Coral health and disease assessment in the central Republic of Maldives

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Coordinatore: Prof.ssa Giovanna Lucchini

Anno Accademico 2011-2012
After the magic moment in which my eyes were opened in the sea, I wasn't more possible to see, think and live as before.

(Jacques-Yves Cousteau)
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Finally, I would like to dedicate this work to my loving parents (mamma Maria e papà Francesco), to my brother Daniele and to my wonderful girlfriend Anna.
Currently, it has been estimated that coral reefs, the most diverse of all marine ecosystem, are in severe decline and the most reliable estimates suggest that worldwide 27% have already been lost, with another 16% at serious risk of loss. Coral disease is a significant factor contributing to this decline. However, despite an increasing number of reports of diseases affecting corals and other marine taxa worldwide, and further increases predicted as a consequence of climate change, there has been comparatively little research focused on diseases of Indo-Pacific reef corals. For this reason, given that the Indo-Pacific encompasses 91% of the world’s coral reefs, which are geographically more extensive and specious, knowledge of coral diseases in this region has considerable ecological importance.

The Republic of Maldives consists entirely of coral reefs that are significant on a global scale as well, being the 7th largest in terms of area covered, contributing up to 5% of the world's reef area. Unfortunately, the Maldives have been among the most affected areas in the world, with 60 to 100% coral mortality reported due to an unprecedented coral bleaching event in 1997-1998. So, while the coral bleaching phenomenon and the impact on the coral community around the world are well known, the study of the coral diseases is still in its infancy at least in the Indo Ocean region, and especially in the Republic of Maldives.

This study aims to fill this gap in knowledge through the identification of the diseases affecting reef-building corals and assessing their distribution, host range and prevalence in the Maldivian Archipelago. Principal findings of this study demonstrate that biotic threats identifiable in corals diseases and algal overgrowth represent a serious risk for the coral community and
associated organisms in the Maldivian reefs ecosystem. During the survey period from October 2010 to April 2012 seven islands, belonging to four atolls, were sampled and a total of eight coral diseases were reported for the first time, named: Brown Band Disease (BrBD), Skeleton Eroding Band (SEB), Ulcerative White Spot (UWS), Black Band Disease (BBD), White Syndrome (WS), Black Disease, coral tumors and the not yet described *Porites* White Patch Syndrome. All coral diseases observed affected in total 17 scleractinian genera belonging to 8 different families, representing about the 25 % of the whole scleractinian genera described in this area. In general, the Siderastreidae, Faviidae and Pocilloporidae families were between the most susceptible. Also the Acroporidae results one of the most affected family, but the greater abundance of this family in the Maldivian reef reduce significant the disease prevalence levels. A specie-specific investigation must be made to identify the real extent of the problem. However, although all diseases are present with very low overall disease prevalence (< 1%), which is in contrast with several other studies on reef, our study reveal as WS, UWS and especially BBD resulted widespread in the surveyed area and relatively diffused in the coral community. Malè North displays the higher overall disease prevalence suggesting a probable influence of human activities on pathogen virulence. Considering the numerous studies that related positively anthropogenic disturbance and increase in disease prevalence we think that this result must be keep in consideration.

The overall coral diseases prevalence in the Maldivian Archipelago was estimate around 1.3 %, similar to the level of prevalence reported for the GBR (1.32 %), but the value was lower than the one already reported in the Philippines (4.64 %) for the Indo-Pacific region and Mexican Yucatan (8.3 %) in the Caribbean. Furthermore, even if standardized survey methods and
permanent monitoring sites could provide enough information to determine interannual variability in disease prevalence, our level fall approximately in the range of 3 to 5% estimated for the Indo-Pacific region.

In summary, our study have provided for the first time baseline information on the status of coral diseases affecting reefs in the Republic of Maldives. The present study has also revealed that coral diseases are established and can become prevalent on coral reefs of this archipelago. Furthermore, given that levels of coral diseases are an important indicators of coral reef health and that have been correlated with anthropogenic activities and climate warming events, the prevalence values for multiple diseases reported in this study provide a baseline level of diseases prevalence that could be used to monitor the health of Maldivian reef-building corals as gauge for future change.
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Chapter 1

General Introduction
1.1 Republic of Maldives

The Republic of Maldives consists completely of coral reefs, the most diverse of all marine ecosystems. It represents the main part of the Laccadive-Maldives-Chagos ridge, and extends more than 2000 km from 7°07’ N to 0°40’S in latitude. The Maldives is a chain of coral atolls located in the central Indian Ocean. There are 26 geographical atolls, composed of approximately 1,190 individual coral structures (Zahir 2000). The atolls of the Maldives vary in shape from circular to pear-shaped, and lagoon waters vary in depth from 40 to 60 m. Lagoons contain micro-atolls, faros, patch reefs and knolls. The islands are built of bioclastic sediments and differ in size from 0.5 to several square kilometres. Maximum recorded height above mean sea level in the Maldives is around 3 m and approximately three quarters of available land area is not higher than 1 m above mean high tide (Shareef 2010). The Maldives has a warm and humid tropical climate, dominated by two monsoon periods: the South-West monsoon from April to November and the North-East monsoon from December to March. The annual mean temperature is 28°C, with a maximum average of 32°C and a minimum of 25°C. The average annual rainfall of approximately 1980 mm is evenly distributed throughout the year. This archipelago is built upon coral reefs well-known for their aesthetic magnificence and species diversity. These reefs sustain terrestrial ecosystems that are fragile yet biologically rich and diverse. However, the small size, insularity and fragility of the Maldivian small island ecosystems means that biological diversity is continually under menace. Increasing populations and associated demands of the natural resources are increasing, leading to environmental degradation and loss of biological diversity, especially coral reef decline and habitat
degradation (Zhair 2010). Ecosystems in the Republic of Maldives can be categorized as island, reef, mangrove, swamp, sea grass and open ocean ecosystems, where the coral reefs form the major ecosystem.

1.2 Maldivian coral reefs

The coral reefs of the country are significant on a global scale as well, being the 7th largest in terms of area covered, with a total area of 8,920 km² and contribute up to 5% of the worlds reef area (Spalding et al. 2001). Documented information on marine biodiversity of the Maldives is very limited compared to the richness of marine life occurring in the country. The most comprehensive information on marine invertebrate species diversity can be obtained from the records of the Gardiner Expedition which was undertaken over a century ago. While there are only a few studies conducted, the information on many of these studies are not readily available in the Maldives and are difficult to access (Shareef 2010).

The reefs associated with islands have the general features described by Bianchi et al. (1997). The islands itself are made of sand, changing to coral rubble as the reef edge is approached. The outer slopes are very steep and area down to about 15m is covered with luxuriant coral on a healthy reef. The outer reef slope is characterized by a series of reef terraces at depths of 3-6m, 13-30m, and a deeper one at 50m representing past sea level still stands. The modern coral growth is veneer over older reef rock, but the existing community is constructional down to a depth of at least 50m. In the upper levels, reef is dominated by zooxanthellate corals. In deeper zones, reef is sometimes occupied by azooxanthellate branching coral. Corals are
one of the groups of marine life that is relatively well studied in the Maldives. The coral communities of the Maldives have been investigated only irregularly after the pioneer studies made in 1958 during “Xarifa expedition” (Wallace & Zahir, 2007). Sheppard (1987) recognized 166 species of corals from the Maldives, but a more recent analysis of Indian Ocean corals recognizes 180 species of corals from a recent expedition in the Maldivian Archipelago (Pichon & Benzoni 2007). The latter study reported new records that increase the total number of coral species to 248 species belonging to 57 genera identified from the Maldives (Pichon & Benzoni 2007). The highest species diversity in the Maldives has been recorded within the family Acroporidae.

1.3 Climate change and anthropogenic threats

Several threats currently that endanger worldwide coral reef integrity and biodiversity. All this threats can be classified as biotic when caused by biological factors as coral diseases (Harvell et al. 1999) and/or for example when caused by outbreaks of the crown-of-thorns starfish (COTS) Acanthaster planci (Ciarapica & Passeri 1993) or abiotic when caused by environmental and environmental-human induced factors (e.g. temperature stress, sedimentation, toxic chemicals, nutrient imbalance, ultraviolet radiation). In this case one of the most dangerous threat to coral reefs worldwide is coral bleaching due to anomalous increase of sea surface temperatures (SSTs). However, at present, the main direct threats to marine biodiversity are related to the negative impacts of human activities and human-induced climate change.
In the Republic of Maldives there are a great number of human activities dangerous for the reef ecosystems: threats to living marine resources are represented directly by exploitative uses such as new fisheries, coral and sand mining, non-exploitative but damaging (such as anchoring on reefs) reef uses.

Maldivians usually mine coral for purposes such as building, making lime, or constructing religious structures. Coral mining targets massive corals, which are the longest lived species and form an essential element of the reef structure (Shareef 2010). This results in a loss of topographic complexity, diversity of corals and reef fish and leaves an unconsolidated substrate, which is subject to further erosion. Reef recovery from such physical disturbance is limited by the lack of suitable surfaces for new recruitment (Brown & Dunne 1988). Coastal zone modifications including hard structures such as seawalls, breakwaters and jetties have become the standard in many inhabited islands, as a direct consequence of the advance of human settlement to progressing towards island beaches. These structures, in turn, adversely affect island beaches and ecosystems, leading to severe erosion on some islands. These human activities and human induced changes to the near shore system constitute serious threats to the conservation of biodiversity, especially reef and mangrove ecosystems. Tourism in the Maldives relies on environmental quality and has been developed in a form that is appropriate for small island developing states. Although tourism facilities were initially basic and with low impact on the environment, recent expansion has developed a diverse range of options where more elaborate infrastructures require to support the facilities. As a result, a number of critical environmental issues arising from construction of resorts, harbors
and other infrastructures in the coastal zone have been identified (Shareef 2010).

### 1.4 Coral bleaching: the case of the Maldivian Archipelago

The phenomenon of coral bleaching is characterized by a whitening of corals due to the loss of symbiotic algae (genus *Symbiodinium*) and/or their pigments (Brown 1997). Coral bleaching is triggered by a number of stressful events including tidal exposure, reduced salinities, lack of light, high irradiance, turbidity and starvation (Glynn 1993, 1996; Hoegh-Guldberg 1999; Wilkinson 2000). A major factor responsible for coral bleaching is elevated sea water temperature (Coles & Jokiel 1997; Glynn & D’Croz 1990, Goreau & Hayes 1994; Hoegh-Guldberg 1999). Short-term warming events of 3-4 °C above ambient or long-term warming of 1-2°C may lead to coral bleaching (Jokiel & Coles 1990). Up to date, coral bleaching has occurred in the Caribbean, Indian and Pacific Ocean on a regular basis. Six major episodes of ‘mass bleaching’ of corals, coincident with El Nino Southern Oscillation (ENSO) events, have been reported since 1979, representing one of the most dangerous threats for the coral reefs (Hoegh-Guldberg, 1999). In 1997-1998 there was an unprecedented bleaching followed by the increase in mortality of corals reported from many areas around the world (Berkelmans & Oliver 1999, Hoegh-Gudlberg 1999) with particularly severe bleaching and high mortality reported from the Indian Ocean (McClanahan 2000, Sheppard 1999). The Maldives have been among the most affected areas in the world, with 60 to 100% coral mortality reported (Ciarapica & Passeri 1999, Longo et al. 2000,
Zahir 2000). Significant reductions in live coral cover were seen at all natural reef surveyed, with average live coral cover decreasing from about 42% to 2%, a 20 fold reduction from pre-bleaching event (Edwards et al. 2001). Branching corals and especially those of the genera Acropora, Montipora, Pocillopora and Millepora had completely disappeared at most survey sites by 1999. Only massive and submassive Porites, Pavona and Astreopora are reported to have survived best after 1998 and became the dominant species on reefs of the central atolls of the Maldives (McClanahan, 2000; Zahir, 2000; Loch et al. 2002, 2004). Seven-eight years after the mass mortality event, coral cover was found to be only 20%. Even if it represents a tremendous increase in hard coral cover if compared to the value observed shortly after the 1998 mass mortality episode, coral cover is still low (Lasagna 2008) with a great number of Maldivian reefs that are in an ecological regressive stage (Lasagna 2010).

1.5 What is a coral disease?

The study of coral diseases is a novel field of research that has quickly developed ever since the first coral disease sign was reported in the early 1970s (Antonious 1973). The most general definition of disease is any impairment (interruption, cessation, proliferation, or other disorder) of vital body function, system, or organs from normal state of health (Stedman 2000). This definition might suit animals whose normal state of health can be expected. However, for corals there is currently no available information on what constitutes a normal state of health, or whether such a normal fixed state of health actually exists. Coral disease causation may be attributed to
pathogens, environmental stressor, or a combination of biotic and abiotic factors. Biotic diseases are caused by pathogenic microorganism such as, protists, fungi, bacteria and viruses, while abiotic diseases result from both natural and human-induced environmental stressor including exposure to pollutants or change in ambient conditions (Peters 1997, Kinne 1980). Currently, it has been estimated that coral reefs are in severe decline and the most reliable estimates suggest that worldwide 27% have already been lost, with another 16% at serious risk of loss (Wilkinson 2002), in which the coral disease is a significant factor contributing to this decline (Porter et al. 2001). Epizootics have been reported for several coral species (Goreau et al. 1998, Harvell et al. 1999, 2001) and evidence is growing of substantial decline in the biodiversity and abundance of reef-building corals, particularly in the western tropical Atlantic Ocean (Green & Bruckner 2000, Sutherland et al. 2004, Weil 2004) and also globally (Willis et al. 2004, Aeby 2005). At present, the number of reported coral disease has increased to 29 in the Caribbean (Weil 2004) and to seven in the Indo-Pacific Ocean (Willis et al. 2004), with four of them reported globally (Sutherland et al. 2004). Explications for the recent increase in the prevalence of coral disease are often related to ocean warming (Bruno et al. 2007, Harvell et al. 2007), although the abundance of coral disease is also known to be enhanced by stressors, such as increased nutrients, sedimentation and pollution (Harvell et al. 2009). However, despite this rapid emergence and impact of diseases on coral reef ecosystems, their etiology (casual agents), pathology (sings and physiological effects and mechanism producing host mortality) and their epizotiology (prevalence, incidence and rate of spread in natural populations) remain poorly understood (Richardson 1998, Sutherland et al. 2004, Weil et al. 2006). Without this knowledge, little can be achieved in
determining the actual health of coral reefs and even less in preventing epizootics from spreading across the oceans.

