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The impact of urbanisation on coral reef ecosystems

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The impact of urbanisation on coral reef ecosystems

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Ao meu Pai

"Set your goals high, and don't stop till you get there."

Bo Jackson

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Summary

Over the last century the main form of human settlement switched from rural to urban. More than half of the world's population is at present settled around coastal zones, making them particularly vulnerable to all sorts of environmental stress (e.g., sedimentation, agricultural runoff, deforestation, oil spills, untreated sewage, siltation, eutrophication, persistent organic pollutants). Coral reefs, one of the most important productive and diverse coastal ecosystems in the world, have already experienced massive destruction. This is largely caused by anthropogenic land-based pollution, which can adversely affect the health of these ecosystems by modifying influencing the microbial communities present in coral reef sediment, water and fauna. Microbial communities are crucial in oligotrophic ecosystems located along major urban centers since they play major roles in the remineralization of organic matter and nutrient recycling. Disturbances play a critical role in structuring community composition. Understanding the response of important reef taxa to disturbances facilitates the design of meaningful conservation strategies that aim to protect coral reefs. This thesis focuses on gaining a better understanding of the main natural and anthropogenic disturbances and how they affect corals reefs adjacent to the cities of Jakarta and Makassar (Indonesia). This was achieved through field surveys, satellite imagery and molecular techniques. An assessment was made of the amount of variation in composition of ecologically important taxa (corals, sponges, foraminifera, bacteria and archaea) explained by environmental and spatial variables. The spatial variable was the distance between transects while the environmental variables were determined in the field (substrate variables) and were derived from ocean colour satellite imagery (Remote sensing reflectance at 645 nm, coloured dissolved organic matter index and chlorophyll-a) and thermal infrared imagery (sea surface temperature). The results showed environmental variables and especially CDOM (coloured dissolved organic matter index) as the most important explanatory variables. In addition to the above, pirosequencing and in silico metagenome (PICRUSt) analyses were used to access the composition, diversity and function of archaeal communities in six different coral reef habitats (sediment, seawater and four different sponge species *Stylissa massa*, *Stylissa carteri*, *Xestospongia testudinaria* and *Hyrtios erectus)*. The biotope explained more than 70% of the variation in archaeal composition revealing the importance of the biotope in structuring archaeal community composition. In general, *Crenarchaeota* dominated the archaeal community of sponge species and sediment whereas *Euryarchaeota* dominated the seawater community. In terms of function, significant differences were observed among the different biotopes. The 'Energy

methabolism' functional subcategory was significantly enriched in sponge biotopes but some of its individual pathways were significantly enriched in seawater namely the methane metabolism. A more detailed analysis of the Nitrogen metabolism revealed the use of different ammonia assimilation strategies by the distinct biotopes. Overall, these results draw attention for the importance of maintaining a diverse coral reef ecosystem in order to maintain a functionally diverse microbial community. Furthermore, they suggest that these should be achieved by taking into consideration different management approaches when designing effective coral reef conservation strategies.

Resumo

No decorrer de um século a principal forma de ocupação humana passou de rural a urbana. Atualmente, mais de metade da população mundial vive em zonas costeiras, tornando-as particularmente vulneráveis a todos os tipos de impactes ambientais (sedimentação, escoamento agrícola, desflorestação, derrames de óleo, esgoto não tratado, assoreamento, eutrofização, poluentes orgânicos persistentes). Os recifes de coral, um dos mais importantes, produtivos e diversos ecossistemas costeiros do mundo, estão a sofrer uma destruição massiva. Isto deve-se essencialmente à poluição antropogénica de origem terrestre que inflige impactes agudos e/ou crónicos na saúde destes ecossistemas, influenciando nomeadamente as comunidades microbianas presentes no sedimento, coluna de água e fauna. As comunidades microbianas são cruciais em ecossistemas oligotróficos localizados junto de grandes centros urbanos, uma vez que estas comunidades desempenham um papel fundamental na remineralização de matéria orgânica e reciclagem de nutrientes. As perturbações ambientais desempenham um papel importante na estruturação da composição de comunidades. Perceber a resposta dos grupos taxonómicos funcionalmente importantes a essas mesmas perturbações facilita a concepção de estratégias de conservação que visam proteger os recifes de coral. Esta tese teve por objectivo compreender melhor quais as principais perturbações naturais e antropogénicas e como estas afectam, em particular, os recifes de coral adjacentes às cidades de Jacarta e Makassar (Indonésia). Estes objectivos foram atingidos através da realização de trabalho de campo, da análise de imagens satélite e do uso de técnicas moleculares. Foi analisada a variação na composição de grupos taxonómicos ecologicamente importantes (corais, esponjas, foraminifera, bactéria e archaea) explicada por variáveis ambientais e espaciais. A variável espacial consistiu na distância entre transectos enquanto as variáveis ambientais foram determinadas *in situ* (variáveis relacionadas com o substrato) e derivadas de imagens satélite no visível da cor do oceano (reflectância a 645 nm, matéria orgânica dissolvida colorida (CDOM) e clorofila) e ainda de imagens satélite de infravermelhos térmicos (temperatura à superfície). Os resultados mostraram as variáveis ambientais, e em especial o CDOM, como as variáveis explanatórias mais importantes. Para além do referido, análises de dados de pirosequenciação e metagenoma in silico (PICRUSt) foram usados para aceder à composição, diversidade e função da comunidade de Archaea em seis biótopos diferentes (sedimento, água e quatro espécies de esponjas (*Stylissa massa*, *Stylissa carteri*, *Xestospongia testudinaria* and *Hyrtios erectus*). O biótopo explicou percentagens de variação de composição em Archaea acima dos 70% revelando a importância do

biótopo na estruturação da composição desta comunidade. De uma forma geral, o filo *Crenarchaeota* dominou a comunidade de Archaea das várias espécies de esponja e do sedimento enquanto o filo *Euryarchaeota* dominou a comunidade planctónica. Também ao nível da função, diferenças significativas foram observadas entre os diferentes biótopos. A categoria funcional "Metabolismo energético" mostrou-se significativamente enriquecida nas esponjas mas alguns dos seus "pathways" individuais foram significativamente enriquecidos na coluna de água, nomeadamente o metabolismo do metano. Uma análise mais detalhada do metabolismo do Azoto revelou o uso de diferentes estratégias na assimilação de amónia pelos distintos biótopos. De uma forma geral, os resultados deste estudo salientam a importância da manutenção de um ecossistema de recife de coral com uma diversidade taxónomica elevada de forma a manter uma comunidade microbiana funcionalmente diversa. Igualmente sugerem que, para tal, deve ter-se em consideração diferentes abordagens de gestão no desenvolvimento de medidas de conservação efectivas de sistemas de recife de coral.

Chapter 1: General Introduction

Watercourses, both maritime and fluvial have always been a focal point for human settlement. Many delta systems are, however, now seriously degraded due to chronic disturbance associated with human settlement. For the first time in human history, in 2006, the percentage of the world's population in cities rose to more than 50% (Wilby and Perry 2006). Coastal development is changing in character as more people now live in large urban areas than ever before and the percentage of humanity inhabiting moderate to large conurbations will continue to increase through the course of the present century. Currently, 14 cities have more than ten million inhabitants and 300 cities have more than one million inhabitants; these numbers are predicted to increase rapidly over the next few years (United Nations 1993; 2001). This will have an enormous impact on surrounding ecosystems. Coral reefs in particular, have been shown to be susceptible to local anthropogenic disturbances including overfishing, sedimentation, eutrophication, heavy metal, pollution, logging, dredging, land-based run-off and urban effluents (Jackson et al. 2001; Aronson et al. 2002). These ecosystems are among the most valuable ecosystems for human society. A large proportion of coastal populations relay on coral reef goods and services (e.g., food, building materials, coastal protection); and several economic activities are based on coral reef resources (e.g., fisheries, tourism, pharmaceutics, jewelry, aquarium and live fish trade) (Bryant et al. 1998). In addition to the environmental degradation associated to coastal development, this excessive and unmanaged pressure on coral reef resources is turning coral reefs into functionally-at-risk (with very limited capabilities to support coastal communities) or even non-functional ecosystems. For example, in the Great Barrier Reef the amount of sediments reaching the inner zone is now five to 10 times greater than before European settlement (McCulloch et al. 2003). In addition to the above, global disturbances like warming, intense El Niño Southern Oscillation (ENSO) events and rising concentrations of dissolved CO_2 are also adversely impacting coral reefs by increasing coral bleaching and reducing coral calcification rates (Buddemeier et al. 2004). Some studies have focused on reductions in live coral cover and phase shifts in dominant species (Jackson et al. 2001) as the most perceptible responses of coral reefs to these stresses. The World Resources Institute in the Reefs at Risk Revisited report (Reytar et al. 2011), alert us to the fact that more than 60% of the word's reefs are under direct threat from local sources. This value increases up to 75% if we also add the impact of global threats (e.g., thermal stress). Southeast Asia is the coral reef region with the highest percentage of threatened reefs (95%) and Indonesia is the country with the largest area of reefs at risk. These conditions enforce the need for more long-term studies on coral reef diversity in this region.

Kepulauan Seribu and Spermonde coral reef system are two of the most important Indonesian coral reef ecosystems and are located adjacent to two major conurbations namely Jakarta and Makassar. Jakarta is the capital city of Indonesia and home to more than 10 million inhabitants whereas Makassar is a city with more than 1 million inhabitants.

1.1. Objectives

The main objectives of this thesis are: 1) to understand which anthropogenic and natural disturbances have been affecting Spermonde and Kepulauan Seribu coral reef systems; 2) to infer how these disturbances impact these coral reef ecosystems and, more specifically, affect ecologically important taxa, and 3) to characterize the distribution, composition and function of one of the less studied but crucially important coral reef taxa – Archaea. To this end, this thesis investigated: how different taxa (corals, sponges, foraminifera, archaea and bacteria) respond to spatial and environmental variables linked to anthropogenic disturbances (Chapter 3); how archaeal communities inhabiting different biotopes differ in composition, phylogeny and function (Chapter 4); how the nitrogen functional pathway differs between biotopes (Chapter 5). A final conclusion and future research directions are provided in Chapter 6.

1.2. References

Aronson RB, Macintyre IG, Precht WF, Murdoch TJT, Wapnick CM (2002) The expanding scale of species turnover events on coral Reef in Belize. *Ecological Monographs* 72: 233-249.

