/Importance	(1)	(2)	(3)	(4)	(5)	N	Mean	SD	Skewness	Kurtosis	Normality*
Fish abundance	0	0	3	6	39	48	4.750	0.564	-2.216	3.963	False
Fish variety	0	0	0	5	42	47	4.893	0.055	-2.638	5.178	False
Coral cover	0	1	3	7	36	47	4.659	0.836	-2.190	4.480	False
Reef size	0	8	10	6	31	47	4.446	0.910	-1.018	-0.743	False
Reef variety	0	0	3	10	35	48	4.666	0.771	-1.635	1.711	False
Reef colors	0	1	3	6	38	48	4.687	0.830	-2.349	5.186	False
Reef accessibility	0	0	8	8	32	48	4.500	0.878	-1-158	-0.272	False
Information on house reef	0	1	3	9	35	48	4.625	0.838	-1-998	3.375	False

Table S6.5. Satisfaction on reef attributes – Residents.

Not Satisfied = 1, Slightly Satisfied = 2, Moderately Satisfied = 3, Very Satisfied = 4, Very Satisfied = 5.

^{*: &#}x27;False'= not normal distribution

Attribute/Satisfaction	(1)	(2)	(3)	(4)	(5)	N	Mean	SD	Skewness	Kurtosis	Normality*
Fish abundance	0	2	19	20	6	47	3.617	0.795	0.003	-0.391	True
Fish variety	0	4	8	22	14	48	3.958	0.898	-0.651	-0.153	True
Coral cover	1	13	16	11	6	47	3.170	1.049	0.234	-0.772	True
Reef size	0	8	13	16	11	48	3.625	1.023	-0.166	-1.056	True
Reef variety	2	5	20	13	8	48	3.416	1.027	-0.195	-1.154	True
Reef colors	7	9	17	8	7	48	2.937	1.278	0.057	-0.879	True
Reef accessibility	0	2	14	18	13	47	3.893	0.865	-0.208	-0.818	True
Information on house reef	3	7	10	13	15	48	3.625	1.248	-0.537	0.746	True

Name:	o.o – CLIEN IS Number:	QUESTIONAL		Age:						
Email:	vuiliber:		Nationality:							
1.	How many time	es have you visi	ted the Maldi	ves, including	this visit?					
	☐ 1 ☐ 2 ☐ More than 2	·		,						
2.	What is the ma	in purpose of y	our current v	isit?						
	☐ Snorkeling☐ Scuba Diving	bration (honeym		,						
3.	What interests	you the most?								
	☐ Fish ☐ Coral ☐ Other (please	e specify)								
4.	-	ur level of satis	faction with r	espect to the	following iter	ns, on a scale of				
	1 to 5	Not Satisfied	Slightly Satisfied	Moderately Satisfied	Satisfied	Highly Satisfied				
Fish abu	ındance	1 🗆	2 🗆	3 □	4 🗆	5 □				
Fish var	riety	1 🗆	2 🗆	3 □	4 □	5 □				
Coral co	over	1 🗆	2 🗆	3 □	4 □	5 □				
Reef siz	e	1 🗆	2 🗆	3 □	4 □	5 □				
Reef var	riety	1 🗆	2 🗆	3 □	4 □	5 □				
Reef co	lors	1 🗆	2 🗆	3 □	4 □	5 □				
Reef acc	cessibility	1 🗆	2 🗆	3 □	4 🗆	5 □				
Informa		on1 □	2 🗆	3 □	4 □	5 □				
house re Briefing excursion	g provided	on1 □	2 🗆	3 □	4 🗆	5 🗆				
5.	Please state ho	w important, in	your opinion	, are the follo	owing items o	on a scale of 1 to				
		Not	Slightly	Moderately	Important	Very				
Fish ahı	ındance	Important 1 □	Important 2 □	Important 3 □	4 П	Important 5 □				