### 1.6 Coral diseases in the Indo-Pacific Ocean

Despite an increasing number of reports of diseases affecting corals and other marine taxa worldwide (Ward & Lafferty 2004), and further increases predicted as a consequence of climate change, there has been comparatively little research focused on diseases of Indo-Pacific reef corals. Indeed, in the past, coral diseases were mainly studied in the Caribbean region, which is considered a global hot spot for diseases (Harvell et al. 1999, Green & Bruckner 2000), while, in contrast, disease’s effects in the Indo-Pacific region (which is geographically more extensive and specious) are poorly documented. However, the coral reefs of the Indo-Pacific are the most diverse in the world. A total of 581 species of scleractinian corals have been reported from this region. In contrast, the Western Atlantic has 62 reported species (Veron 2000). This diversity could be predicted to lower the spread of infectious diseases that are limited in host range, as the probability of pathogens encountering a host would be reduced in high-diversity community. Alternatively, more diverse reefs could harbor a greater diversity of pathogens (Raymundo et al. 2005). For this reason, given that the Indo-Pacific encompasses 91% of the world’s coral reefs, knowledge of coral diseases in this region has considerable ecological importance. In the Indo-Pacific, significant coral diseases have been reported in the Philippines (PH) (Raymundo et al. 2005), Guam (Myers & Raymundo 2009) and the Great Barrier Reef (GBR) (Willis et al. 2004) and might underlie or
at least contribute to the increasing rate of coral decline in this region (Bruno & Selig 2007). In addition, reports on coral diseases presence and outbreaks from Hawaii (Aeby 2005), East Africa (McClanahan 2004), Indonesia (Haapkylä et al. 2007), the Gulf of Eilat (Barash et al. 2005), Japan (Weil 2012) and Maldives (Montano 2012) have contributed to the acknowledgement on the coral diseases in the Indo-Pacific and poses a serious threat to coral populations. Although, the majority of these reports describe single point-in-time observation and a more in-depth investigations needed, a few diseases have been documented quantitatively.

1.7 The aims of this study

Coral reefs of the Maldives-Chagos ridge are one of the lowest human influenced areas in the Western Indian Ocean (McClanahan et al. 2000) and, therefore, changes in these reefs are likely an indicator of global change. Nevertheless, coral bleaching and diseases are problems rapidly emerging on coral reefs (Harvell et al. 1999, 2004; Weil et al 2004) and they are becoming the predominant threats to their ecological health (McClanahan 2002; Weil 2004). While the coral bleaching phenomenon and its impact on the coral community around the world is well known, the study of coral diseases is still in its infancy at least in the Indo Ocean region. Indeed, prevalence, distribution and host range of coral diseases has not yet been determined for many parts of the world, and especially almost nothing is known regarding coral diseases affecting the reef-building coral in the Republic of Maldives.
This PhD study tries to fill this gap in knowledge through investigation that addresses the following specific aims:

1. To identify coral diseases affecting reef-building corals on the Maldivian reefs

2. To determine prevalence, host range and distribution of the coral diseases in the central part of the Republic of Maldives.

3. To estimate a baseline coral diseases prevalence that will represent historical levels for this archipelago

1.8 References


Antonius A (1973) New observations on coral destruction in reefs. Abs Assoc Isl Mar Lab Caribb 10:3


Stedman TL (2000) Stedman’s medical dictionary, 27th edn. Lippincott Williams & Wilkins, Baltimore


Veron, JEN Corals of the World vols 1–3 (Australian Institute of Marine Science, Townsville, Australia, 2000).


Chapter 2

Biotic stresses in the Maldivian Archipelago: coral diseases and algal overgrowth
Preface

Several threats are responsible of the coral reefs’s decline. Despite their spectacular and widespread biodiversity the Maldivian marine life lacks of detailed study related to the natural impact present in the coral community. Here in we report the presence of two biotic threats not well studied and not yet reported for this country: algal overgrowth and coral diseases. Both were observed in the waters around Magoodhoo Island, in Faafu Atoll, where in 2010 the University of Milano Bicocca opened a Marine Research Station (MARHE centre) that provided support to logistics and field work. This research allowed me to work in partnership with other colleagues to investigate the expression of 60-kDa heat shock protein (Hsp60) in corals subjected to algal overgrowth and the skeleton eroding band disease. This study has been published as research paper on Marine Environmental Research 78(2012) 34-39. (Appendix 1)
2.1 First report of coral diseases in the Republic of Maldives

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Abstract
Little is known about coral diseases in the Indian-Ocean region, especially in the Republic of Maldives. This study is aimed at documenting the presence of coral diseases in the archipelago of Maldives. Surveys for lesions in scleractinians conducted at 8 sites around Magoodhoo Island (Faafu Atoll) in October and November 2010 led to the identification of five coral diseases and one anomalous pigmentation response affecting 8 hard coral genera. White syndrome, skeleton eroding band disease, black band disease and Porites dark discoloration response were the most commonly observed conditions. In contrast with several reports of other reef systems, the overall observed prevalence of coral diseases was rather low(< 2 %), with individual prevalence ranging from 0.7 % for skeleton eroding band to 0.18 % for Porites dark discoloration response. These data represent the first report of coral diseases for the Republic of Maldives.
2.1.1 Introduction

Coral disease epizootics have become a major threat to reef ecosystems globally, and an increasing number of newly emerging syndromes has been reported over the past 20 years (Harvell et al. 1999, Raymundo et al. 2005, Sokolow 2009). Because climate change is predicted to amplify host susceptibility, host range, pathogen survival and disease transmission (Ritchie et al. 2001, Myers & Raymundo 2009), outbreaks are expected to increase worldwide in the future (Willis et al. 2004, Bruno et al. 2007).

The coral reefs of the Maldivian archipelago are among the most diverse in the Indian Ocean, and are known to host more than 180 zooxanthellate coral species belonging to 51 genera (Pichon & Benzoni 2007). The major reef structures occupy an area of about 21,000 km², 21.1% of which can be categorized as marine productive reef habitats (Naseer & Hutcher 2004).

Around the world researchers are growing alarmed about the potential negative effects of infectious diseases on reef communities (Bruno et al. 2007). Temporary shifts from acroporid- to agaricid-dominated reefs caused by disease-induced mortality have already occurred in Belize (Aronson et al. 2002), and outbreaks of coral diseases caused a significant loss of coral cover in the Caribbean Sea and on the Great Barrier Reef (Willis et al. 2004, Weil et al. 2009).

The Maldivian archipelago has been heavily affected by 1998 coral bleaching event which led to a coral mortality of up to 100%, with varying effects depending on species and locality (Bianchi et al. 2003). The living coral cover was of 2-8% immediately after the mass mortality event, and have increased up to 12-37% in the following eight years (Lasagna 2008).
qualitative change in coral communities, with a shift from *Acropora* dominated reefs to *Porites* dominated reefs (Goreau 2000).

The ecological impact of bleaching on coral communities, and particularly its ability to increase coral susceptibility towards infectious disease, is well known (McClanahan 2009). However, there is no available information about the presence of coral diseases in Maldives. Here, we try to fill this gap, by reporting the results of a survey conducted to investigate the occurrence and prevalence of diseases affecting reef-building corals in an island of the Republic of Maldives.

### 2.1.2 Materials and Methods

Underwater surveys were conducted during October and November 2010 in order to investigate the presence and prevalence of infectious diseases affecting scleractinian corals in the waters around the inhabited island of Magoodhoo, Faafu Atoll, Republic of Maldives (3°04’ N; 72°57’ E) (Fig. 2.1). The island measures 900 x 450 m and it is located on the south-east part of the atoll rim, about 140 km South of the capital Malè. Magoodhoo reef is approximately 2.9 km long and 1.55 km wide, and exhibits the features of a typical low-energy reef with a luxuriant growth of coral and gently slope to all sides. Sites were selected haphazardly from those accessible.

During sampling period, which fell within the wet season (mid-May to November), the local monthly mean sea surface temperature (SST) was of 29,1 ± 0,12 °C, and temperature variation among seasons did not exceed 1 °C (http://disc.sci.gsfc.nasa.gov/techlab/giovanni/). Analyses were conducted by snorkeling at shallow sites (n= 4, 0-5m) and by scuba diving at
deep ones (n= 4, 10-20 m) (Fig. 2.1c). For each site we performed an exploratory qualitative analysis aimed to compile a complete list of the hard coral diseases occurring in the area. Additionally, quantitative information about coral disease prevalence was obtained by performing, at each site, three randomly placed 25 × 1 m belt transects (total = 24 transects) spaced 10 to 20 m apart. Colonies on the belt margin were counted only when 50% or more of the colony lay within the belt. The selected transect size was chosen as the most suitable in relation to field logistics and the size of the surveyed area.

In both qualitative and quantitative analyses, all corals were identified in situ at genus level (according to Veron 2000). Diseases were identified in situ as well (according to Rosenberg 2004). Small samples of both healthy and infected coral tissue were collected for further laboratory identification. Visible symptoms of disease or stress not ascribable to those reported from available literature were described and photographed when encountered. Micro photographs (32x) of infected colonies were obtained using a StemiDIV4 stereomicroscope paired with a Canon G11 camera.

All diseased colonies within each belt transect were noted, and the number of diseased and healthy colonies was counted in order to compute disease prevalence, which was calculated as the number of infected colonies quoted by the total number of colonies. Taxon specific prevalence was calculated as the number of cases of a specific disease or syndrome divided by the number of appropriate host encountered. The average total disease prevalence for each site was calculated by averaging the prevalence of all belt-transsects.
2.1.3 Results

2.1.3.1 Occurrence and prevalence of observed diseases

Our survey of the reefs of Magoodhoo Island revealed the presence of 5 syndromes affecting different genera of reef-building corals, namely black band disease, ulcerative white spot disease, white syndrome, skeleton eroding band, and brown band disease. Additionally, we observed a dark discoloration response on *Porites* spp. loosely similar to that typical of dark spot disease, to which we will refer as *Porites* dark discoloration response, in order to distinguish it from any common disease and tissue pigmentation response known for this genus (Raymundo et al. 2005).

In total we counted 2,761 colonies belonging to 19 genera. Among them, disease-induced lesions were observed on 64 colonies (belonging to 8 genera), 50 of which (belonging to 6 genera) were found within transects. Thus, the resulting overall prevalence of coral diseases on the reef was lower than 2%. Individual prevalence of each investigated coral disease was lower than 1%, ranging from 0.7% (skeleton eroding band) to 0.18% (*Porites* dark discoloration response). Although qualitative surveys revealed the presence of brown band disease and ulcerative white spot, the two diseases were not found within transects. For the other diseases (black band disease, skeleton eroding band and white syndromes) as well as for *Porites* dark discoloration response we report the number of occurrences across all the found coral genera (Table 2.1). All the diseases observed were found in the shallow site, while in the deeper sites *Porites* dark discoloration response was not found. A preliminary assessment of disease prevalence among affected coral genera is reported in Table 2.2.
2.1.3.2 Description of observed diseases

We found evidence of coral disease in five scleractinian families, namely Acroporidae, Poritidae, Faviidae, Pocilloporidae and Agariciidae. More than half (54.7 %) of the diseased colonies belonged to the Acroporidae, which resulted the most affected family. The other diseased colonies belonged to the Poritidae (17.3 %), the Siderasteridae (12.5 %), the Faviidae (6.2 %), the Pocilloporidae (6.2 %) and the Agariciidae (3.1 %). Among affected genera, Acropora hosted the highest number of coral diseases (n=5), while the remaining genera were affected by a maximum of two diseases.

Black band disease (Figure 2.2a), that is constituted by a mat of microbes dominated by the cyanobacterium Phormidium corallitycum (Rützler & Santavy 1983)(Figure 2.2b), showed the largest host range, affecting 5 different coral genera and particularly Psammocora and Goniopora genera.

Ulcerative white spot disease (Figure 2.2c), which was found mainly on Porites genus, was characterized by discrete, bleached round foci of 3–5 mm in diameter, coherently to the description given by Raymundo (2003) (Figure 2.2d). Lesions that resembled ulcerative white spot were also observed on some colonies of Acropora spp. (Figure 2.2e). These lesions were characterized by discrete, multifocal round foci that revealed an underlying intact skeleton. These injured areas were mainly located in the basal portion of the colonies. Similarly to the description given by Work and Aeby (2006), the lesions due to white syndrome (Figure 2.2f) consisted of large, diffuse bands of tissue loss that revealed a bare, white, intact skeleton. The lesions were mildly to severely extended and the tissue loss involved the coenosarc and the polyps of the colonies belonging to the Acropora genus. We closely examined all the colonies affected by white syndrome and ulcerative white
spot, and we found no evidence suggesting an involvement of coral predators in tissue death.

Skeletal eroding band (Figure 2.2g), which is associated with the ciliate *Halofolliculina corallasia* (Figure 2.2h) (Antonius 1999, Willis et al. 2004) was found on genera *Acropora*, *Pocillopora* and *Goniastrea*, while brown band disease (Figure 2.2i), that is caused by a mobile ciliate (Figure 2.2j) such as the recently described *Porpostoma guamense* (Lobban 2011) was found only on branching *Acropora* spp.. Members of the genus *Porites*, affected by dark discoloration response, were characterized by small to large areas of brown discoloration with indistinct undulating borders (Figure 2.3); in addition, diseased colonies showed lesions both focally and diffusely distributed at peripheral areas.

### 2.1.4 Discussion

The present study provides baseline information on the status of diseases affecting scleractinian corals in a previously unsampled region. Our surveys documented the presence of five different coral diseases and one anomalous pigmentation response in the reefs of Magoodhoo Island. All the pathologies observed in this study have been previously reported from the Indo-Pacific region. Nonetheless, the data presented here constitute the first records of coral diseases in the Maldives Archipelago.

All observed coral diseases were present apparently with low prevalence, which is in contrast with several other studies on reef systems (Myers & Raymundo 2009, Weil et al. 2012). The most commonly observed diseases were skeleton eroding band, black band disease (coherent with the
assumptions of their circumglobal distribution, see Croquer et al. 2006 and Myers & Raymundo 2009) and white syndrome. However, white syndrome showed a much lower prevalence than that already observed in similar ecosystems (see, for example, Willis et al. 2004 and Hobbs & Frish 2010 for the Indo-Pacific, and Sutherland et al. 2004 for the Caribbean).

Our surveys revealed also the presence of one anomalous pigmentation response which we defined *Porites* dark discoloration response and that resulted relatively widespread in the area of study. We observed no skeleton or tissue damage by scars, tumors or other known lesions associated to the condition. This suggests that the response may be triggered by unidentified chemical and/or microbial agents, or may be interpreted as a hypermelanization response to a pathogen (Petes et al. 2003). Further investigation would improve our knowledge of this response or disease. We recorded a few cases of brown band disease and ulcerative white spot which extend the documented geographic range for the two diseases (Weil et al. 2012). In particular we extend the range of brown band disease westward from Philippines and Great Barrier Reef (Raymundo et al. 2003, Willis et al. 2004). Interestingly, we noticed signs of infections similar to those due to ulcerative white spot on several *Acropora* colonies. Although still to be confirmed by more detailed analyses, this finding may suggest a possible increase in host range for the disease, that, up to date, has been reported mainly from *Porites* spp. (Raymundo et al. 2003; Kaczmarsky 2006).