Bryant D, Burke L, McManus J, Spalding M (1998) Reefs at risk: a map-based indicator of threats to the world's coral reefs. World Resources Institute, Washington DC.

Buddemeier RW, Kleypas JA, Aronson RB (2004) Coral reefs & global climate change: potential contributions of climate change to stresses on coral reef ecosystems. Pew Center on Global Climate Change.

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA and others (2001) Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293: 629−637

McCulloch M, Fallon S, Wyndham T, Hendy E, Lough J, Barnes D (2003) Coral record of increased sediment flux to the inner Great Barrier Reef since European settlement. *Nature* 421: 727-730.

Reytar K, Spalding M, Perry A (2011) Reefs at risk revisited. Washington, DC: World Resources Institute.

United Nations (1993) United Nations Statistical Yearbook, New York.

United Nations (2001) United Nations Statistical Yearbook, New York.

Wilby RL, Perry GLW (2006) Climate change, biodiversity and the urban environment: a critical review based on London, UK. *Progress in Physical Geography* 30: 73-98.

Chapter 2: General Information

Sections of this chapter have been previously published in A.R. Polónia, M. Figueiredo, D. F. R. Cleary, N. J. de Voogd and A. Martins, "Sea surface temperature and ocean colour (MODIS/AQUA) space and time variability in Indonesian Sea coral reef systems from 2002 to 2011", Proc. SPIE 8175, 817502 (2011); doi:10.1117/12.901820

2.1. Socio-economic context

Originating almost entirely from volcanic activity, Indonesia is the largest archipelago in the world with about 18000 islands, which only represent a quarter (1.43 million km^2) of the whole Indonesian dominion (6 million km^2); the remaining 3 quarters (5.57 millions) is water (UNEP 2005).

Indonesia is the fourth most populous country in the world (Farida et al. 2014). As a whole, in 2000 Indonesia had a population of about 225 million (Population Reference Bureau 2006). There are five major islands in Indonesia namely Sumatra, Java, Kalimantan, Sulawesi and Papua. However, the total population is not equally distributed among the different islands; Java, representing approximately 7% of the whole Indonesian land area, houses about 60% of the total Indonesian population (Firman et al. 2007). From 1980 to 2000 the population of Java grew rapidly (32.8 million in 1980; 55.4 millions in 1990; 85.2 million in 2000; Population Reference Bureau 2006); in the remaining territory the increase was only 16 million. In 2000, 48.7% of the Java population lived in urban areas (Firman et al. 2007) and, according to UN (2002), 140 million inhabitants lived within 60 km of the coast.

2.1.1. Jakarta

Since the 12th century and until the 16th century Jakarta was essentially a Port center. In the beginning of the 17th century Jakarta became Batavia. At the end of the 17th century the population of Jakarta was about 27000 inhabitants. Some years later, in the middle of the 18th century, Jakarta was characterized as a very dirty city with several wastewater problems, leading the Europeans to leave the city and move further south (Cybriwsky and Ford 2001).

In the beginning of the 20th century the population increased to 115000. In 1945 Indonesia became independent and in 1949 Batavia was renamed Jakarta and was designated the

capital city. Sukarno, president of Indonesia between 1945 and 1967, had as main objective to transform Jakarta into a huge city, thus starting an urbanization process (Cybriwsky and Ford 2001).

This post independence growth resulted in an increase of migrants from the inner parts of Jawa and from the other Islands, who sought work and better living conditions in Jakarta (Cybriwsky and Ford 2001). In 1950 Jakarta reached a population of about one million (Firman et al. 2007), resulting in a significant deterioration of people´s living conditions. In 1970, under Suharto (president of Indonesia between 1967 and 1998), the government attempted to control this growth (not allowing migrants in the city) but this policy failed and in 1971 the province of Jakarta (constituting several municipalities; also called DKI Jakarta) reached 4.5 million inhabitants (Cybriwsky and Ford 2001). Despite the attempt made by Suharto to control the population, he mantained the same growth ambition shown by his predecessor and during his governance the metropolitan area was enlarged with several new towns (where the manufacturing industries were also transferred). This transformed the DKI Jakarta into a province particularly specialized in services and several other economic activities (Cybriwsky and Ford 2001).

The highest growth rate was reached in the 1980's, through the increase in urban population (8.36%) and in total population (1.97%). During the 1990´s growth rates decreased slightly (4.4% in urban population and 1.35% in total population) (Firman et al. 2007). This reduction occurred simultaneously with the increase of population in districts adjacent to large cities. This was reflected primarily as a movement of people from the center of major cities to outlying urban fringes; carried out essentially by middle and higher income residents (Browder et al. 1995). For example, the Bekasi, one of the adjacent Jakarta districts, had an annual population growth rate of 4.13% over the period of 1990-2000 (West Java Office of Central Board of Statistics 2001) in which about 60% of the new population in the district came from the core of Jakarta City.

From 1980 to 1985 and 1990 to 1995 only about 35% of the urban growth resulted from natural population increase. The remaining 65% was related to rural-urban migration and reclassification from rural to urban areas (Firman 1997, 2004; Firman et al. 2007). During the period 1999- 2005 the number of urban localities in the Jakarta Metropolitan Area increased by about 305 (*i.e.,* from 730 to 1035) (Firman et al. 2007). Currently, the Jakarta metropolitan area represents an area of 6418 km^2 and encompasses the cities of Jakarta, Bogor, Tangerang and Bekasi (Cybriwsky and Ford 2001). As a whole, this area has a

population of about 21 million (Winarso 2011) from which half of the population lives in Jakarta city (> 10 millions) (UNEP 2005; Nadarajah and Yamamoto 2007).

2.1.2. Makassar

Since Indonesian independence, Makassar, one of the largest cities of Indonesia and the capital of Sulawesi, experienced an enormous growth. In the early 19th century Makassar, with an area of 21 km^2, had less than 15000 inhabitants; in 1980 its population increased about 47 times (708465) (Anwar 2004). From 1970 to 2000 Makassar was the Indonesian city with the highest growth rate, whith annual rates of 5.5 % in the first decade, 2.91 % in the second decade and 1.46 % in the third decade. Actually, between 1990 and 2000 only Makassar and Palembang grew more than the average national population growth rate of 1.35 % per year *i.e.,* 1.46 % and 2.30 %, respectively (Firman 2004).

In 1999, Makassar had 134 urban localities and eight rural localities; in 2005 the number of urban localities increased to 137 and the number of rural localities decreased to six (Firman et al. 2007). At present, Makassar, with an area of 175.77 km^2 has a population of approximately 1.2 million (Sattar et al. 2012). Of the total surface area, 60% are residential areas, 15% are related to industrial areas and 25% involve open spaces (Nas and Nas 2003).

The big proxy for this growth was the construction of the Makassar sea harbour; which was considered the most important Port in East Indonesia (Augustinus 2001). The possibility of exporting their goods through the harbour led most of the industries to settle in or around Makassar (Augustinus 2001). These economic opportunities in addition to the settlement of several economic and social facilities (which were lacking in rural areas) attracted people to the city (Anwar 2004). The impacts caused by the Port are numerous, not only in the post-construction phase (pollution from ports; oil spills; ship-based sewage (ballast and bilge discharge, garbage and solid waste); physical impacts from groundings and anchor damage) but also during the construction phase (dredging and opening up channels to improve navigation) (Burke et al. 2002). The industrial activities, even of those industries that are not settled in the coastal areas, exert their impact through the fluvial input of waste substances. Until 2001, there were no water treatment systems in Makassar to deal with contaminated sewage (Augustinus 2001).

The river discharges, such as the Jene Berang near the city of Makassar (Ujung Pandang) and the Maros in the north, strongly affect the water transparency through the input of terrigenous sand, silt and land based pollution. The ecosystems close to the coast are

therefore, severely affected. Furthermore, human populations can have a remarkable impact on coral reef ecosystems located near the islands (Moll 1983). About 54 of the 120 islands that make up the Spermonde archipelago are densely inhabited (Glaser et al. 2010). The sewerage derived from the islands villages can be quite extensive affecting the closest reefs (Moll 1983).

2.2. Researched Reefs

2.2.1. Kepulauan Seribu coral reef system

Jakarta, the capital city of Indonesia, is located on the north Coast of Java Island. The 105 islands that constitute Jakarta Bay and Kepulauan Seribu ecosystem (Figure 2.2.1) are dispersed over a chain of 80 km extending from Java to the northwest into the Java Sea. With a population of more than 12 million inhabitants (Renema 2010), Jakarta exerts a strong impact over this ecosystem. Jakarta bay is subjected to strong discharges originating from numerous rivers and from Jakarta sewage system which represents a high input of organic and inorganic suspended matter, sediments, chemical and industrial pollutants (Rees et al. 1999; Cleary et al. 2006; Renema et al. 2010).

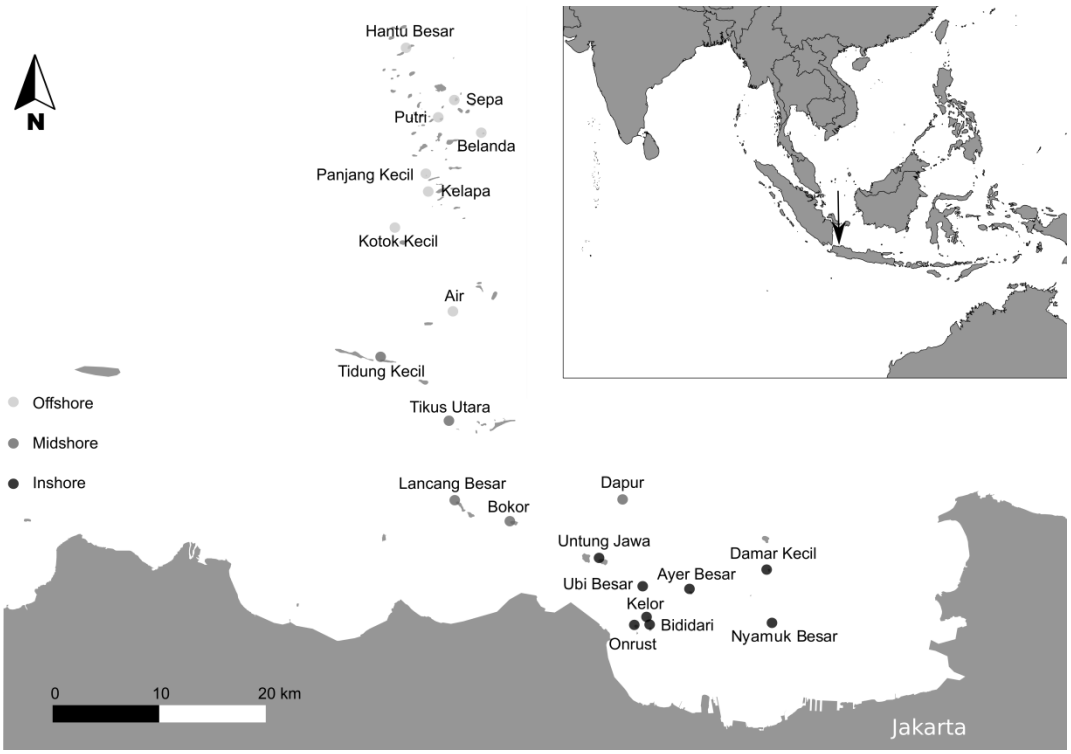

Figure 2.2.1 - Kepulauan Seribu Coral Reef System (Cleary et al. 2014)

Based on distance to the shore and geomorphologic and geographic characteristics, Kepulauan Seribu coral reef system has been divided in three distinct shelf zones: inshore, midshore and offshore, representing an on-to-offshore gradient of anthropogenic disturbances (De Vantier et al. 1998; Cleary et al. 2006, 2008, 2013).