Fish variety	1 🗆	2 🗆	3 □	4 🗆	5 □
Coral cover	1 🗆	2 □	3 □	4 🗆	5 □
Reef size	1 🗆	2 🗆	3 □	4 🗆	5 □
Reef variety	1 🗆	2 🗆	3 □	4 🗆	5 □
Reef colors	1 🗆	2 □	3 □	4 □	5 □
Reef accessibility	1 🗆	2 🗆	3 □	4 🗆	5 □
Information available house reef	on1 □	2 🗆	3 □	4 🗆	5 🗆
Briefing provided excursion(s)	on1 □	2 🗆	3 □	4 🗆	5 □

6.	Would you like to	receive more	information on	our Coral Project?

7. Would you be interested in participating to our Coral Project?

8. Do you have any improving suggestions (topics of presentations, information provided, projects, etc.)?

S6.7 – RESIDENT QUESTIONNAIRE

Occup	(optional): pation (optional): (optional):			Age: Gender: Nationality:					
1.	How many yea	ars have you v	vorked in the	Maldives?		•			
	How many year	rs have you wo	orked in the to	ırism industry (i	f Maldivian)?				
2.	Can you swim?	?							
	□ Yes □ No								
3.	Can you dive?								
	□ Yes □ No								
3.	What interests	you the most	t?						
	☐ Fish ☐ Coral ☐ Being open-a ☐ Doing sports ☐ Other (please	3							
4.	Please state yo	ur level of sa	tisfaction with	respect to the	following ite	ng items, on a scale of			
		Not Satisfied	Slightly Satisfied	Moderately Satisfied	Satisfied	Highly Satisfied			
Fish a	bundance	1 🗆	2 🗆	3 □	4 🗆	5 □			
Fish v	ariety	1 🗆	2 🗆	3 □	4 □	5 □			
Coral	cover	1 🗆	2 🗆	3 □	4 □	5 □			
Reef s	ize	1 🗆	2 🗆	3 □	4 □	5 □			
Reef v	ariety	1 🗆	2 🗆	3 □	4 □	5 □			
Reef c	colours	1 🗆	2 🗆	3 □	4 □	5 □			
Reef a	ccessibility	1 🗆	2 □	3 □	4 □	5 □			
Inforn house	nation available reef	on1 □	2 🗆	3 🗆	4 🗆	5 🗆			
5.	Please state ho	w important,	, in your opini	on, are the foll	owing items o	on a scale of 1 to			
		Not	Slightly	Moderately	Important	Very			
Fish a	bundance	Important 1 □	Important 2 □	Important 3 □	4 🗆	Important 5 □			

Fish var	iety	1 🗆	2 □	3 □	4 🗆	5 □			
Coral co	over	1 🗆	2 □	3 □	4 □	5 □			
Reef siz	e	1 🗆	2 🗆	3 □	4 □	5 🗆			
Reef var	riety	1 🗆	2 🗆	3 □	4 🗆	5 🗆			
Reef col	ours	1 🗆	2 🗆	3 □	4 □	5 🗆			
Reef acc	cessibility	1 🗆	2 🗆	3 □	4 🗆	5 □			
Informa house re	tion available o eef	on 1 □	2 🗆	3 🗆	4 🗆	5 🗆			
6.	Do you know a	nything about	Coral Restor	ation Projects?	•				
	□ Yes □ No								
7.	Would you be	interested in p	articipating to	a Coral Resto	ration Project	t?			
	□ Yes □ No								
8.	Do you have any improving suggestions (information provided, subjects to include in our research?)								