Among coral genera *Acropora* hosted the highest number of coral diseases. This supports the hypothesis that fast-growing corals might have a weaker disease resistance than slow growing corals (Willis et al. 2004; Palmer et al. 2008, 2010; Mydlarz et al. 2010). Overall, less than half of the sampled
genera resulted affected by at least one coral disease. This could suggest a low spread of host-specific coral diseases (Raymundo et al. 2005).

Despite Maldives and hence the area of study have been affected by a mass coral bleaching in 1998 (Longo et al. 2000, Zahir 2000), the currently available data do not make possible to individuate a relationship between the past thermal stress and the current diffusion of coral diseases. However the expected future increase in sea surface temperatures (Kleypas et al. 1999) could lead not only to new bleaching events, but also promote the spread of coral pathogens by increasing their growth rate and virulence (Ben-Haim et al. 2003b) and by reducing immune response in coral hosts (Alker et al. 2001; Mydlarz et al. 2009; Palmer et al. 2011). Considering the current state of regression of many reefs in the Maldives (Lasagna 2010), a better understanding of the actual and potential impact of infectious diseases on coral ecosystem dynamics is fundamental to conservation planning.

Although we cannot exclude that our results may be affected by sample size, number of replicates and local variation, and that they may be not representative of large scale patterns valid for the whole Magoodhoo reef, we hope that our preliminary study could stimulate the interest of coral pathologists and promote future in-depth investigations focusing on coral diseases in Maldivian reefs.

2.1.5 References


Rosenberg E, Loya Y (eds) 2004 Coral health and disease. Springer-Verlag, Berlin


2.1.6 Table and Figures

Table 2.1. Frequency of occurrence of the coral diseases at the genus level. BBD= black band disease; SEB= skeleton eroding band; WS= white syndromes; PDDr= *Porites* dark discoloration response; BrBD= brown band disease; UWS= ulcerative white spot; ?>= to be confirmed; ( ) = not found within transect.

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Table 2.2. Total prevalence and depth distribution of observed coral diseases. LL 95% and UL 95% bootstrap lower and upper confidence limits.

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Fig. 2.1. Map of the study area including the 8 sampling sites. A: Republic of Maldives; B: Faafu Atoll; C: Magoodhoo Island
Fig. 2.2. Photographs illustrating the coral diseases found in Magoodhoo reef. a Black band disease on Psammocora digitata colony; b close up of the mat forming the black band; c ulcerative white spot on a massive Porites colony; d areas of white
tissue discoloration with discrete margins; e signs similar to ulcerative white spot on an *Acropora* spp. colony; f *Acropora* plate coral with white syndrome; the white band is the lesion area, with live coral on the right (LC) and dead coral (colonized by filamentous algae) on the left (DC); g skeleton eroding band (pointed by the arrow) on a branching *Acropora muricata* colony; h *Halofolliculina corallasia*: note the large peristomial wings coming out of the lorica; i brown band disease (pointed by the arrow) on a branching *Acropora muricata* colony; j details of ciliate clustering constituting the band. Photos: S. Montano

**Fig 2.3. Porites** dark discoloration response. (H) shows the regular color of the healthy part of the colony. (D) shows the anomalous pigmentation response founded in Magoodhoo island. It consists in an abnormal dark-like purple discoloration of the coral tissue. Photos: S. Montano
2.2 Acropora muricata mortality associated with extensive growth of Caulerpa racemosa in Magoodhoo Island, Republic of Maldives

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Caulerpa racemosa, a common and opportunistic species widely distributed in tropical and warm-temperate regions, is known to form monospecific stands outside its native range (Verlaque et al. 2003). In October 2011 we observed an alteration in benthic community due to a widespread overgrowth of C. racemosa around the inhabited island of Magoodhoo (3°04’N; 72°57’ E, Republic of Maldives). The mats formed a continuous dense meadow (Fig 4a) that occupied an area of 95 x 120 m (~11,000 m²) previously dominated by the branching coral Acropora muricata. Partial and total mortality (Fig 2.4 b-c) were recorded on 45% and 30% of A. muricata colonies, respectively. The total area of influence of C. racemosa was however much larger (~25,000 m²) including smaller coral patches near to the meadow, where mortality in contact with the algae was also observed on colonies of Isopora palifera, Lobophyllia corymbosa, Pavona varians, Pocillopora damicornis, and Porites solida. Although species of the genus Caulerpa are not usually abundant on oligotrophic coral reefs, nutrient enrichment from natural and/or anthropogenic sources are known to promote green algal blooms (Lapointe and Bedford 2009). Considering the current state of regression of many reefs in the Maldives (Lasagna 2010) we report an unusual phenomenon which could possibly become more common.
References


Figure

Fig 2.4. (a) Extensive meadows of *Caulerpa racemosa* overgrowing colonies of the dominant coral *Acropora muricata*. (b-c) Partial and total mortality of *A. muricata* following algal overgrowth.
Chapter 3

Distribution, prevalence and host range of the Maldivian coral diseases
3.1 Introduction

Coral diseases are increasing worldwide and pose a severe threat to the future of coral reefs (Sutherland et al. 2004). Some of them have already been devastated by diseases, and outbreaks are predicted to raise in the future. Some coral disease outbreaks in the Caribbean and Pacific Ocean have been linked to changes in environmental conditions (e.g. increased water temperature; Patterson et al. 2002). Worldwide, the anomalous increment of sea surface temperature has affected, often with greater severity, coral reefs in the Indian Ocean (Goreau et al. 2000, Graham et al. 2006). Nevertheless there have only been preliminary reports of coral diseases in the Indian Ocean and the prevalence, distribution and impact of these and other unreported diseases on Indian Ocean reef communities are largely unknown (McClanahan 2004).

However, establishing these values for coral diseases is considered a priority (Harvell et al. 2002) as it helps to identify the origins, reservoirs, modes of transmission and potential causes of diseases outbreaks (Willis et al. 2004). For this reason, given that the coral diseases have caused widespread destruction to Caribbean reefs and are increasing rapidly in prevalence in the Pacific Ocean (Willis et al. 2004), it is imperative to assess the impact of coral disease on reefs in the Indian Ocean.

My previous study (Montano et al. 2012) represents the first report of coral disease in the Republic of Maldives aimed to identify the disease affecting reef-building coral in this archipelago. We observed some of the most dangerous Indo-Pacific coral diseases named: Brown Band Disease (BrBD), Skeleton Eroding Band (SEB), White Syndrome (WS), Ulcerative White Spot (UWS) and Black Band Disease (BBD) (see chapter 2 for their
morphological description). The mentioned study represents only a single-in-time investigation, and can not be representative of the whole Maldivian reef ecosystem. Nevertheless it is extremely important to improve our knowledge about the epidemiology of the diseases in this geographic area, considering that local population utilizes an enormous amount of resources deriving from this ecosystem. The main aims of this study are to assess for the first time prevalence, distribution and host range for skeleton eroding band disease, brow band disease, white syndrome and ulcerative white spot disease, identified in 2010, and comparing their disease prevalence between depth, islands and atolls.

### 3.2 Materials and Methods

The study was conducted between October 2010 and April 2012 in order to record distribution, prevalence and host range of infectious disease affecting scleractinian corals in the waters of central Republic of Maldives. During sampling period, which fell within both the wet and dry seasons, the local monthly mean sea surface temperature (SST) was of 29,1 ± 0,12 °C, and temperature variation among seasons did not exceed 1,7 °C ([http://disc.sci.gsfc.nasa.gov/techlab/giovanni/](http://disc.sci.gsfc.nasa.gov/techlab/giovanni/)). In total we investigated seven island named Ihuru and Vabbinfaru (Malè Atoll), Adanga and Magoodhoo (Faafu Atoll), Athuruga and Thudufushi (Ari Atoll), and Kunfunadhoo (Baa Atoll) (Fig. 3.1) through 4 to 8 sites for island. The islands, including land and related coralline reefs, measures from 0.5 km of Ihuru to 3.5 km of Thudufushi and exhibits the features of a typical low-energy reef with a luxuriant growth of coral and gently slope to all sides.
Sites were selected haphazardly from those accessible and surveys were carried out by snorkelling at the shallow sites (n= 25, 0-5m) and by scuba diving at the deep ones (n= 24, 5-15 m). Quantitative information about coral disease prevalence were collected by performing at each site three randomly placed 25 × 1 m belt transects (total = 147 transects) spaced 10 to 20 m apart. The selected transect size was chosen as the most suitable in relation to field logistics and the size of the surveyed area (Montano et al. 2012). All corals were identified at genus level in situ, according to Veron (2000) and colonies on the belt margin were counted only when 50% or more of the colony lay within the belt.

All diseased colonies within each belt transect were noted, and the numbers of diseased and healthy colonies were counted so that disease prevalence could be calculated as the number of infected colonies /total number of colonies. Taxon specific prevalence was calculated as the number of cases of a disease divided by the number of appropriate host encountered. The average total disease prevalence for each site was calculated by averaging the prevalence of all belt-transects. Coral disease recorded in this study were identified by macroscopic characteristic of lesions according to photographs and description in Beeden et al 2008 and Willis et al 2004. Micro photographs (32x) of infected colonies were obtained using a Leica EZ4 D stereomicroscope.

Statistical comparisons of the disease prevalence of Skeleton eroding band, Brown band disease, White syndrome and Ulcerative white spot between depth, Islands, Atolls and scleractinian families were each made using Mann-Whitney U-test and Kruskal-Wallis test when non-normally distributed data were encountered (Zar 1999). Because the assumptions of a parametric test could not be met (due to non-normally distributed data), a
Sperman’s rank correlation was used to examine if diseases and taxon specific prevalence was related to host and overall coral density for each coral disease. Statistical analyses were performed using SPSS computer software, and a significant difference was defined as p < 0.05. All data are presented as arithmetic means ± standard error (SE) unless otherwise stated.

3.3 Results

During a field activities from October 2010 to April 2012 a total number of 49 sites around 7 islands, belonging to 4 different Atoll, were sampled in the central Archipelago of Maldives. Colonies affected by at least one coral disease were found on all islands. In the investigated area we counted 34’480 colonies belonging to 23 scleractinian genera.

3.3.1 Brown Band Disease

Sites with colonies affected by BrBD were 15 (corresponding to the 30.6% of the total). Forty-nine scleractinian colonies resulting affected by brown band disease (Fig. 3.2), with a mean overall prevalence for sites of 0.18 ± 0.05 (mean ± S.E.). The highest BrBD prevalence (1.43 %) was observed in a shallow site of Vabbinfaru. The total deeper sites (with 0.22 ± 0.7) showed higher overall mean prevalence than the total shallow sites (0.14 ± 0.7) but a statistically significance difference were not found between the two different depth (Mann Whitney U test p > 0.05).
Islands showed a similar prevalence (less than 0.5% each) with Vabbinfaru Island that showed the highest disease prevalence (0.40 ± 0.19) and Ihuru the lowest (0.02 ± 0.02), and a statistically significance difference was found for them (Kruskal-Wallis test p < 0.01). Between atolls, Ari showed the highest BrBD prevalence (0.35 ± 0.1) (Fig. 3.3) with Ari and Faafu atolls that showed an higher BrBD prevalence in deeper water than shallow ones. Opposite scenario was observed in Malè Nord atoll. A statistically significance difference was found between atoll (Kruskal-Wallis test p < 0.05) and between two different depth in Ari atoll (Mann Withney U test p < 0.05) (Fig. 3.4).

BrBD exclusively affects scleractinian corals on the Maldivian reef, with no observation of BrBD affecting soft or hydroid corals. In total cases of BrBD were recorded from one scleractinian genus Acropora. To genus level mean Acropora BrBD prevalence range from 0 to 2.71 % in a deep site in Thudufushi island, Ari Atoll.

Positive correlations were found between overall BrBD prevalence and Acropora density (Spearman’s rho ρ= 0.4 p < 0.01), and between overall BrBD prevalence and overall coral density (Spearman’s rho ρ= 0.35 p < 0.05).

3.3.2 Skeleton Eroding Band

Sites with colonies affected by SEB were 32 (corresponding to the 65.3 % of the total). Total number of the transects sampled were 147 (= 3675 m²), 54 of which affected by SEB and 93 without diseased colonies (respectively the 37 % and 63 %). Regarding the transect affected, the 56 % of them were in shallow water and the 44 % in deeper one.
Sixty-eight scleractinian colonies resulting affected by skeleton eroding band (Fig. 3.5), with a mean overall prevalence for sites of 0.29 ± 0.05 (mean ± SE). The highest SEB prevalence (2.01 %) was observed in a deep site of Magoodhoo. The total shallow sites (with 0.27 ± 0.06) showed higher overall mean prevalence than the total deeper sites (0.30 ± 0.09) but no statistically significance difference was found between the two different depth.

Six Islands showed a prevalence less than 0.5% each. By contrast, Magoodhoo island showed the highest disease prevalence (0.74 ± 0.23) but no statistically significance difference was found for them (Kruskal-Wallis test p > 0.05) (Fig. 3.6). Between atolls, Faafu showed the highest SEB prevalence (0.55 ± 0.17) and no statistically significance difference was found for them (Kruskal-Wallis test p > 0.05) (Fig 3.7). No depth distribution patterns were observed, with Faafu and Ari that showed the higher SEB prevalence in the deeper sites than the shallow ones, while Malè North and Baa atolls showed the opposite scenario (Fig. 3.8). No statistically significant differences in SEB prevalence were observed between two different depth in each atolls.

SEB exclusively affects scleractinian corals on the Maldivian reef, with no observation of that disease affecting soft or hydroid corals. In total cases of SEB were recorded from 2 scleractinian genera (Acropora and Pocillopora) (Tab. 3.1) belonging to different scleractinian families (Acroporidae, Pocilloporidae). Single Goniastrea spp. diseased colony was not found within the transect so it was not considered in the analyze.

To genus level Pocillopora showed the higher mean SEB prevalence with 3.23 ± 0.7 with a maximum prevalence value of 33.3% in a deep site in
Vabbinfaru island (Malè North Atoll), while the genus *Acropora* showed a mean SEB prevalence of 0.4 ± 0.06. No correlations were found between overall SEB prevalence and overall coral density (Spearman’s rho $\rho = -0.24$ $p = 0.09$) as well as between overall SEB prevalence and *Pocillopora* density (Spearman’s rho $\rho = -0.09$ $p = 0.51$) or *Acropora* density (Spearman’s rho $\rho = -0.186$ $p = 0.2$).