The inshore zone is located between the coast and 21 km (Cleary et al. 2008) and comprises the reefs within Jakarta bay: Nyamuk Besar, Onrust, Bididari, Kelor, Ayer Besar, Ubi Besar, Damar kecil and Untung Jawa. Due to its proximity to Jakarta city these reefs have been seriously affected by land based pollution (euthrophization, rubbish accumulation, coral mining, high heavy metal, pesticide sewage and petroleum contamination; Ongkosongo 1986; Cleary et al. 2006). As a consequence, these reefs have extremely low coral cover and extremely high sand cover percentages.

The midshore zone is located between 22 and 40 km offshore from Jakarta (Cleary et al. 2008) and comprises the mid region reefs: Dapur, Bokor, Lancang Besar, Tikus Utara and Tidung Kecil. Here, the influence of land-based pollution is less intense. However, during the Southeast monsoon, the prevalent winds push the polluted plume from Jakarta bay to the north over this zone (Cleary et al. 2006).

The offshore zone is located more than 40 km offshore from Jakarta (Cleary et al. 2008) and consists of the outer-region reefs: Air, Kotok Kecil, Kelapa, Panjang Kecil, Belanda, Sepa, Putri and Hantu Besar. This zone is minimally affected by land based pollution associated to Jakarta city, however, other disturbances such as: dredging activity, poison and blast fishing, outbreaks of *Acanthaster planci* (a coral predator) and high temperatures associated with ENSO phenomena (De Vantier et al. 1998; Vail and Thamrongnawasawat 1998; Cleary et al. 2006) have been reported. The first marine park of Indonesia - Pulau Seribu National Marine Park - was established within this zone (Farhan and Lim 2012; Cleary et al. 2013).

2.2.2. Spermonde Archipelago

The Spermonde Archipelago (Figure 2.2.2) is situated on the coast of the southwest peninsula of Sulawesi (Celebes), which lies in the Wallacea region.

Located at 4° 27'00" - 5° 29'00" south latitude and 119° 2'00" 119 33'00" east longitude the Spermonde Archipelago comprises a group of 160 islands (de Voogd et al. 2006; Pet-Soede and Erdmann 1998) and shallow banks. According to Umbgrove (1930), the Spermonde Archipelago, with a total area of 400,000 ha consists of a group of submarine

reefs, patch reefs and cays distributed over a submarine plateau that are, from a geological point of view, in different stages of development (Umbgrove 1930).

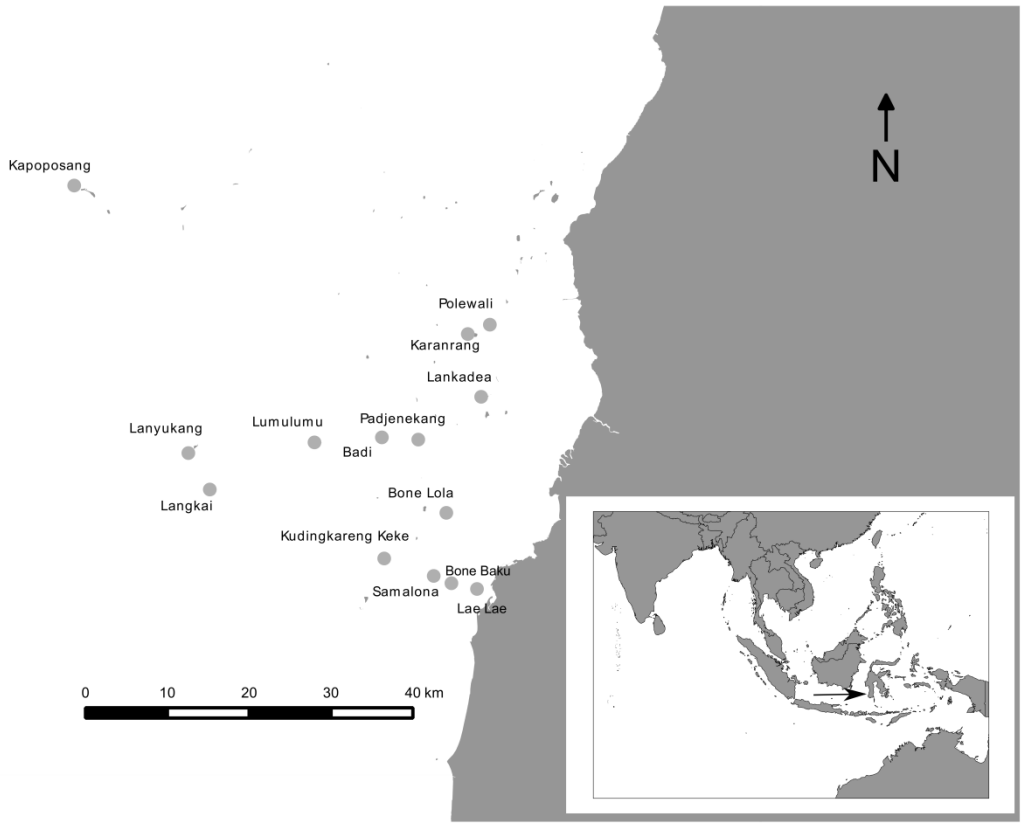

Figure 2.2.2 - Spermonde Coral Reef System

On the western side of the reefs, the development of reef flats is broadest and coral growth is generally vigorous due to powerful currents and restricted sedimentation (Moll 1983). Conversely, the eastern side reef flats are narrower and very sandy and, despite the weaker currents than in the west, this reef side presents poorer coral growth due to high rates of sedimentation. Although waves can apparently promote reef destruction, wave action seems to have a stronger role in reef development. The southern reef flat is usually well developed but not as extensive as the western sides (Wijsman-Best et al. 1981). The reef edges can be quite skewed in the northern and eastern sides, while in the southern and western sides the inclination is usually smoother (Moll 1983).

All Spermonde reefs are cay crowned reefs lying on a carbonate shelf (Umbgrove 1929; 1930; Guilcher 1988 in Renema and Troelstra 2001; Cleary et al. 2005; Renema and Troelstra 2001) which increases in depth with distance from the coast (Renema and Troelstra 2001; de Voogd et al. 2006; Hoeksema 2012). The westernmost islands rely on

a higher rim beyond which the shelf abruptly drops to depths exceeding 800 meters in the Makassar Strait (Moll 1983). According to several authors (de Voogd et al. 2006; Hoeksema 1990; Pet-Soede 2000) this shelf can be divided into four distinct shelf zones parallel to the coast. The distinct zones differ between them in biotic and abiotic parameters derived from their bathymetry, geography, geomorphology and distance to the shore (Renema and Troelstra 2001).

The first zone, also called the inshore zone, has a maximum depth of 20 m and is located between 0 and 5 km offshore (Becking et al. 2006). It comprises the islands: Lae Lae, Lankadea, Polewali, Karanrang, Gusung and Barangbaringan. Due to its proximity to Makassar city, major river discharge reaches this zone with similar intensities during both seasons (wet and dry). The high concentrations of organic and inorganic nutrients (Troelstra et al. 1996) and clay/silt content (Erftemeijer 1993) result in the lowest diversity of stony corals registered in the Spermonde archipelago (Hoeksema 1990). In 1993, Erftemeier (1993) reported chlorophyll-a concentrations of 2.9±1.5 µg/l (dry season) and secchi depths of 0.5-2.5 m (wet season) and 2.5-5 m (dry season) around Lae Lae (Renema and Troelstra 2001). In this zone the water energy is low (Troelstra et al. 1996).

The second zone, the middle-inner zone, has an average depth of 30 meters, is located between 5 and 12.5 km offshore from the mainland (Chozin 2008) and is composed of the Islands Samalona, Pajenekang, Bone Lola Barang Lompo, Bone Batang and Bone Bako. Here, the influence of the Jene Berang River is not too strong; however, during the wet season the river plum can reach this zone causing poor water transparency (Renema and Troelstra 2001). During the dry season, Erftemeier (1993) observed chlorophyll-a concentrations of 0.5±0.2 µg/l and Secchi depths of about 10-17 m. In the wet season, Secchi depths were reduced up to 1-5 m (Renema and Troelstra 2001). The water energy varies with the season, with high values on the exposed sides (north and west) during the wet season and moderate during the dry season (Verhey 1993; Troelstra et al. 1996).

The middle-outer zone is composed of the islands Kudingareng Keke, Lumulumu, Badi and Bone Tambung. This zone is located between 12.5 and 30 km offshore from the coast and presents depths ranging from 30 to 50 meters. Here, as in the fomer zone, the water energy is stronger during the wet season (Chozin 2008). As the river influence is much smaller, the chlorophyll-a concentrations are also lower (1.0±0.1 µg/l in the dry season) while the Secchi depths are depper (7.5-20 m in the wet season and 10-30 m in the dry season) (Renema and Troelstra 2001). These conditions make this zone the most diverse zone in terms of stony corals (Hoeksema 1990).