- CHAPTER 7 -

7.1 General conclusions

In the Anthropocene, the co-occurrence of multiple anthropogenic and natural stressors is altering the diversity of coral reef communities (Fine et al., 2019; Hughes et al., 2019; Rice et al., 2019). Although natural disturbances, such as corallivory, may be beneficial under particular conditions. For example, parrotfish may benefit coral recruits by the algae-grazing activity as reported in the Caribbean (see Mumby, 2009), both Acanthaster spp. and Drupella spp. are attracted by diseased or damaged corals meaning that they may feed on weak individuals, thus, in non-outbreaking condition, they may support an healthy reef (Pratchett, 2001; Morton and Blackmore, 2002). Moreover, their feeding preferences focus on fast-growing coral, as shown also by this study, such as corals of the genus Acropora and Pocillopora, favoring slow growing corals potentially exposed to competition for light and space. However, as the pressure from global warming, overfishing and pollution increase, the predation of corals became a driving force to the biodiversity decline (Rice et al., 2019). The pressure from corallivores has been underestimate in the last decades in the Maldives, but recent events of coral mortality, such as temperature-induced bleaching in 1998 and 2016, have resulted in the increasing attention in the dynamics of interaction between predation and induced coral mortality. In accordance with other studies in different locations (Hoeksema et al., 2013; Pratchett et al., 2017; Scott et al., 2017; Raj et al., 2018), our study found that corallivores in Maldives may alter coral community by acting sinergically with coral bleaching events. Especially, with regards of outbreaks of Acanthaster planci and the predation pressure of Culcita spp. on coral recruits in the aftermath of coral mortality event. Differently, Drupella spp. didn't show severe predatory pressure during this study. Corallivory may also interact with local stressors, such as marine debris, increasing incidence of diseases and hence coral mortality. Plastic debris may stuck among branches of structurally complexed coral causing wounds and promoting microbial colonization by pathogens (Lamb et al., 2018). Further, corallivores, such as Acanthaster spp. and Drupella spp., may be attracted by diseased coral or represent pathogen vectors enhancing diseases incidence and thus coral mortality (Nicolet et al., 2018). However, density, distribution and effects of anthropogenic marine debris (AMD) on Maldivian reef ecosystem is still poorly assessed.

Specifically, debris accumulation on shore and effects on marine life are also poorly studied. Mostly, logistical limitation makes difficult to create a proper disposal, causing the dumping of debris in the ocean. The presence of high level of plastic-derived substances and contamination to marine invertebrates has been recently reported in the Maldives by Saliu et al. (2018, 2019). However, the rate at which anthropogenic marine debris (AMD) accumulate on shores is still unknown. Methodological gaps makes collection of data and comparisons among areas difficult, further, time-effectiveness is fundamental to optimize effort to survey at large temporal and geographical scale to obtain a global overview. The use of drone and Artificial Intelligence (AI), through the PlasticFinder software presented in this study, may help to address such limitations. By the development of technologies, surveys of AMD will rapidly reveal the alarming rate of accumulation and future effects on marine and land ecosystems. Information on presence, density and accumulation rate will help the on-going developing Maldivian waste management plan (MoE, 2018). Certainly, with tourism arrival projected to double by 2021 as a result of the increasing market (MoT, 2013, 2015, 2018) it is likely that anthropogenic disturbances will increase impact on coral reef in the near future. Thus, it is of priority from resorts and government to apply strategies to prevent anthropogenic disturbances such as nutrients pollution and sediments accumulation (Cowburn et al., 2018). It is also highly recommended that a minimal standards for sustainable tourism are developed in the resorts, such as respectful practices for tourists while snorkeling or diving, a waste management plan, boat uses, coastal erosion and beach management, insects control and social responsibilities (Cowburn et al., 2018). Currently, some resorts are starting to improve environmental management: resorts run by Soneva, Four Seasons, Planhotel are developing strategies such as recycling in loco, use of local food or production of water through desalinization plant to reduce plastic bottles usage (Excellencemagazine.luxury, 2019; National Geographic, 2019). Such suggestions are intended to persecute the purpose of minimizing environmental damage which can exacerbate effects of natural disturbances such as corallivory (Rice et al., 2019) and/or anthropogenic temperature-induced bleaching events (Perry and Morgan, 2017; Pisapia et al., 2017, 2019). Due to the continued decline of coral reef the last chance to assist in reef recovery after a coral mortality event is represented by the coral restoration activity (Rinkevich 1995, 2008) which may buffer the effects of natural and anthropogenic disturbances, such as coral predation and AMD