### 3.3.3 White Syndrome

Sites with colonies affected by WS were 32 (corresponding to the 65.3 % of the total). Total number of the transects sampled were 147 (= 3675 m$^2$), 67 of which affected by WS and 80 without diseased colonies (respectively the 45.5% and 54.5%). Regarding the transects affected, the 53.7 % of them were in shallow water and the 46.3 % in deeper one. One hundred-twenty one scleractinian colonies resulting affected by white syndrome (Fig. 3.9), with a mean overall prevalence for sites of 0.42 ± 0.06 (mean ± S.E.). The highest WS prevalence (2.60 %) was observed in a deep site of Vabbinfaru, Male North Atoll. The total shallow sites (with 0.51 ± 0.14) showed higher overall mean prevalence than the total deeper sites (0.34 ± 0.07) and no a statistically significance difference was found between the two different depth (Mann Whitney U = 295 $p = 0.9$). Islands showed a mean WS prevalence varying from the lowest of Kunfunadhoo (0.09 ± 0.06) to the highest showed by Ihuru Island (0.84 ± 0.17). No statistically significance difference was found for them (Kruskal-Wallis test $p > 0.05$) (Fig. 3.10). Between Atolls, Male North showed the highest WS prevalence (0.69 ± 0.17) and a statistically significance difference was found
for them (Kruskal-Wallis p < 0.05) (Fig. 3.11). However no any spatial pattern about depth distribution was recognizable for that disease both to Island than Atoll spatial scale (Fig. 3.12 and 3.13).

WS exclusively affects scleractinian corals on the Maldivian reef, with no observation of WS affecting soft or hydroid corals. In total cases of WS were recorded from 5 scleractinian families (Acroporidae, Pocilloporidae, Faviidae, Siderastreidae, Agariciidae), and 5 scleractinian genera (Tab. 3.2). Several Echinopora spp. and Psammocora spp. diseased colonies were not found within the transects so they were not considered in the analyze. Species within the Acroporidae family were by far the most susceptible, with 117 diseases colonies on 121 of all WS infection founded on species within this family. To genus level (Tab. 3.2) the mean WS prevalence range from 0.11 ± 0.07 of Pavona to 1 ± 0.2 of the Acropora genus, with Pocillopora genus that showed a maximum prevalence of 16.6 % in a deep site in Vabbinfaru Island, Malè North Atoll. No correlations were found between overall WS prevalence and overall coral density (Spearman’s rho ρ= - 0.09 p = 0.51) as well as between overall diseases prevalence and Acropora density (Spearman’s rho ρ= 0.05 p= 0.7), Pavona density (Spearman’s rho ρ= -0.02 p= 0.82), and finally Pocillopora density (Spearman’s rho ρ= - 0.19 p= 0.18). Further no correlations to genus level between host density and host prevalence were found for the three host genera.

3.3.4 Ulcerative White Spot

Sites with colonies affected by UWS were 28 (corresponding to the 57.1 % of the total). Total number of the transects sampled were 147 (= 3675 m²),
53 of which affected by UWS and 94 without diseased colonies (respectively the 36% and 64%). Regarding the transect affected, the 47.2% of them were in shallow water and the 52.8% in deeper one.

Hundred and thirty one scleractinian colonies resulting affected by ulcerative white spot disease (Fig. 3.14), with a mean overall prevalence for sites of 0.51 ± 0.14 (mean ± S.E.). The highest UWS prevalence (5.13%) was observed in a deep site of Vabbinfaru, Malé North. The total shallow sites (with 0.53 ± 0.21) showed a similar overall mean prevalence of the total deeper sites (0.51 ± 0.19) but no statistically significance difference was found between the two different depth.

Islands showed different prevalence varying from 0 of Magoodhoo island to 1.47 ± 0.44 of Kunfunadhoo that showed the highest disease prevalence and a statistically significance difference was found for them (Fig. 3.15). Further between Atolls, Baa showed the highest UWS prevalence and a statistically significance difference was found for them (Kruskal-Wallis test p < 0.001) (Fig. 3.16). Significant differences between two different depth were found in Malé North atoll (Mann Whitney U test= 12.5 p< 0.5) and Baa atoll (Mann Whitney U test= 10 p < 0.5)(Fig. 3.17).

UWS exclusively affects scleractinian corals on the Maldivian reef, with no observation of that disease affecting soft or hydroid corals. In total cases of UWS were recorded from 4 scleractinian families (Faviidae, Poritidae, Agaricidae, Merulinidae), and 6 scleractinian genera (Tab 3.3). Several Acropora spp. colonies showed similar injuries but they were not found within the transect so it was not considered in the analyze. Species within the Faviidae family were by far the most susceptible, with 49.5% of all infection founded on species within this family. Forty-nine percent of UWS infections were recorded from species in the family Poritidae, the rest
percentage was recorded from Agariicidae (0.75%) and Merulinidae families (0.75%).

To genus level the mean UWS prevalence range from 0.02 ± 0.02 of *Hydnophora* to 3.79 ± 1.59 of the *Goniastrea* genus, with *Leptoria* genus that showed a maximum prevalence of 83.3% in a shallow site in Kunfunadhoo Island, Baa Atoll. Statistically significance difference in coral density were found between shallow and deep sites (Mann Whitney U test = 129.5 p < 0.05), with the shallow one that showed the highest coral density (Mann Whitney U test = 38.5 p < 0.01) but no correlations was found between overall UWS prevalence and overall coral density (Spearman’s rho \( \rho = -0.154 \) p= 0.291). Positive correlation to genus level between host density and host prevalence was found only for *Gonistrea* (Spearman’s rho \( \rho = 0.433 \) p= 0.002), with the last genus that showed a statistically significance difference between depth (Mann Whitney U test =201 p < 0.05).

### 3.4 Discussion

Coral diseases are an emerging issue in coral reef ecosystems, but little fundamental knowledge exist on their morphology, their cause or options for their management (Work et al. 2008). This lack of information is amplified in the Indian Ocean, where the most recent observations of coral diseases were reported in East Africa (McClanahan 2009, Weil & Jordân-Dahlgren 2005) and around Christmas and Cocos Islands (Hobbs and Frisch 2010). Consequently, while some Caribbean and Great Barrier Reef regions show a great number of quantitative and detailed studies on diseases affecting their
reef ecosystems (Willis et al. 2004, Weil et al. 2009), the Indian Ocean, despite its enormous corals biodiversity, is almost not completely surveyed. The archipelago of Maldives presents one of the most important world’s coral reefs, with more than 21,000 km$^2$ occupied from this ecosystems. Unfortunately the reefs of this country were heavy affected by coral bleaching in 1998 causing a dramatic loss of hard coral cover (Longo et al. 2000). Even if now the recovery is going on, all the studies aimed to assess this ecological trend trying to observe the coral recruitment, coral size and coral richness (Loch et al. 2002, Lasagna et al. 2010, Pichon & Benzoni 2007), but no attention has been given to the coral diseases before 2010 (Montano 2012). Our results represent the first coral diseases assessment for the Republic of Maldives, constituting a baseline level of disease prevalence that could be used in the future to monitor the health state of Maldivian reef-building corals. During the surves, from October 2010 to April 2012, seven islands, belonging to four atolls were sampled by 147 belt transects counting 34’480 scleractinian colonies in the central part of the Maldivian Archipelago. In total we report the data of the coral diseases named Brown Band Disease (BrBD), Skeleton Eroding Band (SEB), Ulcerative White Spot (UWS) and White Syndrome (WS), already reported both in the Indo-Pacific region and Republic of Maldives (Willis et al. 2004, Kackzmarsky 2006, Montano et al. 2012).

Two of them (BrBD and SEB), caused by protozoan ciliates, showed the lowest mean disease prevalence in the investigated area. Brown band disease was observed only on Acropora genus as already reported in other studies (Haapkylä et al. 2010). This could suggest an higher disease susceptibility of this genus, but also it could imply an elevated resistance to the pathogen by all other scleractinian genera. The mean BrBD Acropora prevalence was
relatively high in Thudufushi (Southern Ari Atoll), where an elevated number of diseased plate corals was observed. The recent building of water-villas for touristic scope and the consequential coral damage, as well as the consequent nutrient and/or energy input could have enhanced the pathogen proliferations. Further, Ari Atoll (with the larger contribute given by Thudufushi Island) showed the highest disease prevalence between atolls and a significant preference in depth distribution was observed for BrBD disease. Even if didn’t confirmed statistically, this scenario was observed also in Faafu Atoll suggesting a peculiar pattern of this disease in this geographic area. However the mean disease prevalence was extremely low (< 0.5 %) both in the whole area and in single islands. The distribution of that disease in the central archipelago lacks of diseased colonies in Baa Atoll. Likely this pattern is due to an extremely low abundance of the genus Acropora, confirming the extremely genus-specific preference of that pathogen. To confirm this pattern, positive correlations were found between mean BrBD prevalence and overall coral density as well as Acropora density. However, the absence of BrBD from Baa Atoll is more likely to reflect its low abundance on this reef, rather than its complete absence in this area. The potential to miss a disease when its prevalence is low high-lights the need for temporally and spatial replicated surveys to gain accurate distributional ranges of coral diseases. Further, during our survey we reported the probable presence of different categories of mobile ciliates infesting Acropora colonies. From our preliminary observations we think that different Acropora growth morphologies could host different kind of pathogen ciliates. To confirm that morphological and genetic studies need to discover if, and eventually how many, different ciliate species are involved
in the disease pathogenesis (as previously reported by Lobban et al 2011, Sweet & Bythell 2012). The skeleton eroding band showed a widespread distribution in the investigated area and it was found in all four atolls surveyed, with Faafu atoll and related Maghoodoo Island that showed respectively the highest Atoll and Island mean SEB prevalence. SEB is one of the most diffused disease in the Indian Ocean (Sutherland et al. 2004), and recently ciliates infestation similar to SEB were observed also in the Caribbean sea (Cròquer et al. 2006a), even if probably caused by different *Halofolliculina* species (Cròquer et al. 2006b). The wide distribution of SEB throughout the Indo-Pacific (Antonious 1999, Antonious and Lipscomb 2001, Willis et al. 2004), in combination with the recent discovery of a similar disease in the Caribbean, suggests that halofolliculinid infections may be endemic in coral population worldwide (Page & Willis 2008). SEB showed a very similar disease prevalence of BrBD, as well as similar host range. Usually that disease is reported on several scleractinian genera (Sutherland et al. 2004), but in this case only two scleractinian genera were found infested within the transects. The total number of hosts in the central republic of Maldives increases to three considering some *Gonisatrea* colonies found infested. Although usually this pathogens is found in a wide number of genera (Page & Willis 2008), and sometimes occurs as secondary infections, more sampling need to identify if this pattern is authentic of this ecoregion or if this disease is most diffused than already reported in this first assessment. However, one of the genera affected is still *Acropora* genus, but in this case with lower prevalence than *Pocillopora*. Moreover no correlations were found between mean SEB prevalence and overall coral density as well as between mean SEB prevalence and *Acropora* or *Pocillopora* density and
none specific depth distribution pattern was identified. This could be due to a particular and generalist features of that disease, since its presence could be attributed to numerous non-ecological factors not yet investigated.

White syndrome and Ulcerative white spot are the two diseases morphologically recognizable by the features of the white injuries (Beeden et al. 2008). White syndrome in which the pathogen agent has been identify in the GBR (Sussman et al. 2008), but not yet in the Indian Ocean (Hobbs & Frisch 2010), could be also constituted by a different diseases that create similar aspects of the injuries. The white diseases in the Caribbean and those that comprise WS in the Pacific Ocean have been the most destructive diseases on coral reefs and are increasing in prevalence (Green & Bruckner 2000, Willis et al. 2004). The actual study confirms that WS is also impacting coral reefs in the Republic of Maldives. WS showed the wider spatial distribution with about 70% of the sites sampled hosting the mentioned disease. Malè North Atoll and Ihuru Island showed respectively the highest mean WS prevalence between atolls and islands. WS resulted most abundant in shallow site than deeper, but is not known if prevalence varies with depth. A plausible explanation is that environmental conditions in shallow water (such as greater light intensity or higher water temperatures) promote the occurrence of WS. Moreover, considering that the Maldivian capital Malè is not so far from the two sampled islands (Vabbinfaru and Ihuru), we can not exclude an amplified pathogen virulence due to the human activities on the Island. Indeed is well known as the current anthropogenic impact is directly linked to some disease outbreaks in Caribbean and Great Barrier Reef (Harvell et al. 1999, Bruno et. Al. 2003). This could suggest that local environmental factors could play a major role in the disease prevalence and distribution. For this reason the analyze of
possible relationship between human land use and WS prevalence will be indispensable to define the epidemiology of that disease. The scleractinian genera affected were five in total, but three founded within the transects. The genus *Acropora* is still the most affected, as reported in many other studies (Hobbs & Frisch 2010), which indicates that causative agent(s) responsible is host specific. Indeed *Acropora* genus hosted the higher number of diseased colonies compared both within and between each coral disease sampled. This result confirms its high susceptibility and point out the requirement of more detailed information on disease risk for this genus. This supports also the hypothesis that fast-growing corals might have a weaker disease resistance than slow growing corals (Willis et al. 2004; Palmer et al. 2008, 2010; Mydlarz et al. 2010). Although direct comparisons between this and other studies are difficult without knowing the exact causative agent(s) responsible for WS, the data presented here indicate the extent of coral diseases in the Indian Ocean.

Ulcerative white spot is characterized by discrete, bleached, round foci 3 to 5 mm in diameter, which may result in ulcerations that coalesce and cause tissue loss and colony mortality (Raymundo et al. 2003). Distributed in the whole investigated area, UWS disease affected the higher number of scleractinian colonies showing the highest mean disease prevalence between all disease found. Although the highest UWS mean prevalence was observed in a deep site in Vabbinfaru, Male North Atoll, the highest atoll and island mean UWS prevalence were observed respectively in Baa atoll and Kunfunadhoo Island, in Baa atoll. That disease seems relatively widespread in the coral community, with 6 scleractinian genera belonging to 4 families found affected. To family level Faviidae and Poritidae showed the higher susceptibility between the families with the 98% of diseased colonies. That
disease is mainly reported in the Poritidae family, and although it could be quite normal (Raymundo et al. 2003, 2005), it is alarming if we consider that this hard, slow-growing, robust genus is a dominant component of Indo-Pacific reefs. So, the potential for significant impact on reef structure may be quite high. Furthermore the elevated diseased colonies discovered in the Faviidae family remains a distinctive feature for this family. This result is due to the surveys done in Kunfunadhoo (Baa Atoll) where we found an elevated number of colonies belonging to *Goniastrea* genus showing UWS-like lesions. Here we found a positive correlation between mean disease prevalence and *Goniastrea* density. Nevertheless, many of these disease/syndromes can be recognized in the field by using traditional observation methods, such as viewing cards and underwater photography (Bedeen et al. 2008), especially because to the human eye, there are not many ways in which coral tissue can exhibit signs of stress. Despite, corallivores, including fishes, gastropods, and other invertebrates could produce predation scars that could be easily confused with disease signs. For this reason we can not completely exclude that some of these signs were caused by predations act or other pathogen agent. In-depth studies of disease pathogenesis, differential species susceptibilities, host defensive capabilities, etiology and causative agents are absolutely urgently needed.