The outer zone is located beyond the platform rim (>30 kilometer offshore from Makassar) and is composed of the barrier reef. This zone has depths ≥ 50 meters and consists of the islands Langkai, Lanyukang and Kapoposang. The hydrodynamic energy as well as the water transparency is higher compared to the other zones, especially during the wet season (Troelstra et al. 1996). Chlorophyll-a concentrations of 0.7±0.1 µg/l were registered during the dry season (Erftemeier 1993; Renema and Troelstra 2001).

Despite differences in stony coral (Hoeksema 1990; Moll 1983) and sponge diversity (de Voogd et al. 2006) between the two outermost zones, the same is not true for foraminifera. Renema and Troelstra (2001) did not observe differences between the two outer shelf zones and combined them into a single zone (the outer zone). However, these authors could distinguish two near shore zones: one in the North and another in the South. Foraminifera were less diverse close to Makassar city, *i.e.,* in the southern part of the inshore zone, where the reef base fauna was absent.

2.3. Coral reef Anthropogenic Stressors

High population growth rates require a larger food supply. These demands were essentially fulfilled by agricultural and fishery intensification. The increase in the cultivation of annual crops was achieved by expanding agricultural land-use through deforestation and by the application of higher amounts of chemical fertilizers. This land clearing resulted in increased soil erosion and in the concomitant transport of nutrient and chemical contaminated sediments by the local rivers into the sea (Augustinus 2001). Agricultural runoff threatens mangrove and coral reef ecosystems. As transitional ecosystems between marine and terrestrial environments, mangroves act as buffers and protector for both of them. Mangroves protect terrestrial ecosystem from the power of meteorological processes (storms, tsunamis, cyclones, wind and wave action), and they protect the marine environment from sediments and organic materials derived from the terrestrial environment through their ability to filter and trap.

However, in addition to massive rain forest deforestation, the deforestation rate of mangroves has also grown considerably. In 1983, the Indonesian government prohibited trawling operations in the whole Indonesian oceans due to their negative impact on coral reef ecosystems (Sano 2000). Afterwards shrimp production shifted to tambaks (brackish water fish ponds). South Sulawesi became the Indonesian province with the highest area

of tambaks. From 1979 to 1996 the number of tambak households increased from 9873 to 26698. However, this alternative to the damage caused by trawling activities became itself a threat to the ecosystem. The tambaks were mostly constructed by clearing mangrove forests and were the most direct cause of mangrove destruction (Nurkin 1994). According to Nurkin (1994), the south of Sulawesi lost 76000 ha of mangrove between 1950 and 1980. In 1980 the South Sulawesi area of tambaks was 57858 ha and in 1996 this increased to 84832 ha (Sano 2000); only 22.88% of the original total area of mangrove (110000 ha) remained.

This mangrove destruction leads, among other things, to the loss of coastal protection and the consequent increase of coastal erosion, and to the increase of coral reef contamination by the inland runoff of nutrients and sediments. Coral reef ecosystems are capable of growing under very low nutrient concentrations. This is largely due to their photosynthetic endosymbionts known as zooxanthellae, which provide corals with oxygen and organic material. High nutrient concentrations tend to adversely affect corals by giving algae a competitive advantage that allows them to overgrow corals. Additionally, high nutrient loads are associated with high chlorophyll concentrations and phytoplankton blooms. Edinger et al. (1998, 1999) found that chlorophyll-a concentration is negatively correlated with both live coral cover and coral species diversity. De'ath and Fabricius (2010) likewise, in a study in the Great Barrier Reef, found a reduction in hard coral and phototrophic octocoral richness with increasing chlorophyll-a concentrations and an increase in the cover of macroalgae.

High sedimentation rates can severely reduce the euphotic zone thereby inhibiting photosynthesis in the symbiotic zooxanthellae and smothering coral polyps (Aerts et al. 1997; Bell 1992). According to Rogers (1990), persistent high concentrations of sediments lead to the reduction of: coral species number, live coral cover, coral growth rates, coral recruitment, calcification and rates of reef accretion.

In addition to these impacts, mangrove deforestation results in a loss of the marine hatcheries and refuges for a wide variety of off-shore marine life, many of which, are commercially important (fish, shrimp, crabs, clams).

This, in addition to the aforementioned increase of food demand due the regional population explosion, led to the overexploitation of Indonesian fish stocks (Burke et al. 2002). South Sulawesi is the most important Indonesian fishery province for sea-fish (Pet-Soede 2000). From 1975 to 2007 the fish capture increased on average 5600 tons per year (122649 tons in 1975 (Bailey et al. 1987) and 301.549 ton in 2007 (Badan Pusat

Statistik Republik Indonesia 2009)). According to Pet-Soede (1999), this increase was not supported by an increase in total effort (number of trips or number of fishing boats) but by an increase in the effectiveness of a fishing trip. The use of motorized boats allowed the increase in fishing time and the exploitation of different fish stocks. However, these values could be under-estimated since some species are mostly exported and sold directly to exporting companies no longer being landed at the local auctions where official data are recorded (Pet-Soede et al. 1999). In addition to the traditional mode, fishing is often carried with the use of destructive fishing techniques namely blast and poison fishing (Burke et al. 2002).

Blasting is one of the most devastating causes of reef destruction and affects even the most remote islands. This destructive fishing technique was already noted by Wijsman-Best et al. (1981) in several reefs. A single blast is capable of destroying several square meters of coral reef leaving the surrounding corals vulnerable to longer-term periods (Moll 1983). Furthermore, this technique kills innumerable target and non-target reef inhabitants changing or even destroying the reef community (Steer and Walton 2003).

The collection of fish by poisoning is the predominant method for the capture of live food and ornamental fish in Southeast Asia (Burke et al. 2002). The main component used in this method is the anesthetic sodium cyanide. In contact with this chemical the fish can become easier to capture not only for the divers but also for their predators. When in contact with the cyanide, corals can bleach or even completely die (Burke et al. 2002).

Due to the export of 1000 tons of coral per year in the early 1990s and 500 tons per year in 2001 the Indonesian archipelago was considered, by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the world's largest exporter of corals. Indonesia itself was responsible for 41% of all coral exports worldwide since 1985 (Spalding et al. 2001).

Coral bleaching events have been related to temperature anomalies (particularly related to severe ENSO events) but also with UV radiation (e.g., Jokiel and Coles 1990; Brown, 1997; Ayoub et al. 2009). During severe temperature anomalies coral can expulse their endosymbionts leading to partial or full colony mortality. Lesser (1997) noted that in corals exposed to elevated temperatures zooxantellae produce high concentrations of superoxide radicals and hydrogen peroxide that diffuse through biological membranes into coral tissue, thereby resulting in an increased level of oxidative stress in the host. In order to avoid these high concentrations of oxygen radicals, corals expel their endosymbionts. When this happens corals lose their typical pigmentation and are left in a bleached (white) state. The reduction of the ozone layer and the consequent increase in UV radiation has

been subjecting marine environments and more particularly coral reefs (smaller zenith angle regions) to higher rates of stress. Coral bleaching, DNA damage, productivity reduction and consequent alterations in species diversity have been associated with UV stress (Gleason and Wellington 1993; Hader et al. 2007; Kuwahara et al. 2010) while photooxidative stress (related to coral diseases) have been linked with high photosynthetically active radiation (PAR) (Ayoub et al. 2012).

2.4. Remote sensing

Obtaining all the environmental information necessary to monitor key physical parameters influencing the coral reefs condition is almost impossible without the use of remote sensing. Remote sensing allows the detection of spatial and temporal patterns, very important in terms of reef management, but practically and financially prohibitive to collect manually (Eakin et al. 2010). Satellite-based ocean color instruments provide valuable derived data products (e.g., chlorophyll, remote sensing reflectance, colored dissolved organic matter index) that can be used as proxies for important coral reefs threats, namely eutrophication, sedimentation and runoff. The ocean color is essentially defined by the inherent optical properties (IOP) of the water constituents (*i.e.*, absorption, scattering and backscattering coefficients).

The result of the scattering and absorption of the light by the pure water is the emergence of a blue color (Martin 2004). Since the pure water forms a constant background optical property, its contribution to the water-living signal is not taken into account (IOCCG 2000). Each of the below described seawater constituents constitute a source of color.

2.4.1. Living organisms

Virus and bacteria are the smallest organisms in this group, with sizes varying between 10 nm to 1µm. These characteristics along with the fact that they tend to co-vary with phytoplankton led, for reasons of simplicity, to their incorporation in the phytoplankton fraction (Martin 2004). However, recent studies have demonstrated that these organisms could have a strong role in the back-scattering observed in phytoplankton (Morel and Ahn 1991; Stramski and Kiefer 1991; Ulloa et al. 1992 in IOCCG 2000).

Phytoplankton, like the Greek origin of the name suggests, are free-floating (plankton) plants (phytoe) living in the oceanic euphotic layer and producing carbohydrates through photosynthesis - a process where carbon dioxide is transformed in organic compounds using sunlight energy. They represent the basis of the oceanic and freshwater food web. In an optical point of view, with sizes greater than the visible wavelengths (2 to 200 µm) (Martin 2004), phytoplankton are detected through their main light harvesting pigment - chlorophyll-a (hereafter Chlor_a) (Richardson and LeDrew 2006). Despite the existence of other pigments in phytoplankton cells such as chlorophyll b, c and caratenoids, Chlor_a is the only one present in all phytoplankton species, and is therefore, used as an index of biomass (Martin 2004). Chlorophyll-a absorb specially at 440nm and at 665nm and present an absorption close to zero between 500 and 550 giving to the waters with high Chlor_a concentrations a greenish color. In addition to these patterns, the absorption peak of Chlor_a near 665 nm is three times shorter than the absorption peak near 440 nm and at 683 nm Chlor_a has a fluorescence emission peak (Martin 2004).

The next trophic level in the food chain of aquatic ecosystems *i.e.,* zooplankton (the second trophic level), fishes and mammals (the third and further trophic levels) despite having sizes ranging from 100 µm to 10 m, occur at such small concentrations that their impact on the absorption or scattering can be considered negligible (Martin 2004).