or their interactions. However, in a hospitality context, such as the resorts in the Maldives, it may be difficult to allocate properly financial support to effectively increase reef resilience by restoration. To obtain an optimization of the allocation of financial support, it is necessary to include in the coral restoration assessment socio-economical attributes in order to obtain reef recovery and satisfaction of reef users. Reef use is directly connected with social and economic values such as recreational activities, fishing and, in general, tourisms (Woodhead et al., 2019). Thus, resorts may take advantage from an healthy reef which makes users (clients) satisfied, rather than from a dead reef which is not rewarding from users. In fact, we found that investment in two resorts in the Maldives may be better used if focused more on the reef rather than on artificial structures and more on a long-term solution. Further, locals and women are likely to be more available in working for conservation of the reef, which means that resorts can invest in training them to obtain efficient team for wider spatial and temporal restoration projects. Such investment can be used to increase restoration efficiency, for example, involving voluteers in the direct removal of corallivorous snails from acroporid corals, as reported by Williams et al. (2014), or by removing predators during nursering or outplanting phases (Young et al., 2012; Ladd et al., 2018). Coral reefs in the Maldives resulted more resilient to natural and human pressure over the past decades, if compared to other regions, such as the Caribbean which experience a phase-shift to non-coral dominate reef (Gardner et al., 2003; Bianchi et al., 2006; Jackson et al., 2014; Morri et al., 2015; Pisapia et al., 2017). However, such condition of resilience won't be maintained in the future without proper management, such as preventing disturbances or restore coral communities. In particular, if natural pressures, such as coral predation, will be enhanced by other anthropogenic disturbances such as AMD or coral-induced mortality the future of coral reef will be hopeless. Thus, to avoid the severe risk of losing such a diverse and paradisiacal tropical ecosystem, we finally suggest to improve the understanding of the Maldivian coral reef ecosystem. In particular, starting from our study on the three major corallivores, it is mandatory to deepening the knowledge of their ecology, spawning timing and behavior during non-outbreak periods to prevent predation-induced coral mortality events. Interactions with anthropogenic disturbances need to be also addressed, in addition to the research on effects of AMD on marine organisms. Further, through the use of PlasticFinder, to define the amount and accumulation rate of AMD on shores to push over the attention of the

government and stakeholders in decreasing the production of debris. Sustainable approaches are also suggested, such as the reduction of use of single-use plastic tools (i.e. straws and cutlery) or plastic bottles. We finally suggest to increase restoration efficiency by introducing socio-economic factors in the evaluation of projects at government or private resorts level and promote educational activities for locals and tourists.

Thus, it could be possible to buffer the mortality of corals caused by corallivory, AMD, or their interactions and other natural and anthropogenic disturbances..

7.2 References

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- APPENDIX -

Abstracts and papers peer-reviewed and published during the PhD program

I.1. Climate change, biological invasions, and the shifting distribution of Mediterranean fishes: A large-scale survey based on local ecological knowledge

Azzurro E, Sbragaglia V, Cerri J, Bariche M, Bolognini L, et al. *Global Change Biology* (2019), DOI: 10.1111/gcb.14670

Climate change and biological invasions are rapidly reshuffling species distribution, restructuring the biological communities of many ecosystems worldwide. Tracking these transformations in the marine environment is crucial, but our understanding of climate change effects and invasive species dynamics is often hampered by the practical challenge of surveying large geographical areas. Here, we focus on the Mediterranean Sea, a hot spot for climate change and biological invasions to investigate recent spatiotemporal changes in fish abundances and distribution. To this end, we accessed the local ecological knowledge (LEK) of small-scale and recreational fishers, reconstructing the dynamics of fish perceived as "new" or increasing in different fishing areas. Over 500 fishers across 95 locations and nine different countries were interviewed, and semiquantitative information on yearly changes in species abundance was collected. Overall, 75 species were mentioned by the respondents, mostly warm-adapted species of both native and exotic origin. Respondents belonging to the same biogeographic sectors described coherent spatial and temporal patterns, and gradients along latitudinal and longitudinal axes were revealed. This information provides a more complete understanding of the shifting distribution of Mediterranean fishes and it also demonstrates that adequately structured LEK methodology might be applied successfully beyond the local scale, across national borders and jurisdictions. Acknowledging this potential through macroregional coordination could pave the way for future large-scale aggregations of individual observations, increasing our potential for integrated monitoring and conservation planning at the regional or even global level. This might help local communities to better understand, manage, and adapt to the ongoing biotic transformations driven by climate change and biological invaders.