In summary, our surveys have provided baseline information on the status of coral diseases affecting reefs in the Republic of Maldives. The present study has also revealed that coral diseases are established and can become prevalent on coral reefs of this archipelago. Because climate change is predicted to amplify host susceptibility, host range, pathogen survival and disease transmission (Ritchie et al. 2001, Myers & Raymundo 2009), outbreaks are expected to increase also in this Indian Ocean region. The loss
of coral cover due to coral diseases will not only affect coral community structure, but also other reef organism that depend on this habitat-forming coral for food or shelter. For this reason more detailed surveys are recommended.

3.5 References


### 3.6 Table and Figures

**Tab. 3.1** Mean skeleton eroding band disease prevalence by genera

<table>
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<tr>
<th>genera</th>
<th>mean prevalence (%± S.E.)</th>
<th>max</th>
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<tr>
<td>Acropora</td>
<td>0.4 ± 0.08</td>
<td>5.26</td>
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<td>Pocillopora</td>
<td>3.23 ± 0.7</td>
<td>33.30</td>
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**Tab 3.2** Mean white syndrome prevalence for each scleractinian genera

<table>
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<th>mean prevalence (%± S.E.)</th>
<th>max</th>
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<tr>
<td>Acropora</td>
<td>1 ± 0.20</td>
<td>6.8</td>
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<tr>
<td>Pocillopora</td>
<td>0.33 ± 0.33</td>
<td>16.6</td>
</tr>
<tr>
<td>Pavona</td>
<td>0.11 ± 0.07</td>
<td>2.77</td>
</tr>
</tbody>
</table>

**Tab 3.3** Mean ulcerative white spot disease prevalence for each scleractinian genera

<table>
<thead>
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<th>genera</th>
<th>mean prevalence (%± S.E.)</th>
<th>% max</th>
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</thead>
<tbody>
<tr>
<td>Goniastrea</td>
<td>3.79 ± 1.59</td>
<td>64.23</td>
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<tr>
<td>Porites</td>
<td>3.12 ± 0.97</td>
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<tr>
<td>Leptoria</td>
<td>2.04 ± 1.72</td>
<td>83.33</td>
</tr>
<tr>
<td>Favites</td>
<td>0.68 ± 0.68</td>
<td>33.33</td>
</tr>
<tr>
<td>Pavona</td>
<td>0.17 ± 0.17</td>
<td>8.30</td>
</tr>
<tr>
<td>Hydnophora</td>
<td>0.02 ± 0.02</td>
<td>1.07</td>
</tr>
</tbody>
</table>
Fig 3.1 Map of the sampled area. The island number 1 is situated in Baa atoll, 2-3 in Malè North atoll, 4-5 in Ari atoll, and 6-7 in Faafu atoll.
Fig. 3.2 a) brown band disease (pointed by the arrow) on a branching *Acropora muricata* colony; b-c) details of ciliate clustering constituting the band.
Fig 3.3 Mean (±SE) Brown band disease prevalence for each atoll
Fig. 3.4 Mean (±SE) Brown band disease by atolls for shallow sites (grey bars) and deep sites (black bars).
Fig. 3.5 a) Skeleton eroding band (pointed by the arrow) on a branching *Acropora muricata* colony; b-c) *Halofolliculina corallasia*: note the large peristomial wings coming out of the lorica
Fig. 3.6 Mean (±SE) skeleton eroding band disease prevalence for each Island

Fig. 3.7 Mean (±SE) skeleton eroding band disease prevalence for each atoll
Fig. 3.8 Mean (±SE) skeleton eroding band disease prevalence by atolls for shallow sites (grey bars) and deep sites (black bars).
Fig. 3.9  a) Acropora plate coral with white syndrome;  b-c) the white band (arrows) is the lesion area that separates live coral (LC) and dead coral (colonized by filamentous algae) (DC);  d) close-up of necrosis tissue.
Fig 3.10 Mean (±SE) white syndrome prevalence for each Island

Fig. 3.11 Mean (±SE) white syndrome prevalence for each Atoll
Fig. 3.12 Mean (±SE) white syndrome prevalence by Islands for shallow sites (gray bars) and deep sites (black bars)
Fig. 3.13 Mean (±SE) white syndrome prevalence by atoll for shallow sites (grey bars) and deep sites (black bars).
Fig. 3.14  a) Ulcerative white spot (arrows) on a massive *Goniastrea* colony; b) close up of the bleached round foci; c) signs of ulcerative white spot on *Porites* colony; d) areas of white tissue discoloration with discrete margins
Fig. 3.15 Mean (±SE) ulcerative white spot disease prevalence for each Island

Fig. 3.16 Mean (±SE) ulcerative white spot disease prevalence for each atoll
Fig. 3.17 Mean (±SE) ulcerative white spot disease prevalence by atolls for shallow sites (grey bars) and deep sites (black bars).
Assessment of prevalence, host range and spatial distribution of black band disease in the Maldivian Archipelago
Assessment of prevalence, host range and spatial distribution of black band disease in the Maldivian Archipelago

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Abstract
To date, limited quantitative researches have been conducted on diseases affecting reef-building corals in the Western-Indian Ocean. During 2010 and 2011, a quantitative assessment of black band disease (BBD) was conducted in the central Republic of Maldives. Its distribution, host range and prevalence were determined in six coral islands (Magoodhoo, Adanga, Ihuru, Vabbinfaru, Thudufushi and Athuruga) belonging three different atolls. BBD resulted largely widespread between atolls. Islands showed a prevalence less than 0.5 % each with Magoodhoo island that showed the highest mean disease prevalence. In the whole surveyed area the shallow sites showed higher overall mean BBD prevalence than in the deeper ones. In total cases of BBD were recorded from 6 scleractinian families (Acroporidae, Faviidae, Poritidae, Siderastreidae, Agaricidae, Fungiidae), and 13 scleractinian genera. Two of them, Gardineroseris and Sandalolitha, represent new records for this disease and increase its global host range. The siderastreid Psammocora (4.6 ± 1.2 %) was the most affected genus followed by Goniopora (2.7 ± 1.3 %), both positively correlated to their host density. By contrast, the less affected were Favites and Acropora (both < 0.1 %). Although we observed an extremely low overall disease prevalence in the investigated area (< 1 %), the large number of different scleractinian genera affected and the widespread distribution of BBD represent alarming results. Thus, the health condition of these reefs, the risk of which some species seem to be exposed, and the potential significant long-term effects of BBD, indicate urgent need for further investigations.
4.1 Introduction

Coral diseases represent a serious threat to reef ecosystems. Over the last decades outbreaks of emerging diseases have become more frequent, possibly due to global ocean warming and human activities, contributing substantially to the speed up of coral loss and reef decline (Green and Bruckner 2000; Rosemberg and Ben-Haim 2002; Harvell et al. 2007, 2009) with dire consequences for coral population and associated reef communities (Harvell et al. 2002, Sokolov 2009).

Black band disease (BBD) is considered one of the most widespread and destructive coral infectious disease (Richardson 1998). BBD is characterized by a darkly pigmented microbial mat predominantly composed of cyanobacteria (primarily *Roseofilum reptotaenium* (Casamatta 2012) and secondarily *Geitlerinemia spp.*, *Oscillatoria spp.* *Leptolyngbya* in the Caribbean (Mayers et al. 2007) and *Oscillatoria* and *Pseudoscillatoria* in Indo-Pacific and Red Sea (Sussman et al. 2006 Sato et al. 2009)) and sulphide reducing (*Desulfovibrio spp.*) and oxiding (*Beggiotoa spp.*) bacteria (Rutzler & Santavy 1983; Richardson 1997; Frias-Lopez et al. 2003). As the disease progresses, a consolidated band that separates living tissue from recently denuded skeleton becomes evident (Sutherland et al. 2004). BBD causes structural damages to coral tissue, producing widespread necroses that constitute the main mechanism of cellular death (Ainsworth et al. 2007). BBD is known to affect 64 scleractinian species (Sutherland et al. 2004). Although it is generally characterized by low prevalence (Edmunds 1991, Dinsdale 2000, Weil et al. 2002), BBD is nonetheless considered a serious threat to coral populations worldwide due to its persistence (Kuta & Richardson 1996, Green & Bruckner 2000, Sutherland et al. 2004,
Kaczmarsky 2006, Page & Willis 2006, Sato et al 2009), as it can affect the same reef for decades, thus leading to long-term mortality of susceptible coral species and, in general, producing long-term changes in coral community structure (Bruckner & Bruckner 1997).

BBD was first reported from reefs of Belize and the Florida Keys (Antonious 1973), while the first records from the Indo Pacific (Philippines and Red Sea) date back to the beginning of the eighties (Antonious 1985b). The most extensive losses of reef-building corals caused by BBD outbreaks have been reported in the Wider Caribbean Region (Edmunds 1991; Bruckner & Bruckner 1997). BBD is currently known to be in present in most of world's coral reefs, however, there is no detailed information available for its diffusion among Indo-Ocean coral populations, especially in the Republic of Maldives where it was recorded only in 2010 (Montano et al. 2012).

The Maldivian reefs are among the most species rich of the Indian Ocean, being inhabited by more than 180 zooxanthellate coral species belonging to 51 genera (Pichon & Benzoni 2007). The major reef structures occupy an area of about 21000 km$^2$, 21.1% of which can be categorized as marine productive reef habitat (Naseer & Hutcher 2004). Maldivian reefs have been recently affected by harsh environmental phenomena like coral bleaching, that have caused a mass coral mortality (up to 90%) and have promoted the replacement of communities dominated by Acropora with communities dominated by Porites (Goreau 2000). Most of the studies aimed at evaluating Maldivian reef health after these mass mortality events focus on coral cover, recruitment, colony sizes, and species richness (Loch et al. 2002, 2004; Bianchi et al. 2006a, 2006b; Lasagna et al. 2006, 2008; Pichon & Benzoni 2007). By contrast, little attention has been paid to the possible

In this study we quantify the prevalence of BBD on 41 reefs of the Republic of Maldives, distributed among six islands belonging to three different Atolls. This work, that constitutes the first assessment of black band disease in this archipelago, is aimed at: (1) investigating the distribution and host range of BBD; (2) comparing BBD prevalence between island and depth; (3) exploring the differences in susceptibility among genera; and (4) assessing the relationship between BBD prevalence and host density.

4.2 Materials and Methods

The study was conducted in the waters of central Republic of Maldives between October 2010 and October 2011. During this period (that spanned across both the wet and the dry season) the local monthly mean sea surface temperature (SST) was pretty constant, varying from 28.4 to 30.6 °C (mean 29.4 ± 0.2 °C) (http://disc.sci.gsfc.nasa.gov/techlab/giovanni/)

In total we investigated six islands (Fig. 4.1), namely Ihuru and Vabbinfaru (Malè Atoll), Adanga and Magoodhoo (Faafu Atoll), and Athuruga and Thudufushi (Ari Atoll). The islands, including land and related coralline reefs, measure from 0.5 km (Ihuru) to 3.5 km (Thudufushi) and exhibit the features of a typical low-energy reef with a luxuriant growth of coral and gently slope at all sides. We selected 4 to 8 sites per island, choosing them haphazardly among those accessible. We carried out surveys by snorkelling at the shallow sites (n= 21, 0-5m) and by scuba diving at the deep ones (n= 20, 5-15 m). We collected quantitative information about black band disease
(BBD) prevalence by performing at each site three randomly placed 25 × 1 m belt transects spaced 10 to 20 m apart. We chose this transect size as it was the most suitable in respect to field logistics and to the size of the surveyed area (Montano et al. 2012). We identified all corals at genus level in situ, according to Veron (2000). We counted colonies on the belt margin only when 50% or more of them laid within the belt boundaries. For each belt transect, we took note of the total numbers of healthy and diseased colonies in order to make it possible the computation of disease prevalence, that was calculated as the ratio between the number of infected colonies and the total number of observed colonies. For each transect we computed both an overall BBD prevalence value and a series of taxon specific BBD prevalence values, that we calculated as the ratio between the number of diseased coral colonies belonging to a particular genus, and the total number of observed colonies belonging to that genus. Then we computed overall and taxon specific prevalence values for each site by averaging the corresponding prevalence values measured in the three random belt transects.

We identified colonies affected by BBD according to Beeden et al. 2008 and to Willis et al 2004. For documentary purposes we took micro photographs (32x) of infected colonies using a Leica EZ4 D stereomicroscope.

Since observed values of disease prevalence and coral density were not normally distributed, we evaluated how they respectively differed in shallow and deep sites using a Mann-Whitney U test (Zar 1999). Finally, we assessed significance of the difference in disease prevalence between islands or families using a Kruskal-Wallis Test.
4.3 Results

We examined 41 sites distributed on 6 islands belonging to 3 different Atolls. To do that, we performed 123 belt transects (covering ~3075 m²). We found colonies affected by black and disease (BBD) in 46 transects (37.4%) belonging to 27 sites (65.9%) distributed among all islands. Most of the transects where we detected BBD (78.3%) were at a depth ranging from 0 to 5 m, while few (21.7%) were at a depth ranging from 5 to 15.

In the investigated area we examined 30’284 colonies belonging to 23 scleractinian genera. We found BBD in 74 colonies. The mean overall prevalence per site was 0.29 ± 0.06. The highest BBD prevalence (1.94 %) was observed in a shallow site at Magoodhoo Island. We found a significant difference in the prevalence of BBD between shallow and deep sites (Mann-Whitney U test p < 0.01). In particular, the overall prevalence of BBD was much higher at shallow sites (0.42 ± 0.1) than at deep ones (0.14 ± 0.2).

By contrast, we found no significant difference in prevalence between islands and between atolls. We detected similar BBD prevalence (<0.5) in all islands. The highest value was observed in Magoodhoo island (0.42 ± 0.23). As regarding for the atolls, the highest BBD prevalence was recorded in Faafu (0.34 ± 0.16), with significant differences in the prevalence between shallow and deep sites found in this (Mann Whitney U test p < 0.05) and Ari atoll (Mann-Whitney U test p < 0.05) (Fig 4.2).

From a qualitative point of view, we detected BBD in 13 scleractinian genera belonging to 6 families (Acroporidae, Faviidae, Poritidae, Siderastreidae, Agariciidae, Fungidae), but we did not detect it in soft or hydroid corals. We observed diseased colonies belonging to Gardineroseris
spp. and Sandalolitha spp. in the area of study but not within transects, thus we did not include this information in the quantitative analyses. We detected a significant difference in BBD prevalence between families (Kruskal-Wallis H(4)= 10.532, p < 0.05)(Fig. 4.3). The highest mean BBD prevalence was observed in the Siderastreidae (4.55 ± 1.2), with a maximum of 33.3. On the other hand, acroporid corals showed the lowest mean prevalence (0.09 ± 0.03), with a maximum of 1.2. Species belonging to the Siderastreidae were the most susceptible, with 48.6% of all infection founded on species within this family. Most of the diseased Siderastreidae colonies (47.2%) belonged to the species Psammocora digitata. Sixteen percent of BBD infections were recorded from coral species belonging to the Poritidae and the Acroporidae, 12.1% were recorded from species belonging to the Faviidae, while the remaining percentage of infections was recorded from the Agaricidae (4.1%) and the Fungiidae (2.7%).