2.4.2. Not living organisms:

The colored dissolved organic material (hereafter CDOM) mainly consists of humic and fulvic substances that resulted either from decaying land plant material (or originating from mangroves) or by the degradation of phytoplankton by grazing (carried out by zooplankton) or photolysis (Martin 2004; IOCCG 2000; Richardson and LeDrew 2006). These substances are present in greater concentrations in coastal and inland waters. CDOM absorbs strongly in the blue and ultraviolet region (presenting a major absorption peak at 410 nm) and gives a yellow (brownish) color to the water. This explains why these substances are also called "yellow substances" or “gelbstoff” ('yellow material' in German) (Martin 2004).

The suspended inorganic particulate matter (inorganic tripton) consists of clays and sand (1µm-10µm in size) resulting from the resuspension of bottom sediments by wave action or transported by river run-off (IOCCG 2000; Martin 2004; Richardson and LeDrew 2006).

Inorganic tripton has a low absorption and a strong scattering behavior (Richardson and LeDrew 2006) giving normally a brownish yellow color to the water (Martin 2004).

The suspended organic particulate matter (detritus; organic tripton) is a result of cell fragmentation of both phyto and zooplankton and the fecal pallets of the latter (Martin 2004). The organic tripton scattering/absorption behavior is very similar to the CDOM and its size is similar to the phytoplankton albeit without their chlro-a absorption proprieties (Richardson and LeDrew 2006).

In 1977, Morel and Prieur divided oceanic - water into two different types concerning their color: case I and case II waters.

Case I waters (also called "blue") are those waters where the ocean color is, mainly, determined by the phytoplankton concentration since it is the component with major influence on the water optical properties. Despite the existence of other substances in this kind of waters their proportion is very low and is directly related to the phytoplankton, co-varying with it (*i.e*., elements from decomposing phytoplankton debris). Conversely, in case II waters the phytoplankton co-exists in similar concentrations with many other substances that are not directly related to it and thus vary in an independent way (mainly organic and inorganic particles in suspension) (IOCCG 2000).

Ninety percent of the oceans waters fit into the characteristics of the case I waters and are mostly represented by the open ocean waters (Richardson and LeDrew 2006), while case II waters encompass all the other water bodies which can not be characterized as case I waters, or in other words, those waters close to land masses (coastal waters, lakes, estuaries, rivers) (IOCCG 2000).

In case II waters the entire signal received by the sensor is the result of the summation and interaction of the optical water-leaving signal of many factors other than the phytoplankton concentration. What distinguishes case II from case I waters results primarily from three aspects: biological richness (very high concentration of both phytoplankton and many other bio-optically active organisms such as macroalgae, invertebrates etc.); proximity to land (high concentrations of sediments derived from natural river runoff, and dissolved matter resulting from several anthropogenic sources of pollution); and, the possibility of finding areas of shallower depths (*i.e.,* with higher signal magnitude impacts generated by bottom reflectance and by the resuspension of bottom particles) (Richardson and LeDrew 2006).

The contribution of all these factors to the optical water leaving signal of these waters requires a careful analysis in order to infer the weight of each one of them in the final magnitude of the signal and thus avoid the overestimation of phytoplankton (Chlor_a) concentrations (Richardson and LeDrew 2006).

2.4.3. Ocean-atmosphere climate phenomena affecting Indonesia

Indonesia is positioned in the middle of two different continents (Asia and Australia) and two different oceans (Pacific and Indian). No other place in the world, at this low latitude, allows the communication between two different oceans (Kinkade et al. 1997; Susanto et al. 2006).

This geographic position places this archipelago under the influence of various temporal and spatial ocean-atmosphere climate phenomena namely: monsoons (Susanto and Marra 2005); ENSO - El Niño/ La Niñã in the tropical Pacific (Susanto and Marra 2005) and Indian Ocean Dipole - IOD in the Indian Ocean (Susanto and Marra 2005). All these ocean-atmosphere climate phenomena exert a strong impact on the Indonesian climate namely in the precipitation and wind patterns which in turn have a strong influence on important parameters for coral reefs like chlorophyll and sea surface temperature (Susanto et al. 2006).

2.4.4. Asia - Australia Monsoon System

The strong monsoon system felt in this archipelago is due to pressure differences between Asia and Australia. During the wet season (October to March) warm and moist air is transported to the region, as result of the westerlies developed by the formation of a high pressure over Asia - Northwest monsoon. During the dry season (April to September) the opposite happens and the development of a high pressure over Australia leads to the formation of warm and dry easterlies over the region - Southeast monsoon (Susanto and Marra 2005; Susanto et al. 2006). According to Susanto et al. (2006), March, April, May and September, October, November should be considered transition months whereas the months June, July, August and December, January, February should be considered the peaks of the Southeast monsoon and Northwest monsoon, respectively.

2.4.5. ENSO

In normal years, the western Pacific Ocean is warmer than the eastern and the center of atmospheric convection is over Indonesia. This is the result of a normal Walker circulation *i.e*. east-west atmospheric circulation (Sprintall et al. 2003).

During an El Niño episode an alteration of the normal SST pattern in the eastern Pacific triggers a weakening or even a reversal (depending on the intensity) of the Walker circulation; as a result, the atmospheric convection moves eastward and becomes stronger in the central Pacific. This leads to the formation of anomalous surface southeasterlies. If this episode occurs during the dry season these southeasterlies occur with the easterlies typical of the Southeast monsoon and the wind strength is further intensified. These strong southeasterlies reach Indonesia, and as consequence, the archipelago is surrounded by cool surfaces waters and is affected by drought conditions. This cooling further reduces the Walker circulation enhancing its effects. Conversely, during the wet season the Northwest monsoon wind system is reverse (eastward) to the southeasterlies generated during the El-Ninõ. The wind speed is thus attenuated and all the effects of the El-Niño are smoothed (Hendon 2003).

The occurrence of the La Niña phase of the ENSO, in opposition to the El-Niño phase, results in the strengthening of the Walker circulation (Lau and Yang 2003) and the subsequent anomalous surface westerlies (Hendon 2003). If this episode occurs during the dry season the anomalous westerlies act to decrease the local easterlies speed. This results in high precipitation rates and water temperatures over Indonesia that will further enhance the Walker circulation (Hendon 2003; Sprintall et al. 2003)**.** Conversely, the westerlies developed during the wet season (Northwest monsoon) enhance the westerlies generated during the La Niña and the positive SST and precipitation anomalies are smoothed (Hendon 2003).

2.4.6. Indian Ocean Dipole

During the Indian Ocean Dipole (IOD hereafter) opposed temperatures occur in the west and east part of Indian Ocean. In normal years, the atmospheric convection is placed over the eastern Indian Ocean and the wind blows eastward. During the positive IOD event the convection configuration changes to the opposite (west) and as a result anomalous wind easterlies are produced and subsequently a cold SST is found in the eastern and a warm SST in the western Indian Ocean (Sprintall et al. 2003; Webster et al. 1999). Under these

conditions, Indonesia is affected by cold SST and suppressed rainfall. Conversely, a negative IOD period is characterized by warmer than normal SST in the eastern Indian Ocean (near South Sumatra and Java) and cooler than normal water in the west part of the Indian Ocean. As result, high precipitation and SST affect Indonesia (mainly South Sumatra and Java).

2.4.7. Repercussions on Indonesian ocean color

According to Susanto et al. (2006), in a study that used both *in situ* and satellite derived data, highest chlorophyll concentrations are generally observed in the eastern side of Indonesia and during the Southeast monsoon. During the latter event, the typical easterlies have the strongest impact in the Java-Nusa Tenggara Islands (defined as the set of Java, Lombok, Sumbawa, Flores, Sumba, and Timor islands). Here, the easterlies induce upwelling along southern coasts (high satellite derived chlorophyll concentrations) and downwelling along northern coasts (low satellite derived chlorophyll concentration). The same occur in the eastern Banda Sea. As a consequence, low temperatures are observed over these areas. These effects are enhanced by a positive phase of both ENSO and IOD. During an EL Ninõ episode the stronger easterlies and latitudinal changes in the Coriolis parameter extend this plume of high satellite derived chlorophyll concentration northwestward along the Sumatra coast (Susanto 2001; Susanto and Marra 2005).

During the Northwest monsoon high values of chlorophyll are detected in the Malacca Strait, northern and eastern Kalimantan, north and south of the Makassar Strait, Flores Sea and north of the Java-Nusa Tenggara island chain. Conversely, the satellite derived chlorophyll concentrations decay in the eastern Banda Sea and south of the Java-Nusa Tenggara island chain.

High satellite derived chlorophyll concentrations are also detected in the shallow coastal zone of Kalimantan. However, this is likely the result of frequent rainfall and the consequent strong river discharge that is normal in this zone during the wet season. The high concentration of nutrients, inorganic particulate material and colored dissolved organic matter discharged by the local rivers can lead to over-estimations in the satellite derived chlorophyll concentrations (Susanto et al. 2006).

Regarding SST, during the Southeast monsoon cooler SST is observed to the south of the equator, while during the Northwest monsoon cooler SST is observed to the north of the equator, more specifically, in the South China Sea. However, during the Northwest monsoon the reduction in temperature in the South China Sea is not concomitant with the increase in the satellite derived chlorophyll concentrations (Susanto et al. 2006).

2.5. Microbial Ecology

Coral reef environments have been the subject of several cross-shelf distribution and diversity studies (Cleary et al. 2006; Rachello-Dolmen and Cleary 2007). However, most of these studies have focused on *Eukarya* taxa (e.g., corals, fishes, sponges, foraminifera) and there have been relatively few studies of prokaryotes despite their importance on marine food webs and geochemical cycling (Azam and Malfatti 2007; Rodriguez-Brito et al. 2010).

Microbes are the most abundant and diverse group of organisms on Earth. In coral reefs they are present in non-host (seawater and sediment) and host biotopes (e.g., corals, sponges) (DeLong 1994; Hentschel et al. 2002; Wild et al. 2006; Rosenberg 2007). Coral reefs are typically oligotrophic environments which, during the lasts decades have been subjected to increasing anthropogenic pressure, particularly inputs of land based organic matter. The transformation and mineralization of this organic matter (e.g., primary production and nitrification) is essentially mediated by microbes (Herbert 1999). These are present in the different coral reef compartments and are extremely important in both oligotrophic and eutrophic coral reefs.