I.2. Shrimps with a coat: an amphipod hiding in the mantle of *Coriocella hibyae* (Gastropoda, Velutinidae)

Maggioni D, Saponari L, Montano S

Coral Reefs (2018). https://doi.org/10.1007/s00338-018-1688-8

Amphipods can live in association with a variety of molluscs, mostly bivalves, and in a few cases are found in proximity of nudibranchs, mimicking the general appearance of the gastropods (Vader and Tandberg 2013). Here, we report a previously unknown behaviour of an amphipod associated with the lamellarian gastropod Coriocella hibyae (Fig. 1a), observed around Magoodhoo Island, Faafu Atoll, Maldives (3.067N, 72.950E) in March 2018. The amphipod was always found lying on its back (Fig. 1b). Specifically, it laid with the dorsal portion of the body in contact with the gastropod and used the thoracic legs to cling to the host (Fig. 1c). Subsequently, it pushed against the mantle of C. hibyae, burrowing itself in the soft body of the gastropod by bringing the thoracic legs close together (Fig. 1d), as shown in the video in the Electronic Supplementary Material. This behaviour allowed the amphipod to completely hide itself (Fig. 1e), probably finding a safe shelter from predators. This, together with the strong cytotoxic activity of secondary metabolites present in Coriocella species (Cantrell et al. 1999) and likely useful in deterring predation, make the gastropod an ideal refuge for the crustacean. To our knowledge, our findings represent the first report of an association between Coriocella hibyae and other organisms. Notably, the unique behaviour here described was never observed before and provides new insights into the hiding strategies of amphipods.

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I.3. First record of *Phycocaris simulans* (Decapoda; Hippolytidae) from the Republic of Maldives

Saponari L, Galli P, Montano S

Galaxea, Journal of Coral Reefs Studies 20: 11-12 (2018)

Phycocaris simulans (Kemp 1916) is the only species in the genus Phycocaris (family Hippolytidae). It is commonly known as hairy shrimp due to the extreme camouflage that closely resembles the small turf of algae within which it can often be found (Johnson 1989; Gan and Li 2017). Phycocaris simulans was originally described by Kemp (1916) from the Andaman Islands and more recently by Gan and Li (2017) from Hainan Island in the South China Sea, although only a few reports have been published (e.g. Johnson 1989; Preston and Doherty 1990). To date, the known geographic distribution comprises the Andaman Islands (Kemp 1916), Heron Island and Darwin, Australia (Bruce 1983), Kume Island, Japan (Kawamoto and Okuno 2003) and Hainan Island, China (Gan and Li 2017). During a biodiversity survey in April 2016 a single specimen of P. simulans was observed (Fig. 1) at 20 m depth on the south side of the coral reef surrounding Angaagaa Island, Ari Atoll, Republic of Maldives. The reef is a circular-shaped coral formation of around 1 km in maximum diameter with the island on the east side. The reef consists of gentle slopes with around 80% of coral cover mainly dominated by branching and tabular Acropora spp. colonies. The specimen was found motionless on a small turf of filamentous red algae partially covering a dead tabular Acropora sp., which was also coated by green algae, tunicates and sponges. The dead Acropora sp. colony, of around 50 cm in maximum diameter, was lying on the slope in a relatively sheltered area with no direct exposure to the current. The shrimp was photographed for subsequent observations, but could not be collected (Fig. 1a). The original description of Kemp (1916) and the recent description of Gan and Li (2017) were used as guidelines for the identification. The shrimp was identified as Phycocaris simulans due to the presence of hairs at the junction of the cornea and stalk (Fig. 1b-c) which is a unique and remarkable feature reported in both descriptions. Moreover, this morphological characteristic is not known in any other genera of the family Hippolytidae. Thus, to the best of our knowledge this finding represents the first record of P. simulans in the Republic of Maldives and it confirms that this species is more widespread throughout the Indian Ocean than previously thought. Considering the paucity of studies regarding this genus, further investigations are needed to clarify its habitat preferences, distribution and diversity.

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