As regarding for coral genera, BBD prevalence ranged from 0.03 ± 0.03 (Favites) to 4.6 ± 1.22 (Psammocora) (Table 4.1). The highest prevalence was observed in Goniopora (41.6), and particularly at a shallow site in Ihuru island (Malè North Atoll). Psammocora was the only genus that showed a significant difference between depth (Mann Whitney U test p < 0.001).

In general, we found a significant difference in coral density between shallow and deep sites (Mann-Whitney U test p < 0.05), with the firsts showing the highest coral density. We detected no significant relationship between overall BBD prevalence and overall coral density, but we found a significant positive correlation between overall disease prevalence and host density (Spearman’s rho ρ=0.807 p < 0.001). At genus level, however, positive correlation between host density and host prevalence was detected only for Goniopora (Spearman’s rho ρ=0.376 p < 0.05), Isopora.
(Spearman’s rho $\rho=0.420 \ p < 0.01$) and *Psammocora* (Spearman’s rho $\rho=0.452 \ p < 0.05$).

### 4.4 Discussion

Qualitative and quantitative patterns of coral disease presence, host range, prevalence and epidemiology are largely unexplored in the Indian Ocean (Weil et al. 2012). The present study is the first assessment of BBD in the Republic of Maldives, that should be considered an area of particular interest due to the peculiar ecological features of the West Indo Pacific Realm (Obura 2012).

We found coral colonies affected by BBD in all the six Islands (belonging to three different atolls) that we investigated. Considering that this study represents a first assessment and that we selected sampling sites completely at random in respect to environmental and ecological features, our results may indicate that BBD is widely distributed in the Maldivian archipelago. From a quantitative perspective, our survey revealed a very low BBD prevalence, which is consistent with the general low coral disease prevalence already reported in most reef regions (Dinsdale 2000, Weil et al. 2002, Page and Willis 2006, Myers & Raymundo 2009). We observed the highest prevalence in Magoodhoo, that is an inhabited island belonging to Faafu Atoll. This is not surprising, considering that reefs around Magoodhoo should be considered in a current state of regression, being partly degraded and affected by a recently detected overgrowth of macro algae (Montano et al. 2012). In general, this can be partly attributed to the fact that impacts resulting from human activities conducted on islands inhabited by natives
(such as overfishing, land use, water pollution, etc.) may promote reef degradation (Harvell et al. 1999, 2007). This effect is expected to be weaker in resort islands and, obviously, in uninhabited ones, which, again, is consistent with our observations.

From an ecological perspective, we found that shallow coral colonies are more susceptible to diseases than deep ones. Indeed, it is well known that temperature and light represent two important environmental factor affecting distribution patterns and progression of BBD (Kuta & Richardson 2002; Boyett et al. 2007; Sato et al. 2010), especially when water temperatures are higher than 28.4 °C (Miller & van Woesik 2011). Note that during our survey, water temperature has always been higher than that threshold.

The fact that we did not find BBD in soft or hydroid corals could be related to the low coverage of these categories (< 3%, McClanahan 2011). However, further investigation focused on these benthic categories would be ausplicable to verify this hypothesis.

Among families, the Acroporidae was the less affected. Despite their relative abundance, the Pocilloporidae did not show any diseased colony. This is consistent with what already observed in the Caribbean (Rutzler et al. 1983, Antonious 1988a), where a very low BBD prevalence was observed in both the Acroporidae and the Pocilloporidae. Still, this result is quite remarkable, considering that these two families, and especially the Acroporidae, are usually highly susceptible (Sutherland et al. 2004, Moreno et al. 2012), being sometimes heavy affected by BBD (see for example the case of the Great Barrier Reef, as reported by Dinsdale (2000), and Page & Willis (2006)). On the other hand, the Siderastreidae was the most affected family in our survey. Again, this is consistent with patterns already observed in both the Caribbean and the Indo-Pacific region (Moreno et al. 2012).
In total we detected BBD in 13 scleractinian genera (representing about the 25% of the total number of known genera for the entire archipelago). Up to date BBD was not reported from the genera *Gardineroseris* and *Sandalolitha* (Sutherland et al. 2004), thus our results extend its global host range. The less affected genus was *Favites*, immediately followed by the fast growing coral *Acropora* genus. This is in contrast with other studies, and confirms the known variability in the response of *Acropora* to diseases (Page & Willis 2006), possibly mediated by natural and/or anthropical local features (Bruno et al. 2007, Garren et al. 2009). However, the differences in susceptibility observed for the genus *Acropora* in different geographic areas could be simply explained by local differences in species composition. Thus, although we are aware of the technical difficulties that limit most of the current investigation on coral diseases (including the present study) to the genus level, we would like to stress the importance of acquiring information on disease susceptibility at the species level in order to orient future management and conservation plans.

*Acropora* genus is the dominant in the Maldivian reefs, with a coverage percentage higher than 40% (Tkachenko 2012). Moreover, it displays several different growth morphologies, is present a wide depth range (from 0.5 to 20 m) (Bianchi 1997), thus giving a fundamental contribution to the 3D complexity of reef structure (Lasagna 2010). This stresses the importance of keeping *Acropora* health status monitored, even if our results characterize it as not much susceptible to BBD.

By contrast the genus *Psammocora* is one of the less abundant in the archipelago and, at the same time, one of the most affected ones (with almost all of the diseased colonies belonging to the species *Psammocora digitata*). Interestingly, we detected a positive correlation between the prevalence of
BBD in *Psammocora* and *Psammo* *cora* density, that may suggest the existence of some auto-regulatory ecological mechanisms. Finally, the overall ecological vulnerability of *P. digitata* (that is ranked by IUCN red list 2011 as “Near Threatened”) should not be underestimated. In general, the present results, together with those provided by other recent studies (Montano et al. 2012, Seveso et al. 2012), highlight the need for further studies aimed at better understanding of the coral epidemiological dynamics going on in the Maldivian archipelago.

4.5 References


Antonius A (1973) New observations of coral destruction in reefs. Assoc Isl Mar Lab Caribb, Mayaguez 10:3


Mc Clanahan T.R.(2011) Coral reef fish communities in management systems with unregulated fishing and small fisheries closures compared with lightly


Veron, J. E. N. Corals of the World vols 1–3 (Australian Institute of Marine Science, Townsville, Australia, 2000).


Fig 4.1 Map of the area surveyed in the central Republic of Maldives. A= Malè North Atoll (A-1/A-2 Ihuru and Vabbinfaru islands); B= Ari Atoll (B-1/B-2 Athuruga and Thudufushi islands); C= Faafu Atoll (C-1/C-2 Magoodhoo and Adanga islands). The solid line is the limit of the reef, the black area are the lands, and the grey area represent the enclosed lagoons. Scale bars: 0.5 Km
Fig. 4.2 Mean (±SE) black band disease prevalence for every affected scleractinian family.
Fig. 4.3 Mean (±SE) BBD prevalence by depth for each atoll. Grey bars represent shallow transect and the black ones the deeper.
### Table

#### Tab 4.1. Total black band disease prevalence to genus level.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>BBD prevalence %</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>maximum</td>
<td>Mean ± SE</td>
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<tr>
<td>Acroporidae</td>
<td><em>Acropora</em></td>
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</tr>
<tr>
<td></td>
<td><em>Isopora</em></td>
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</tr>
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<td></td>
<td><em>Montipora</em></td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td><em>Porites</em></td>
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<tr>
<td></td>
<td><em>Goniopora</em></td>
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</tr>
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<td>Siderastreida</td>
<td><em>Psammocora</em></td>
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<td><em>Pavona</em></td>
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<td>4.72</td>
</tr>
<tr>
<td></td>
<td><em>Sandalolitha</em></td>
<td>x</td>
</tr>
</tbody>
</table>
- Chapter 5 -

General Discussion
5.1 Global assessment of the Maldivian coral diseases

Currently an increasing number of studies are revealing that coral diseases occur across a range of locations in the Pacific Ocean and that the prevalence of some diseases have increased much as 150-fold in 5 years (Sutherland et al. 2004, Willis et al. 2004, Bruno and Selig 2007). Known difference between the Caribbean and Pacific Ocean, with respect to disease ecology, etiology end epidemiology have led to the conclusion that coral diseases must be examined on a region-by region basis (Willis et al. 2004). Of course this has to be applied also to the Indian Ocean, as well as to the Republic of Maldives, as some specific ecological coral disease patterns could exist.

This study is the first coral health and diseases assessment in the Republic of Maldives. Principal findings of this study demonstrate that biotic threats identifiable in corals diseases and algal overgrowth represent a serious risk for the coral community and associated organisms in the Maldivian reefs ecosystem. Over the 5 coral diseases and one anomalous pigmentation previously described in chapter 2, we report for the first time the observation of other diseases and syndromes in this archipelago. Those are named Black Disease caused by the sponge Terpios hoshinota (Rützler & Muzik 1993), coral tumors and the not yet described Porites White Patch Syndrome (Serè et al. 2012). Those were not reported in the analysis because they have been very rare in the investigated area, with individual o very few coral colonies affected. However, this result rises to eight the total number of diseases and syndrome affecting reef-building corals in this archipelago.

All coral diseases observed affected in total 17 scleractinian genera belonging to 8 different families, representing about the 25 % of the whole scleractinian genera described in this area. Although all diseases are present
with very low overall disease prevalence (< 1%), which is in contrast with several other studies on reef systems (Myers & Raymundo 2009, Weil et al. 2012), WS, UWS and especially BBD resulted greatly widespread in the surveyed area and relatively diffused in the coral community. The high number of colonies belonging to the siderastreid *Psammocora digitata* affected by BBD, and the number of coral diseases hosted by *Acropora* genus were two of the most important results. The first one was been recently inserted in the IUCN Red list for the threatened species, and more in-depth monitoring need to estimate the impact of the reported coral diseases for this endangered species. *Acropora* genus, instead, represents the dominant genus in the whole archipelago. Despite Maldives and, hence, the area of study have been affected by a mass coral bleaching in 1998 (Longo et al. 2000, Zahir 2000), the currently available data make it impossible to identify a relationship between the past thermal stress and the current diffusion of coral diseases. However the expected future increase in sea surface temperatures (Kleypas et al. 1999) could lead not only to new bleaching events, but also promote the spread of coral pathogens by increasing their growth rate and virulence (Ben-Haim et al. 2003b) and by reducing immune response in coral hosts (Alker et al. 2001, Mydlarz et al. 2009, Palmer et al. 2011). For this reason an alert monitoring focused on the health of this specific and dominant genus will be indispensable to better understand the actual and potential impact of infectious diseases on coral ecosystem dynamics, and it will be fundamental to conservation planning. Finally, we found a negative correlation between overall disease prevalence and overall coral density (Spearman’s rho $\rho = -0.4 \ p < 0.01$) that it is different from data observed in all previous studies. The Indian Ocean harbor a great number of scleractinian species and that this feature could be
lower the spread of coral diseases. Although Maldivian reefs showed a similarities to the whole Indian Ocean reef ecosystem, the negative trend observed could be explained from the regressive stage in which are many Maldivian reefs (Lasagna 2010). In effect the compromised state of many Maldivian reefs could enhance the pathogens virulence, without regard to corals biodiversity or abundance. Malè North Atoll displays the highest overall diseases prevalence suggesting a probable influence of human activities on pathogen virulence. Considering the numerous that many studies related positively anthropogenic disturbance and increase in disease prevalence (Harvell et al. 1999, 2002), that result should be keep in consideration. Although we cannot exclude that our results may be affected by sample size, number of replicates and local variation, and that they may be not representative of large scale patterns valid for the whole Republic of Maldives, we believe that the data presented in this study can be considered the first ecology assessment of the reported coral diseases.

5.2 Maldives vs. other regions

Levels of coral disease prevalence are an important indicators of coral reef health and have been correlated with anthropogenic activities (Aeby et al. 2011) and climate warming events (McClanahan 2004, Bruno et al. 2007, Maynard et al. 2008, 2011, Muller & van Woesik 2009). Climate variability may impact severely sensitive coral reefs breaking the symbiosis between the corals and its endosymbiotic algae, allowing the persistence of pathogens at higher abundance than in past decades and reducing the coral resistance to pathogens (Hoegh-Guldberg et al. 2007, Harvell et al. 2009, Carpenter et al.
The prevalence values for multiple diseases in the Republic of Maldives reported in this study provide a reference point to measure future change.

All coral diseases observed in the investigated area displayed a low level prevalence (< 1%) that results extremely low if compared with other reefs ecosystem. Indeed, white syndrome showed a much lower prevalence than the one already observed in similar ecosystems (see, for example, Willis et al. 2004 and Hobbs & Frish 2010 for the Indo-Pacific, and Sutherland et al. 2004 for the Caribbean). In general, the Siderastreidae, Faviidae and Pocilloporidae families were between the most susceptible as already reported by Moreno (2012). Also the Acroporidae results one of the most affected family, but the greater abundance of this family in the Maldivian reef reduces significantly the disease prevalence levels. A specie-specific investigation must be made to identify the real extent of the problem.

The overall coral diseases prevalence in the Maldivian Archipelago was estimated around 1.3 % and was similar to the level of prevalence reported for the GBR (1.32 %), but it was lower than already reported in the Philippines (4.64%) for the Indo-Pacific region and Mexican Yucatan (8.3 %) in the Caribbean (Moreno et al. 2012). Although more sites need to be sampled in the Republic of Maldives to produce a comprehensive picture, previous works suggested that the higher mean disease prevalence in the Caribbean is a general pattern. Furthermore, even if standardized survey methods and permanent monitoring sites could provide enough information to determine interannual variability in disease prevalence, our level of overall disease prevalence fall approximately in the range of 3 to 5 % estimated for the Indo-Pacific region (Moreno 2012). For this reason our overall coral diseases prevalence can be considered the real baseline levels.
for the Maldives and should be used to detect future change in disease level as well as realize future management plans for the Maldivian coral reefs.

5.3 Future researches on coral diseases management

The understanding of coral diseases is still in its infancy in the Maldivian Archipelago and nearly any investigation is likely to be a useful contribution to the region. Coral diseases and related outbreaks are known to be important factors affecting coral reef communities around the world (Harvell et al. 2007), and in the future will certainly represent a serious risk for the Maldivian reefs. These threats are also often amplified by coral bleaching events due to the anomalous increment of sea surface temperatures. For example, reduced energetic reserves in bleached or otherwise compromised corals (Grottoli et al. 2004) may limit investments in boundary maintenance or impair wound healing (Meesters & Bak 1993, Fine et al. 2002) increasing the susceptibilities of coral to infectious disease. While the coral bleaching effects on the coral host populations are well-documented, more detailed coral diseases prevalence surveys are needed to accurately estimate the impact of diseases on coral populations and assemblages in this region. Therefore, the degree to which disease drives coral community structure especially on Pacific and Indian Ocean reefs is largely unknown (Sutherland et al. 2004; Bruno and Selig 2007) and probably under-estimated since it is likely that mortality caused by disease may be attributed to other disturbances. The likelihood of an increase of coral disease frequencies will be augmented not only by climate change but by also regional and local scale anthropogenic stressors (Bruno et al. 2003). The latter, several human
activities already available in the Maldives, like anthropogenic nutrient input by land use, pressure on herbivores fishes (Mumby et al. 2006), and physical damage due to anchoring and/or divers and snorkelers (McManus et al. 1997) could enhance the competitive advantage of macroalgae and consequently reduce coral recovery rates, increasing pathogen virulence and coral susceptibility (Willis et al. 2004).