Of all marine organisms, marine sponges (e.g., Figure 2.5.1) are probably the most common hosts for microbial communities (Turque et al. 2010). Sponges are very abundant benthic invertebrates in coral reef systems where they are usually attached to solid substrates (rock, sediment, and coral).They feed by filtering organic particles from the water. Although sponges may also feed on microbes (Lee et al. 2001; Hentschel et al. 2006) they harbor a remarkable variety of microorganisms in their tissues. Very little is known about this relationship (Lee et al. 2001; Hentschel et al. 2002; Holmes et al. 2007); however, it is generally believed that this interaction has a symbiotic nature and could result in the development of a resistance by the microorganisms to the sponge digestion and/or of sponge capacity of selective absorption (Hentschel et al. 2002; Turque et al.

2010). Microorganisms contribute to sponge nutritional process, metabolic waste processing, offer a more stable skeleton and a more efficient defensive system (*i.e.,* protection from ultraviolet light, chemical and predators). Sponges in turn offer a safe, stable and nutrient-rich habitat to the microorganisms (Hentschel et al. 2002; Holmes et al. 2007).

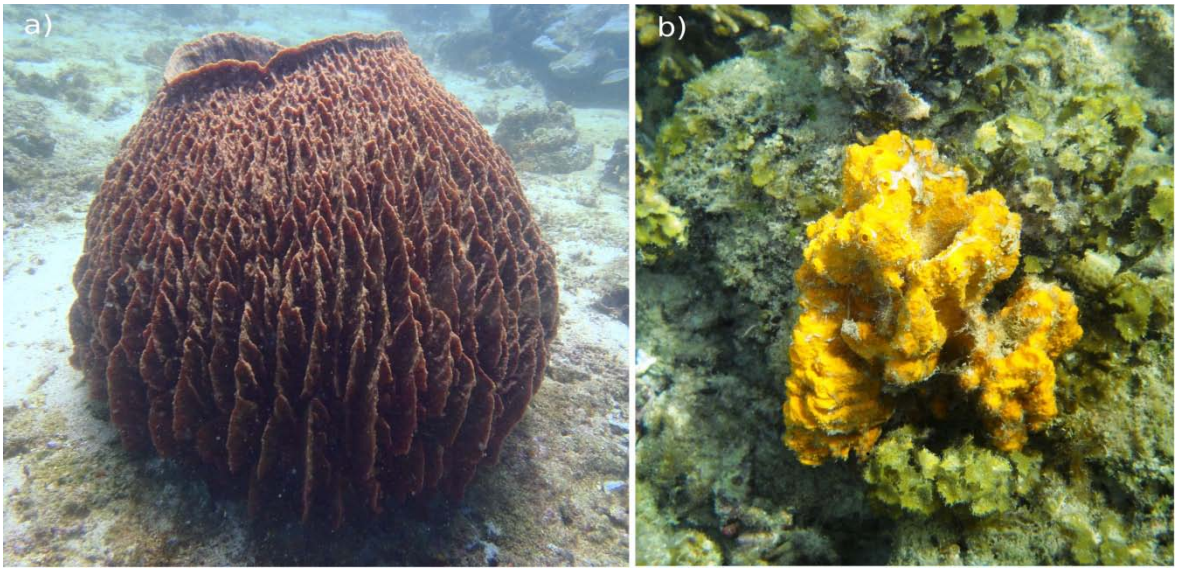

Figure 2.5.1 - Marine sponges: a) *Xestospongia testudinaria* (Photographer: Rossana Freitas); b) *Stylissa massa* (Photographer: Ana R. M. Polónia).

2.5.1. Sponge-associated microbe-host interactions

Sponges living in the same habitat can greatly differ in the abundance of their associated microorganisms. The term 'high microbial abundance' sponges (HMA) represents sponges with microbial abundances of about 10^{10} cells per gram wet weight of sponge (orders of magnitude higher than concentrations in seawater); while the 'low microbial abundance' sponges (LMA) exhibit densities of about 10^{6} cells per gram (similar to densities in seawater) (Kamke et al. 2010; Hentschel et al. 2006). This difference can represent distinct strategies: LMA sponges base their nutrition on filtering huge amounts of water and absorbing particulate organic matter, while HMA sponges have slower pumping rates and rely on their huge number of microorganisms to acquire dissolved organic matter (Weisz et al. 2007).

In addition to being abundant, the microbial community present in sponges has also been suggested to be uniform and taxon-specific. The existence of a "uniform sponge-specific microbial community" was proposed by Hentschel et al. (2002). This thesis is essentially

based on the fact that sponges from different species and/or locations share similar 16S rRNA gene sequences (uniform), and at the same time are distinctly different from non-sponge sources (specific). In recent years, several studies have shown that different host species (even from different locations) share the same sponge-specific microbial community that in turn was not found in the surrounding sediment or water column (Hentschel et al. 2002; Taylor et al. 2007; Schmitt et al. 2008; Kamke et al. 2010; Simister et al. 2012).

In other to understand the extent of uniformity between the microbiota of different sponges Schmitt et al. (2012) defined three microbial community categories: (i) the core microbial community, consisting of microbes found in at least 70% of the analyzed sponges; (ii) the variable microbial community, consisting of microbes found in more than one sponge and less than 70% of the analyzed sponges; and (iii) the species-specific community, consisting of microbes found in only one sponge. When analyzing the microbiota of five Mediterranean sponges, these authors found that the core, variable, and species-specific communities represented 2, 26, and 72% of all operational taxonomic units (OTUs) respectively. This means that the number of unique microbial species in a single sponge species surpasses by far the number of microbial species shared with other sponge species.

According to the same authors (Schmitt et al. 2012) each of the previously described OTU groups can be divided into ‘‘Plus-OTUs’’ and ‘‘Minus-OTUs’. A Plus-OTU represents a tag sequence that during taxonomic assignment, matches to a previously sponge-derived 16S rRNA gene sequence while a Minus-OTU represents a tag sequence that, in the database, matches to a non-sponge-derived 16S rRNA gene sequence. Schmitt et al. (2012) observed that more than 68% of all the sequences belonging to each microbial community group (core, variable and species-specific) were considered Plus-OTUs. The high proportion of Plus-OTUs means that even though each sponge might host unique bacterial species they should still be more similar to each other than to the microbial community of the surrounding non-sponge environment. This study supports the landmark study of Hentschel et al. (2002) entitled “Molecular evidence for a uniform microbial community in sponges from different oceans” and others studies (Taylor et al. 2005; Taylor et al. 2007).

However, some studies obtained different results (Webster et al. 2010 and Erwin et al. 2011). Webster et al. (2010), in a study of sponges from the Great Barrier Reef, found various sequence clusters previously reported as sponge-specific clusters in seawater

samples. Erwin et al. (2011), in turn, verified some bacterial community overlap between the surrounding seawater and the sponges *Hymeniacidon heliophila* (17.0%) and *Haliclona tubifera* (37.8%). Webster et al. (2010) suggested that the conventional metagenome studies were limitated in their ability to detect OTUs belonging to the "rare biosphere" as a possible explanation for this phenomenon. According to these authors, seawater and sponges can, in fact, share some microbes; however their extremely low concentration in seawater makes it impossible to be detected with conventional molecular methods. This led to the assumption of nonexistence of overlapping sequences between seawater and sponges. The rare biosphere present in the surrounding seawater may not only result from sponge incorporation but also from sponge release (spawning or injury) (Schmitt et al. 2008; Webster et al. 2010).

Some studies also shown that sponges from different locations contain different microbial species (despite being closely related to each other). Schmitt et al. (2012), in a study of sponges from eight different locations around the world, reported that microbial communities present in subtropical sponges are more similar to each other than microbial communities present in tropical sponges.

Doubts concerning the concept of "uniform sponge-specific microbial community" have been raised. How this uniformity is maintained in successive generations is probably the main question. Three hypotheses have been proposed: vertical transmission, horizontal transmission and the combination of both. Under the vertical transmission scenario, parents transmit their symbiont microbiota to the next host generation. Over millions of years of evolution, this highly selective transmission through reproductive stages of symbionts should lead to a concerted speciation of both sponge and microbe (cospeciation) resulting in spatially and temporally stable associations. Recent embryo and larvae based studies reported vertical transmission of multiple, phylogenetically diverse microorganisms in a marine sponge (Sharp et al. 2007; Schmitt et al. 2008). In the horizontal transmission thesis, also known as environmental transmission, the hosts acquire their symbionts from the surrounding environment (probably through feeding). The local environment can also exert a selective pressure that can evolve in parallel with the host and form a uniformly shared microbial community (Schmitt et al. 2008). However, this is a time and space constrained uniformity since the symbionts genotypes are not transferred through generations (Vrijrnhoek et al. 2010). The combination of vertical and horizontal transmission combines the advantage of both systems: the vertical element will allow the preservation of the symbiont genotype for some generations while the horizontal component will allow the host to acquire local beneficial symbionts (Vrijenhoek et al. 2010).

In recent years some authors have suggested this mixed system of transmission as the most likely (Taylor et al. 2007; Schmitt et al. 2008; Webster et al. 2010).

However, most of this knowledge was obtained based on bacterial studies and very little is known about the sponge archaeal community.

2.6. References

Aerts L, Vansoest R (1997) Quantification of sponge/coral interactions in a physically stressed reef community, NE Colombia. *Marine Ecology Progress Series* 148: 125–134.

Anwar C (2004) Labour mobility and the dynamics of the construction industry labour market:(the case of Makassar, Indonesia): Cuvillier Verlag.

Augustinus P (2001) Sustainable management of the coastal area of SW Sulawesi, Indonesia. Kaleidoscopic view on social scientific global change research in the Netherlands: 99.

Ayoub L, Hallock P, Coble P (2009) Colored dissolved organic material increases resiliency of coral reefs by controlling exposure to UVR. *Proceedings 11th Internationa*l *Cora*l *Reef Symposium* 1: 579-583.

Ayoub LM, Hallock P, Coble PG, Bell SS (2012) Maa-Like absorbing substances in Florida Keys phytoplankton vary with distance from shore and CDOM: implications for coral reefs. *Journa*l *of Experimenta*l *Marine Biology and Ecology* 420: 91–98.

Azam F, Malfatti F (2007) Microbial structuring of marine ecosystems. *Nature Reviews Microbiology 5:* 782-791.

Badan Pusat Statistik Republik Indonesia (2009) Trends of the selected socio-economic indicators of Indonesia: October 2009. BPS-Statistics Indonesia.