Considering that the whole population of Maldives lives in coral reef islands and that they completely rely on these ecosystems for income, food and shoreline protection, the potential impact due to the coral disease will have significant implications for this social system. For this reason the enhancement our knowledge about the implications of coral disease for coral reefs and their management is an imperative.

Currently, the limited nature of knowledge of the etiology of Indo-Pacific coral diseases is a major impediment to management of potential epizootics. Unfortunately, the causes, the mechanisms by which most diseases kill their hosts, their rates of spread and host mortality, how these parameters vary within and across host species, and a spatial temporal scales, their original sources, vectors and/or reservoirs, or how they are transmitted across colonies of the same species and of different species (Weil et al. 2004) are unknown for many of these diseases (Weil et al. 2004, 2006).

However, even if some researcher think that our scientific contribution to the log-term survival of coral reef communities will be insignificant unless governments and managers increase their effort to reduce the effects of the major stressor (Weil et al. 2004), some particular measure could provide an opportunity to minimize coral disease risk into long-term spatial management plans. For example, outbreaks of some coral diseases are caused by combinations of environmental and ecological conditions that can
be used to assess outbreak likelihood, especially for the temperature-dependent diseases for which bleaching increases disease susceptibility (Mydlarz et al. 2009). This could be done by detecting the early signs of a disease outbreak through a network of observers as many reefs are visited by managers infrequently whereas a disease outbreak can spread quickly (Beeden et al. 2012).

So an effective response to coral diseases depends on knowledge of where they are likely to occur and/or timely receipt of in situ observations of an outbreak. Similarly, removal of disease by physical means (Hudson 2000), aspirating the disease bands on corals affected by band-like diseases and covering the affected area with clay or putty (Raymundo 2010), and traditional strategies like quarantining, vaccination, and antibiotic treatment and culling (Wobeser 2006) could be useful. Though these strategies have strong potential to be vital to managers in the future, all are currently highly experimental and likely to be non prohibitively expensive only to the smallest of spatial scales (10’s–100’s of m$^2$) (Work et al. 2008).

In conclusion, this preliminary study on Maldivian coral diseases is only the beginning. The main recommendation resulting from this study is that this new threats will drive changes in coral reef structure and biodiversity. The documented decline in coral reef cover in this area and the future pressure to coral reef health from ocean warming, acidification and anthropogenic pollution, pose an imminent need to study the role of coral diseases in coral reef deterioration, resilience and recovery. Without an intensive effort among researcher, governments and all the stake-holders to describe the impacts of coral diseases in the Republic of Maldives coral communities as well as coral diseases etiology and epidemiology, our ability to develop
effective management protocols to administer diseases in this archipelago will be confined.

5.4 References


prevalence associated with sea temperature anomalies and local factors. Dis Aquat Org 100:249-261


APPENDIX 1

Up-regulation of Hsp60 in response to Skeleton eroding band disease but not by algal overgrowth in the scleractinian coral Acropora muricata.

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Abstract
Heat shock proteins are biomarkers commonly used to determine the effects of abiotic stresses on the physiology of reef building corals. In this study the effectiveness of the Hsp60 as indicator of biotic stresses in the scleractinian coral Acropora muricata was analyzed, considering the whole holobiont. We focused on two biological interactions recognized to be important contributors to coral reef degradation such as a coral disease, the Skeleton eroding band (SEB) caused by the protozoan Halofolliculina corallasia and the algal overgrowth. In the lagoon of Magoodhoo Island (Maldives) fragments of living tissue of A. muricata exposed to these biotic factors were sampled and proteins subjected to Western analysis. The two different biological interactions trigger diverse responses on Hsp60 level. No detectable effect on Hsp60 modulation appeared in colonies subjected to algal overgrowth. On the contrary, corals displayed a robust up-regulation of Hsp60 in the fragments sampled just above the SEB dark band, where the level of Hsp60 was almost twice compared to the control colonies, indicating that the aggressive behavior of the protozoan causes cellular damage also in coral portions neighboring and along the advancing front of the infection. Portions of coral sampled distant to the SEB band showed a Hsp60 level comparable to that observed in healthy colonies. We propose Hsp60 expression as a promising tool to evaluate physiological stress caused by SEB disease in reef corals.
Introduction

In nature, organisms have developed several mechanisms to withstand environmental stresses, such as physiological regulations and biochemical and cellular specializations (Brown, 1997). The increased importance of determining the effects of stress factors on the physiology of animals have led to an increase of studies investigating stress-inducible proteins in an ecological context (Feder and Hofmann, 1999; Dahlhoff, 2004).

Heat shock proteins (Hsps) are a highly conserved family of stress response proteins which represent one of the most important defense mechanisms of all organisms (Fink, 1999; Kumsta and Jakob, 2009). They function primarily as molecular chaperones, preventing protein aggregation, facilitating proper protein folding and complex assembly, targeting improperly folded proteins to specific degradative pathways and regulating stress-induced apoptosis (Mayer, 2010; Toivola et al., 2010; Vabulas et al., 2010). Hsps are expressed at low levels under normal physiological conditions, but their expression is up-regulated as a consequence of exposure to conditions that perturb cellular protein structure (Dahlhoff, 2004; Richter et al., 2010). High levels of specific Hsps are maintained throughout exposure and protect the organism from a wide variety of stressors.

In literature many works have focused on the expression of coral Hsps (Fang et al., 1997; Sharp et al., 1997; Branton et al., 1999; Robbart et al., 2004; Snyder and Rossi, 2004) particularly on the induction of the chaperonine 60-kDa heat shock protein (Hsp60) under environmental stress factors causing bleaching, such as high temperatures (Choresh et al., 2001; Brown et al., 2002; Kingsley et al., 2003; Chow et al., 2009, 2012), low temperatures (Kingsley et al., 2003), elevated light intensity (Downs et al., 2000; Chow et al., 2009, 2012) and xenobiotics (Downs et al., 2005).

Stress factors that trigger the heat shock response in reef building corals are usually considered to be abiotic. However, in the marine habitat the distribution patterns, spatial relations, growth and health of the populations are affected in a predictable manner not only by natural physical disturbances but also by interactions with other species in the community (Dayton, 1971). Abiotic and biotic stresses often work in concert with one another in driving the physiological ecology of intertidal communities and determining the structure and composition of benthic communities on coral reefs (Lang and Chornesky, 1990; Karlson, 1999). Nevertheless, not much research has been carried out with the aim of assessing the role of Hsps in relation to biotic factors. The study published by Rossi and Snyder (2001) has shown that stress proteins can also be induced solely through biological
stressors, such as competition for space, in two Pacific cnidarians of the Actinaria group. However, to the best of our knowledge no information about Hsps and biotic stress on reef building corals (order Scleractinia) is presented in literature.

Among biotic stresses, coral diseases have been recognized as one of the cause of the coral reefs decline (Harvell et al., 1999; Weil, 2004). In particular, the protozoan disease known as the Skeleton eroding band (SEB) disease, has been the first coral disease described from an Indo-Pacific reef (Antonius, 1999) where it is now one of the most prevalent coral infection having the widest host range documented for any coral disease (Page and Willis, 2008). The organism associated with this syndrome has been identified as *Halofolliculina corallasia* (Antonius and Lipscomb, 2001), a species of folliculinid, heterotrich ciliate, which produces a black band (1–10 cm wide) at the interface between recently exposed skeleton and apparently healthy coral tissue (Antonius, 1999). In addition to coral diseases, another important cause of reef degradation has been attributed to the large increase in the abundance of benthic algae which compete for space and light with scleractinian corals, often overgrowing on them (Jompa and McCook, 2003a). The coral-algal competition is widespread, but the interaction is highly variable in both process and outcome as reported in several studies (reviewed in McCook et al., 2001).

In this study, for the first time, the effectiveness of Hsps as an indicator of biological stress in scleractinian corals has been analyzed. To determine whether Hsp expression patterns could be related to competitive interactions in coral reef habitat, the staghorn coral *Acropora muricata* (Linnaeus, 1758) was chosen for this study, representing one of the most abundant coral species in the Indo-Pacific reef (Veron, 2000) especially in the studied area, the lagoon of Magoodhoo Island, Republic of Maldives (Seveso, personal communication). In particular, we hypothesize that components of the stress response such as the Hsp60 could provide evidence of the intensity and the damage of competitive interactions between the whole holobiont of *A. muricata* and biological agents, such as the protozoan causing SEB disease and the turf/ macroalgae involved in overgrowth of corals.
Materials and Methods

Coral collection

The study was undertaken on coral patches inside the lagoon of Magoodhoo Island (3°04’42”N; 72°57’50”E), in the south east part of Faafu Atoll, Republic of Maldives (Fig. S1 Supplementary Data).

To study Hsp60 expression in corals subjected to SEB disease, infected colonies of A. muricata were located in the lagoon and photographed (Canon A710IS with Ikelite housing). The presence of A. muricata colonies infected by SEB ciliates was confirmed by microscopic analysis (Leica EZ4D, Leica Microsystems, Germany) of coral fragments collected at the dark band (Fig.1A and B). Seven of these colonies were selected and for each colony two different coral fragments were sampled and marked as: “healthy” (H), fragment of a healthy coral branch far from the dark band in an infected colony and “diseased” (D), fragment sampled just above the ciliate dark band, along the disease progression direction, in an infected colony (Fig.1C). All the coral portions were collected using hammer and chisel. To avoid artifacts which might occur when coral fragments were transported under stressful conditions, specimens were immediately frozen at -80°C in the field using an immersion cooler (FT902, JULABO, Labortechnick GmbH). Both the coral fragments of samples H and D should not contain protozoa to avoid interference during the analysis of Hsps, so the total absence of protozoa has been carefully verified by microscopic examination of each frozen sample prior to their homogenization.

To study Hsp60 expression in corals subjected to algal overgrowth, colonies of A. muricata which presented some branches overgrown by filamentous and mixed-species algal turfs were located in the lagoon and photographed. These colonies had some branches with dead coral tissue covered by a thick algal turf which became less dense at the coral tips revealing the living tissue below. To get a confirmation of this, these branches have been carefully examined by microscopic analysis. Other branches of the same colonies were free of algae. Seven colonies were selected and for each colony two different coral fragments were sampled and marked as: “without algae” (WA), fragment of a living coral branch free of algae in a coral colony overgrown by algae, and “algal overgrowth” (AO), fragment of living coral tissue sampled just next to the algal interface that was the area where the turf started to be thinned out (Fig. 2A). All the coral portions were collected and stored as described for samples H and D. For both experiments, as control
seven isolated and entirely healthy colonies of *A. muricata* were likewise sampled in the same zone of the lagoon.

All the coral samples for the controls and the two biotic stresses were collected simultaneously in October 2010 at the same depth, at the same early morning time (08:00 am) and during high tide (coral permanently submerged) to minimize seasonal and/or daily differences in cnidarian behavior and in Hsp60 expression due to changes in water temperature and/or different UV intensity (Chow et al., 2009, 2012), fluctuations in salinity and pH, and other effects that are typical of the intertidal environment. An HOBO pendant data loggers (Onset, UA-002-64) were used to measure temperature of specific locations and seawater samples which were collected in tubes were used for salinity measurements with a refractometer (Milwaukee Instruments, USA).

**Coral species identification**

To confirm that the coral species under investigation was *A. muricata*, coral DNA was extracted using DNeasy® Tissue kit (QIAGEN, Qiagen Inc., Valencia, CA, USA) and a rDNA region of about 500 bp (spanning the entire ITS1, 5.8S, ITS2 and a portion of 28S and 18S) was amplified and sequenced. Amplification was performed using the coral-specific primer A18S (5’ GATCGAACGGTTTAGTGAGG 3’), (Takabayashi et al., 1998) and the universal primer ITS4 (5’ TCCTCCGCTTATTGATATGC 3’), (White et al., 1990). Sequences were compared with known scleractinian corals sequences in GenBank using the BLAST nucleotide search (http://www.ncbi.nlm.nih.gov/BLAST/). BLAST searches showed 94% identity with rDNA sequences of *A. muricata*.

**Protein extracts and Western analysis**

In the laboratory, 1 g of each frozen coral fragment was powdered using mortar and pestle. Proteins of the holobiont were extracted by homogenizing the tissue powder in 400 µl of SDS-buffer (0.0625 M Tris-HCl, pH 6.8, 10% glycerol, 2.3% SDS, 5% 2-mercaptoethanol) containing 1mM phenylmethylsulfonyl fluoride (Sigma) and Complete EDTA free cocktail of protease inhibitors (Roche Diagnostic). Samples were boiled for 10 min and skeleton fragments were removed by a single step of centrifugation (15 min at 12000 rpm, 4°C). Supernatants were clarified (5 min at 12000 rpm) and
then frozen at -20°C until used. Aliquots of the supernatants were used for protein concentration determinations using the Bio-Rad protein assay kit (Bio-Rad Laboratories, California, USA). Protein samples were separated by SDS-PAGE on 8% polyacrylamide gels (18 cm x 16 cm) (Vai et al., 1986). The same amount of proteins (80 μg) was loaded on each lane of the gel. Pre-stained protein markers (range 7-175 kDa, New England Biolabs) were also loaded. Duplicate gels were run in parallel. After electrophoresis, one gel was stained with Coomassie Brilliant Blue to visualize the total proteins (Fig. S2 Supplementary Data), and the other electroblotted onto nitrocellulose membrane at a costant current of 400 mA for 4 h (Vai et al., 1986) for Western analysis. Correct protein transfer was confirmed by Ponceau S Red (Sigma-Aldrich) staining of filters (Fig. S3 Supplementary Data). For each blot, the same amount of recombinant human Hsp60 (StressGen Bioreagents, British Columbia, Canada, ADI-SPP-540) was included. Filters were washed in TBS (0.01 M Tris, pH 7.4, 0.9% NaCl) followed by 1.30 h saturation in TBS containing 0.1% Tween 20 and 5% skimmed milk. Immunodetection was performed with anti-Hsp60 monoclonal antibody (IgG mouse clone LK-2, StressGen Bioreagents, British Columbia, Canada, SPA-807) at 1:1000 dilution in TBS-Tween 20, 5% skimmed milk. After washing in TBS-Tween 20 (10 min, 3 times), membranes were incubated with secondary antibody (diluted 1:10000 in TBS-Tween 20, 5% skimmed milk) antimouse IgG conjugated with horseradish peroxidase (Thermo Scientific). Binding was visualized with the Pierce ECL Western Blotting Substrate followed by X-rays films.