Bailey C, Dwiponggo A, Marahudin F (1987) Indonesian marine capture fisheries: Intl Specialized Book Service Inc.

Becking LE, Cleary DFR, Voogd NJ, Renema W, Beer M, Soest RWM, Hoeksema BW (2006) Beta diversity of tropical marine benthic assemblages in the Spermonde Archipelago, Indonesia. Marine Ecology 27: 76-88.

Bell, PRF (1992) Eutrophication and coral reefs - Some examples in the Great Barrier Reef lagoon. Water Res. 26: 553–568

Browder J, Bohland JR and Scarpadi JL (1995) “Patterns of Development on the Metropolitan Fringe - Urban Fringe Expansion in Bangkok, Jakarta and Santiago”, in Journal of the American Planning Association, Vol. 61: No. 3.

Brown BE (1997) Coral bleaching: causes and consequences. *Cor*al *reefs 16:* S129-S138.

Burke L, Selig E, Spalding M (2002) Reefs at risk in Southeast Asia. World Resources Institute, Washington, DC.

Chozin M (2008) Illegal but common: life of blast fishermen in the spermonde archipelago, South Sulawesi, Indonesia Ohio University.

Cleary DFR, Suharsono, Hoeksema B (2006) Coral diversity across a disturbance gradient in the Pulau Seribu reef complex off Jakarta, Indonesia. Biodivers Conserv 15:3653–3674. doi: 10.1007/s10531-004-4692-y

Cleary DFR, Becking LE, Voogd NJ, Pires AC, Polonia AR, Egas C, Gomes N (2013) Habitat and host-related variation in sponge bacterial symbionts communities in Indonesian waters. FEMS Microbiol Ecol. doi:10.1111/1574-6941.12135

Cleary DFR, De Vantier L, Vail L, Manto P, de Voogd NJ, Rachello-Dolmen PG, Tuti Y, Budiyanto A, Wolstenholme J, Hoeksema BW et al. (2008) Relating variation in species composition to environmental variables: a multi-taxon study in an Indonesian coral reef complex. Aquat Sci 70:419–431. doi: 10.1007/s00027-008-8077-2

Cleary DFR, Becking LE, de Voogd NJ, Renema W, de Beer M, van Soest RWM, Hoeksema BW (2005) Variation in the diversity and composition of benthic taxa as a

function of distance offshore, depth and exposure in the Spermonde Archipelago, Indonesia. Estuarine, Coastal and Shelf Science 65: 557-570.

Cleary DFR, Polónia ARM, Renema W, Hoeksema BW, Wolstenholme J, Tuti Y, de Voogd NJ (2014) Coral reefs next to a major conurbation: a study of temporal change (1985− 2011) in coral cover and composition in the reefs of Jakarta, Indonesia. *Marine Ecology Progress Series 501*: 89-98.

Cybriwsky R, Ford L R (2001) City profile: Jakarta. *Cities 18:* 199-210.

de Voogd NJ, Cleary DFR, Hoeksema BW, Noor A, van Soest RWM (2006) Sponge beta diversity in the Spermonde Archipelago, SW Sulawesi, Indonesia. Marine Ecology-Progress Series- 309: 131.

De'ath G, Fabricius K (2010) Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecological Applications 20: 840e850

DeLong E, Wu K, Prézelin B, Jovine R et al. (1994) High abundance of Archaea in Antarctic marine picoplankton. Nature 371:695–697. doi: 10.1038/371695a0

De Vantier L, Suharsono, Budiyanto A, Tuti J, Imanto P, Ledesma R (1998) Status of coral communities of Pulau Seribu, 1985 – 1995. In: Soemodijhardjo S (ed) Contending with global change. Proceedings: coral reef evaluation workshop Pulau Seribu, Jakarta, Indonesia. 11 – 20 September 1995.UNESCO, Jakarta, Indonesia, pp 1 – 24.

Eakin CM, Nim CJ, Brainard RE, Aubrecht C, Elvidge C, Gledhill D K et al. (2010) Monitoring coral reefs from space. Oceanography 23: 118.

Edinger EN, Jompa J, Limmon GV, Widjatmoko W and Risk MJ (1999) Reef degradation, coral biodiversity and reef management in Indonesia. *Indonesian Journa*l *of Coasta*l *and Marine Resources 2*: 1-16.

Edinger EN, Jompa J, Limmon GV, Widjatmoko W, Risk MJ (1998) Reef degradation and coral biodiversity in indonesia: Effects of land-based pollution, destructive fishing practices and changes over time* 1. Marine Pollution Bulletin 36: 617-630.

Erftemeier PLA (1993) Factors Limiting Growth and Production of Tropical Seagrasses: Nutrient Dynamics in Indonesian Seagrass Beds. Ph.D. Thesis, University of Nijmegen.

Erwin P, Olson J and Thacker R (2011) Phylogenetic diversity, host-specificity and community profiling of sponge-associated bacteria in the northern gulf of Mexico. PloS one 6: e26806. doi: 10.1371/journal.pone.0026806.

Farhan AR, Lim S (2012) Vulnerability assessment of eco - logical conditions in Seribu Islands, Indonesia. Ocean Coastal Management 65: 1−14.

Firman T (1997) Land conversion and urban development in the northern region of West Java, Indonesia. Urban Studies 34:10271046.

Firman T (2004) Major issues in Indonesia's urban land development. *Land Use Policy 21*: 347-355.

Firman T, Kombaitan B, Pradono P. (2007) The Dynamics of Indonesia's Urbanisation, 1980–2006. Urban Policy and Research 25: 433-454.

Farida H, Severin JA, Gasem MH, Keuter M, Wahyono H, van den Broek P et al. (2014) Nasopharyngeal Carriage of Streptococcus pneumonia in Pneumonia-Prone Age Groups in Semarang, Java Island, Indonesia. *PloS one 9:* e87431.

Glaser M, Baitoningsih W, Ferse SCA, Neil M, Deswandi R (2010) Whose sustainability? Top-down participation and emergent rules in marine protected area management in Indonesia. Marine Policy 34: 1215-1225.

Gleason D, Wellington G (1993) Ultraviolet radiation and coral bleaching. Nature 65: 836-8.

Häder DP, Kumar HD, Smith RC and Worrest RC (2007) Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemic*al *and Photobiologic*al *Sciences* 6: 267-285.

Hendon HH (2003) Indonesian rainfall variability: Impacts of ENSO and local air-sea interaction. Journal of Climate 16: 1775-1790.

Hentschel U, Hopke J, Horn M, Friedrich A, Wagner M, Hacker J, Moore B (2002)

Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microb 68:4431–4440.

Hentschel U, Usher K, Taylor M (2006) Marine sponges as microbial fermenters. FEMS Microbiol Ecol 55: 167–177.

Herbert RA (1999) Nitrogen cycling in coastal marine ecosystems. FEMS Microbiology Reviews 23: 563-590.

Hoeksema B (1990) Systematics and ecology of mushroom corals (Scleractinia: Fungiidae): Rijksuniversiteit te Leiden.

Hoeksema BW (2012) Distribution patterns of mushroom corals (*Scleractinia*: *Fungiidae*) across the Spermonde Shelf, South Sulawesi. *Raffles Bulletin of Zoology* 60: 183-212.

Holmes B, Blanch H (2007) Genus-specific associations of marine sponges with Group I *Crenarchaeotes*. Mar Biol 150:759–772. doi: 10.1007/s00227-006-0361-x

IOCCG Report Number 3 (2000) Remote sensing of ocean colour in coastal and other optically-complex waters. p.140. In International Ocean Colour Coordinating Group, ed. S. Sathyendranath

Jokiel PL and Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. *Coral reefs 8:* 155-162.

Kamke J, Taylor MW and Schmitt S (2010) Activity profiles for marine sponge-associated bacteria obtained by 16S rRNA vs 16S rRNA gene comparisons. *The ISME Journa 4*: 498-508.

Kinkade C, Marra J, Langdon C, Knudson C, Ilahudet AG (1997) Monsoonal differences in phytoplankton biomass and production in the Indonesian Seas: tracing vertical mixing using temperature. Deep Sea Research Part I: Oceanographic Research Papers 44: 581-592.

Kuwahara VS, Nakajima R, Othman BHR, Kushairi MRM, Toda T (2010) Spatial variability of UVR attenuation and biooptical factors in shallow coral-reef waters of Malaysia. *Cora*l *Reefs* 29: 693–704.

Lau K, Yang S (2003) Walker circulation. Encyclopedia of Atmospheric Sciences: 2505–2510.

Lee Y, Lee J, Lee H (2001) Microbial symbiosis in marine sponges. J Microbiol 39: 254–264.

Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Cora*l *reefs 16:* 187-192.

Martin S (2004) *An introduction to ocean remote sensing*. Cambridge University Press.

Moll H (1983) Zonation and diversity of Scleractinia on reefs off SW Sulawesi, Indonesia: Leiden.

Morel A and Prieur L, (1977) Analysis of variations in ocean color. Limnol. Oceanogr. 22:709-722.

Nadarajah M, Yamamoto AT (Eds.) (2007) *Urban crisis: culture and the sustainability of cities*. United Nations University Press.

Nas PJM, Nas P (2003) The Indonesian town revisited: LIT Verlag Münster.

Nurkin B (1994) Degradation of mangrove forests in South Sulawesi, Indonesia. Hydrobiologia 285: 271-276.

Ongkosongo, OSR (1986) Some harmful stresses to the Seribu coral reefs, Indonesia. In: Soemodihardjo S (ed) Proceedings of MAB-COMAR Regional Workshop on coral reefs Ecosystems: Their management practices and Research/Training needs, Bogor, UNESCO and LIPI, Jakarta, pp 133–142.

Pet-Soede C (2000) Options for co-management of an Indonesian coastal fishery. PhD Thesis, Wageningen Agricultural University.

Pet-Soede C, Machiels M, Stam M, Van Densen W (1999) Trends in an Indonesian coastal fishery based on catch and effort statistics and implications for the perception of the state of the stocks by fisheries officials. Fisheries Research 42: 41-56.

Pet-Soede L, Erdmann MV (1998) Blast fishing in southwest Sulawesi, Indonesia. Naga, the ICLARM Quarterly April-June: 4-9.

Population Reference Bureau (2006) Washington, DC 20009-5728 USA.