Densitometric and Statistical analyses

Blot band intensities were compared by scanning the X-ray films and analyzing the scans with the Image J free software (http://rsb.info.nih.gov/ij/) of NIH Image software package (National Institutes of Health, Bethesda, Md.). For each blot, the scanned intensity of the Hsp60 bands was normalized against the intensity of the standard Hsp60 protein band. Data were expressed as the mean ± standard error of the means (SEM). One-way analysis of variance (ANOVA) was performed for all the normalized Hsp60 intensity values obtained from the different groups of samples (C, H, D, WA and AO). Since the analysis revealed that the changes in the Hsp60 levels among the five considered groups were significant (F(4,25) = 113.68, p < 0.0000), the Tukey's HSD post hoc tests for pair-wise comparison of means was used to assess significant differences (p < 0.000).
Results

Colonies of *A. muricata* infected by SEB ciliates showed the typical dark band which separates the dead tissue from the healthy tissue. Moreover, the band of ciliates causing SEB might be confused with Black band disease (BBD) caused by bacteria, but microscopic analysis of the coral fragments collected at the level of the dark band, revealed the presence only of the protozoa of the species *Halofolliculina corallasia* responsible for the SEB disease. *H. corallasia* is sessile in a lorica, sac-like with a rounded posterior and a cylindrical neck. The cell body is attached at its pointed posterior end to the base of the lorica. The cell is large and elongated with two conspicuous pericystomial wings (Fig. 1A and B). These protozoa appeared densely packed forming an indistinguishable black mass that cover the dead tissue below (Fig. 1C).

The monoclonal antibody anti-Hsp60 recognized a single specific 62-kDa band in all the coral fragments of all sampled colonies of *A. muricata* (Fig. 1D). No detectable and significant changes in the Hsp60 levels were detected in healthy fragments sampled far from the dark band (H) compared with the control (C). On the contrary, a strong induction of Hsp60 was observed nearby the infected site in fragments sampled just above the ciliate dark band (D), (Tukey’s HSD post hoc tests for pair-wise comparison of means, p < 0.0000), where the Hsp60 level was almost twice compared to the control and samples H (Fig. 1E).

By contrast, no detectable and significant modulation of the Hsp60 expression was detected in coral overgrown by algae. As shown in Fig. 2B and C, in the fragments of living coral branch free of algae (WA) and in the fragments of living coral tissue sampled just next to the algal interface (AO), the level of Hsp60 was similar to the level present in the control. Thus, no modulation of the Hsp60 expression was detected in coral overgrown by algae.

The seawater temperature measured at sampling time (October 2010) was 28.9°C and it appeared in line with the regular mean seasonal trend (± STD Dev) as shown in Fig. S4, Supplementary Data. Moreover, no anomalies regarding the salinity values (~ 35.5‰) were detected.
Figure 1- A. Microscope photo of the protozoan *Halofolliculina corallasia*, a species of folliculinid, heterotrich ciliate responsible of the SEB disease in *A. muricata*. B. *H. corallasia* in a lorica, sac-like. To note the two conspicuous pericytostomial wings. C. Colony of *A. muricata* affected by the Skeleton eroding band (SEB) disease. The infection appears as a dark band (surrounded by a rectangle) with skeleton recently devoid of tissue below. Just above the band, a white part of naked skeleton is visible. Loricae of *H. corallasia* are scattered loosely over this area occupying freely accessible terrain. Above this part, the coral tissue is still healthy and the sampling points are shown. Sample D: fragment of coral collected just above the ciliates dark band and eroded skeleton and tissue. Sample H: fragment of a healthy coral branch in the infected colony. The white arrow indicates the disease progression direction C. Effect of SEB disease on induction of Hsp60 in the different portions (D and H) of the scleractinian coral *A. muricata*. Samples prepared from healthy colonies (C) were also analyzed. Western blot representative of seven experimental repeats is shown. For each blots, the same amount of recombinant human Hsp60 was included D. Hsp60 levels were determined by densitometric analysis as described under Methods. Signals for seven different blots were analyzed. Data are expressed as arbitrary units and as mean±SEM (one-way ANOVA followed by Tukey's HSD multiple pair-wise comparisons, *p < 0.00*).
Figure 2. A. Colony of *A. muricata* overgrown by filamentous and mixed-species algal turf. Sample AO: fragment of live coral tissue sampled corresponding to the algal interface in a colony overgrown by algae. Sample WA: fragment of a coral branch free of algae in a coral colony overgrown by algae. B. Effect of algal overgrowth on induction of Hsp60 in the different portions (AO and WA) of the scleractinian coral *A. muricata*. Samples prepared from healthy colonies (C) were also analyzed. Western blot representative of seven experimental repeats is shown. For each blots, the same amount of recombinant human Hsp60 was included. C. Hsp60 levels were determined by densitometric analysis as described under Methods. Signals for seven different blots were analyzed. Data are expressed as arbitrary units and as mean±SEM.

Discussion and conclusions

The present study investigated the effects of biotic stresses on Hsp expression in a scleractinian coral, comparing the modulation of the Hsp60 levels in response to two different types of biological factors: the coral disease Skeleton eroding band (SEB), whose first record in the Maldives has been recently reported (Montano et al., 2012), and the algal-coral interaction causing the algal overgrowth on corals. Both these different types of biological interactions are recognized to be among the most important contributors to the worldwide decline of coral reefs (Harvell et al., 2002; Gardner et al., 2003; Hughes et al., 2003; Wilkinson, 2004).
To date, Hsp analyses have been predominantly performed in corals exposed to short-term, extreme stress regimes in the laboratory, confirming that corals possess temporally dynamic and responsive cellular machinery to counteract stresses (Van Oppen and Gates, 2006). In particular, Hsp60 is a molecular chaperone known to assist de novo folding, to refold misfolded proteins and to counteract protein aggregation under normal conditions. In response to environmental stresses, the deleterious increase of unfolded proteins triggers the induction of Hsp60 (Richter et al., 2010). In reef building corals the up-regulation of Hsp60 has already been observed under laboratory culture conditions testing stress induced by elevated temperature and light (Chow et al., 2009, 2012). Our analyses, performed on the scleractinian coral A. muricata in the natural environmental habitat of the lagoon of Magoodhoo Island, indicate that two different biological interactions trigger diverse responses on Hsp60 level. In fact, corals displayed a robust up-regulation of Hsp60 in response to the infection of the protozoa H. corallasia which causes the SEB disease, while for the algal overgrowth we did not detect any effect on the modulation of Hsp60 expression. With regard to the low level of Hsp60 present in the control sample of the healthy colonies this indicate that this chaperonine also has an important function under normal physiological conditions of the organism, in agreement with data reported in literature (Choresh et al., 2001; Chow et al., 2009, 2012).

In our experiments the whole holobiont was considered and the antibody used in this study, monoclonal clone LK-2, cross-reacts with a broad group of organisms that include bacteria, yeast, plants and animals. For this reason, the heat shock response could be produced by the coral polyps only, by the holobiont (microbial community, symbiotic zooxanthellae and cnidarians animal) or by the zooxanthellae only. It is important to emphasize that in our experiments all coral tissue samples were free of necrosis and morphologically undamaged. SEB is one of the most common disease of corals widespread in the Indian and Pacific Ocean (Page and Willis, 2008). In the studied area about 3000 colonies belonging to 19 genera were analyzed and the percentage of colonies infected by SEB (about 2%) and other coral diseases were reported in Montano et al. (2012). SEB occurs in sheltered, lagoon-type environments showing the greatest abundance at depths between 0.5 m and 3 m (Antonius and Lipscomb, 2001). Sessile ciliates settled between living tissues and recently exposed coral skeletons and their presence alters the normal body functions of the host starting lysis of the coral tissue and delays and/or reduces tissue regeneration (Rodriguez et al., 2009). Coral mortality is
thought to be caused by spinning and chemical secretions (organic acids) of the asexually produced motile swarming phase of *H. corallasia* (Antonius and Lipscomb, 2001). As a consequence of these processes, once infection has passed over an area the coral tissue dies and the bare coral skeleton loses all fine trabecular limestone structure. Progression rate of the band is very rapid and it has been estimated to change between 1 mm/week and 1 mm/day (Antonius and Lipscomb, 2001) similar to other "band" diseases, such as Black band disease or White band disease (Antonius, 1999; Antonius and Lipscomb, 2001). A study in 2008 found that SEB spread at about 2 mm/day in colonies of *A. muricata*, eventually wiping out 95% of its victims (Page and Willis, 2008).

From the microscopic analyses of the dark band, the total absence of living polyp tissue has been observed, revealing that the host cells were already died. For this reason, in our field sampling we decided to collect fragments of living coral tissue placed at different distances along the advancing front of the SEB band to analyze Hsp60 level, which would be meaningless in the coral fragments collected at the dark band. Our results show that in *A. muricata* infected by SEB disease different levels of stress protein 60-kDa are found in different portions of the colonies. In fact, the coral fragments sampled just above the SEB dark band, on the interface of ciliates progression, displayed a remarkable up-regulation of Hsp60, whose level was twofold higher compared to the control, indicating that the aggressive behavior of *H. corallasia* can cause cellular damage also in coral portions neighboring the infection and suggesting that disease infection causes stress at the cellular level, even in cells not yet infected by ciliates.

In the aquatic organisms, many interactions also involve chemical communication (Brönmark and Hansson, 2000), such as the case of coral SEB disease. The chemicals associated with the unhardened lorica, combined with the mechanical disruption caused by the spinning larvae, appear to damage the coral skeleton and initiate the lysis of the coral tissue (Antonius, 1999). The chemical secretions produced by *H. corallasia* in the infected zone could trigger the induction of Hsp60 in the portions just above the band. In this context, the Hsp60 up-regulation might represent a defense from underlying coral portions colonized by ciliates which excrete harmful substances. Otherwise the up-regulation of the Hsp could represent a strategy/mechanism to stop and circumscribe the infection, preventing it from spreading throughout the coral. However, corals have an immune system based on self/non-self recognition and cellular and humoral processes (Mydlarz et al., 2010). Recognition receptors such as Toll-like receptor (TLR) domain genes (Hemmrich et al., 2007; Miller et al., 2007) have been
characterized in anthozoan corals (Dunn, 2009). Recently, it has been suggested that in mammalian, Hsp60 were implicated in autoimmune disease and antigen presentation since they are potent activators of the innate immune system (Tsan and Gao, 2009). In particular Hsp60 activation appears to be mediated by TLRs ligand (Ohashi et al., 2000). Although no morphological differences were detected in tissues next to SEB band compared to those of healthy colonies, in line with what reported by (Antonius and Lipscomb, 2001) who suggested that the coral polyps immediately ahead of an advancing front of SEB appear undisturbed, our results indicate that physiological processes aimed to counteract the damage caused by infection are active.

The coral fragments sampled distant to the dark band of ciliates had a Hsp60 level comparable to that observed in healthy colonies of A. muricata. This might suggest that the stress response appears confined in a restricted area near the infection even if in a coral colony polyps are linked together by a common tissue named coenosarc or coenenchyme.

The other biological factor analyzed for the Hsp60 expression in A. muricata is the algal overgrowth phenomenon. Different responses of corals to different species of algae or different impacts of algae on corals have been largely documented (McCook, 2001; McCook et al., 2001; Jompa and McCook, 2002; Smith et al., 2006; Diaz-Pulido et al., 2009), suggesting a great variability in the processes and outcomes of coral–algal interactions, even within an algal functional group, algal family, and coral life-forms and genera (Jompa and McCook, 2003a). Also in this case we sampled fragments of living tissue of coral placed at different distances along the progression of the algal turf which caused the death of the backwards coral tissues. In particular, to test whether coral-algal competition may affect the modulation of Hsp60, portions of living coral tissue colonized by a few algal filaments were sampled. Analyzing the expression of Hsp60, no detectable differences have been observed between the healthy and the overgrown colonies and also between the different coral portions of the same colony subjected to algal overgrowth. Different explanations might be envisaged. It’s conceivable that benthic algae and algal turf have light inhibitory effects on A. muricata colonies. In fact some studies have reported minor effects of algal turf on corals, or have even suggested that algal turfs are relatively poor competitors having little effect on corals (McCook et al., 2001) or that corals were competitively superior to the algal turfs (Van Woesik, 1998; McCook, 2001). Nevertheless, algae can actively overgrown on the live coral by exuding allelochemical or secondary substances, as reported in others studies (Littler and Littler, 1997; Jompa and McCook, 2003b).
Presumably, in the fragments sampled next to algal interface, the coral cells have not yet been damaged by algal toxins, and hence have not up-regulated their levels of Hsp60. It is widely known that various species or genera of algae can negatively influence corals (McCook et al., 2001; Jompa and McCook, 2003a, 2003b; Smith et al., 2006) leading to reef degradation. Since further investigations performed five months after the sampling revealed that the same colonies were completely overgrown by algae and turf, this latter scenario appears to be the most likely.

In conclusion, with this study we propose Hsp60 expression might be a useful tool and promising biomarker for the holobiont of scleractinian corals to evaluate physiological stress caused by coral diseases such as SEB, laying the basis for subsequent monitoring in the field of other diseases and other types of biological stresses. Further studies on the different groups of Hsps and their expression in each member of the holobiont association may also be important for the health assessment of scleractinian corals and for the conservation of coral reefs.

References


Dunn S (2009) Immunorecognition and immunoreceptors in the Cnidaria. Invert Surv J 6: 7-14


Jompa J, McCook LJ (2003b) Coral-algal competition: macroalgae with different properties have different effects on corals. Mar Ecol Prog Ser 258: 87-95


Supplementary Data

Figure S1 - Map of Magoodhoo Island (A), located in Faafu Atoll (B), Republic of Maldives (C). The islands are indicated in black, the deeper part of the lagoons in grey and the dotted line indicates the reef edges. An area of approximately 150 m$^2$ and about 70 m distant from the shore in the Magoodhoo lagoon (3$°$ 04' 45.60" N; 72$°$ 58' 02.20" E), was chosen as sampling site to create an easy and short time access area (indicated by the arrow).
Figure S2 - A representative SDS-PAGE on polyacrylamide gel (8%) stained with Coomassie Brilliant Blue to visualize total proteins extracted from different coral fragments is shown. Pre-stained protein markers (M) and two different amount of recombinant human Hsp60 (5 and 10 µg) were loaded on the gel. The abbreviations of the samples are described in the text.
Figure S3 - Representative filter stained with Ponceau used as control for equal protein loading and to ensure a correct protein transfer. Pre-stained protein markers (M) are also shown. The abbreviations of the samples are described in the text.
Figure S4 – Graph showing the trend of sea surface mean temperature for the lagoon of Magoodhoo Island (Maldives) on 2010 (panel above). On panel below, graph reporting the values of standard deviation relative to the average monthly temperature(Source: http://disc.sci.gsfc.nasa.gov/techlab/giovanni/)