Rachello-Dolmen P, Cleary DFR (2007) Relating coral species traits to environmental conditions in the Jakarta Bay/Pulau Seribu reef system, Indonesia. Estuar Coast Shelf S 73:816–826. doi: 10.1016/j.ecss.2007.03.017.

Rees JG, Setiapermana D, Sharp VA, Weeks JM and Williams TM (1999) Evaluation of the impacts of land-based contaminants on the benthic faunas of Jakarta Bay, Indonesia. *Oceanologica Acta 22:* 627-640.

Renema W (2010) Is increased calcarinid (foraminifera) abundance indicating a larger role for macro-algae in Indonesian Plio-Pleistocene coral reefs? *Cor*al *reefs 29*: 165-173.

Renema W, Troelstra S, (2001) Larger foraminifera distribution on a mesotrophic carbonate shelf in SW Sulawesi (Indonesia). *P*al*aeogeography P*al*aeoclimatology P*al*aeoecology* 175, 125+.

Richardson LL, LeDrew EF (2006) Remote sensing of aquatic coastal ecosystem processes. *Science and Management Applications*, 9.

Rodriguez-Brito B, Li L, Wegley L, Furlan M, Angly F, Breitbart M, et al. (2010) Viral and microbial community dynamics in four aquatic environments. *The ISME journal 4:* 739-751.

Rogers CS (1990) Responses of coral reefs and reef organisms to sedimentation. *Marine ecology progress series. Oldendorf 62:* 185-202.

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362. doi: 10.1038/nrmicro1635.

Sano A (2000) Social actors in the global market: socio-economic impacts of shrimp aquaculture in South Sulawesi, Indonesia. *ISS Working Paper Series/General Series*, *316*, 1-70.

Sattar A, Rashid M, Mat R, & Puji L (2012) A Preliminary Survey Of Air Quality In Makassar City South Sulawesi Indonesia. Jurnal Teknologi 57.

Schmitt S, Angermeier H, Schiller R, Lindquist N, Hentschel U (2008) Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. Appl Environ MicroB 74: 7694–7708.

Schmitt S, Tsai P, Bell J, Fromont J, Ilan M, Lindquist N et al. (2012) Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *The ISME Journ*al *6*: 564-576.

Sharp KH, Eam B, Faulkner DJ and Haygood MG (2007) Vertical transmission of diverse microbes in the tropical sponge *Corticium sp*. *Applied and Environmental Microbiology 73*: 622-629.

Simister RL, Deines P, Botté ES, Webster NS, and Taylor MW (2012) Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. *Environment*al *Microbiology 14:* 517-524.

Spalding M, Ravilious C, Green EP (2001) *World atlas of coral reefs*. Univ of California Press.

Sprintall J, Potemra JT, Hautala SL, Bray NA, Pandoe WW (2003) Temperature and salinity variability in the exit passages of the Indonesian Throughflow. Deep Sea Research Part II: Topical Studies in Oceanography 50: 2183-2204.

Steer AD, Walton TE (2003) Indonesia Environment Monitor 2003 Special Focus: Reducing Pollution. Indonesia Air Quality Report. World Banak Indonesia Office.

Stramski D and Kiefer DA (1991) Light scattering by microorganisms in the open ocean. Prog.Oceanogr 28: 343-383.

Susanto R, Marra J (2005) Effect of the 1997/98 El Nino on chlorophyll a variability along the southern coasts of Java and Sumatra. Oceanography-Washington Dc-Oceanography Society 18: 124.

Susanto RD, Gordon AL, and Zheng Q (2001) Upwelling along the coasts of Java and Sumatra and its relation to ENSO. *Geophysic*al *Research L*e*tters 28:* 1599-1602.

Susanto RD, Moore II T, Marra J (2006) An ocean color variability in the Indonesian Seas during the SeaWifs Era. Geochemistry Geophysics Geosystems 7: Q05021.

Taylor M, Radax R, Steger D and Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol Mol Biol R 71: 295–347.

Taylor MW, Schupp PJ, De Nys R, Kjelleberg S, and Steinberg PD (2005) Biogeography of bacteria associated with the marine sponge *Cymbastela concentrica*. *Environment*al *Microbiology 7:* 419-433.

Troelstra S, Jonkers H, de Rijk S (1996) Larger foraminifera from the Spermonde Archipelago (Sulawesi, Indonesia). Scripta Geologica 113: 93-120.

Turque A, Batista D, Silveira C, Cardoso A, Vieira R, Moraes F, Clementino M, Albano R, Paranhos R, Martins O et al. (2010) Environmental shaping of sponge associated archaeal communities. PLoS One 5:e15774.

Umbgrove J (1930) De koraalriffen van den Spermonde Archipel (S. Celebes). Summary in English. Leidsche Geologische Mededeelingen 3: 228-247.

Umbgrove JHF (1929) The influence of the monsoons on the geomorphology of coral islands. In: Proceedings of the 4th Pacific science congress. Baks and Klits, Batavia-Bandoeng, Java, p 49–54.

UN (2002). Johannesburg Summit (2002) Indonesia Country Profile. United Nations Department of Economic and Social Aff airs. Division for Sustainable Development.

UNEP, 2005. Vantier, L. Wilkinson, C. Lawrence, D. and D. Souter (eds.) Indonesian Seas, GIWA Regional assessment 57. University of Kalmar, Kalmar, Sweden.

Vail L, Thamrongnawasawat T (1998) Echinoderms associated with coral reefs in Jakarta Bay and Kepulauan Seribu. In: Soemodijhardjo S (ed) Contending with global change. Proceedings: coral reef evaluation workshop Pulau Seribu, Jakarta, Indonesia. 11 – 20 September 1995. UNESCO, Jakarta, Indonesia, pp 55 – 65.

Verhey E (1993) Marine plants on the reefs of the Spermonde Archipelago, SW Sulawesi, Indonesia: aspects of taxonomy, floristics, and ecology. — Thesis Rijksherbarium, Leiden: 1-320.

Vrijenhoek RC (2010) Genetics and evolution of deep-sea chemosynthetic bacteria and their invertebrate hosts. In The Vent and Seep Biota (pp. 15-49). Springer Netherlands.

Webster N, Taylor M, Behnam F, Lucker S, Rattei T, Whalan S, Horn M, Wagner M (2010) Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. Environ Microbiol 12:2070–2082. doi: 10.1111/j.1462-2920.2009.02065.x.

Webster PJ, Moore AM, Loschnigg JP, Leben RR (1999) Coupled ocean–atmosphere dynamics in the Indian Ocean during 1997–98. Nature 401: 356-360.

Weisz JB, Hentschel U, Lindquist N, and Martens CS (2007) Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. *Marine Biology* 152: 475-483.

West Java Office of Central Board of Statistics (2001) Population of West Java: results of 2000 Population Census', Bandung.

Wijsman-Best M, Moll H, De Klerk L (1981) Present status of the coral reefs in the Spermonde Archipelago (South Sulawesi, Indonesia). Pages 263-267.

Wild C, Laforsch C and Huettel M (2006) Detection and enumeration of microbial cells in highly porous carbonate reef sands. *Marine and Freshwater Research* 57: 415–420.

Winarso H (2011) Urban Dualism in the Jakarta Metropolitan Area. In Megacities (pp. 163-191). Springer Japan.

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Chapter 3: Importance of space and environment to explain the variation in the composition of coral reef taxa in the Spermonde Archipelago, Indonesia: a multitaxon study

Spatial and environmental predictors of coral reef beta diversity: a multi-taxon study in the Spermonde Archipelago, Indonesia

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3.1. Abstract

The spatial variation in the community composition of corals, sponges, foraminifera, bacteria and archaea was assessed at two depths (3 m and 12 m) in the Spermonde Archipelago, Indonesia. Our goal was to assess to what extent this variation could be explained by space (distance between transects) versus environmental variables. The environmental variables consisted of locally measured substrate variables (e.g., live coral cover) and variables derived from ocean colour satellite imagery, such as sea surface temperature (SST), Remote sensing reflectance at 645 nm (Rrs_645; a proxy for total suspended sediment), coloured dissolved organic matter index (CDOM; a proxy for riverine input) and chlorophyll-a (Chlor_a; the main light harvesting pigment of phytoplankton). The amount of total variation in composition explained by the environmental variables ranged from 0 – 74%. For all taxa, with the exception of sediment archaea, this variation was substantially greater for transects at 12 m depth as opposed to 3 m depth. Space explained a marginally significant amount of variation for sponges, corals, sediment bacteria and sediment archaea sampled at 3 m depth, and for sponges sampled at 12 m depth. The most important explanatory overall variable was CDOM, which explained significant amounts of variation in the composition of sponges and sediment bacteria and archaea at 3 m depth and sponges, forams and corals at 12 m depth. The composition of sediment bacteria and archaea was also significantly related to variation in substrate cover at 12 m depth. This reinforces the importance of maintaining a coral reef ecosystem with high substrate diversity in order to maintain microbial diversity. Moreover, these results highlight the importance of the environment in structuring community composition, but also suggest marked differences among different groups in how they respond to environmental processes.

Keywords: Environmental variables; Indonesia; Remote sensing; Spatial variables

3.2. Introduction

Coral reefs are among the most diverse and economically important marine ecosystems in the world (Hughes et al. 2010). They provide coastal defense, food, employment, building materials, bio-active compounds and areas of recreation (Burke et al. 2002). Additionally, they also function as marine hatcheries and refuge for a wide variety of marine organisms, many of which are commercially important (e.g., fish, shrimp, crabs, clams; Burke et al. 2002; Buddemeier et al. 2004). Coral reefs are usually also located in areas of increasing coastal development and subject to relatively high population growth rates (Bryant et al. 1998). This combination of factors threatens their existence and, as a result, the services they provide. Indeed, a majority of coral reefs are now considered vulnerable or seriously degraded (Burke et al. 2011). The latter includes the loss of reef structure, species, and shifts in their community composition (e.g., Bellwood et al. 2004; Graham et al. 2006), as the results of differences in responses of physiologically robust corals versus delicate but opportunistic species (Done et al. 2007), or shifting the balance in the interaction between competing organisms (McCook et al. 2001). For example, many fish species depend on the three dimensional structure provided by mature coral reefs (Moberg and Folke 1999; Pratchett et al. 2008). Losses of key structural components, such as branching Acropora species, can lead to the local extirpation of numerous dependent species (Patton 1994; Pratchett et al. 2008). Importantly, previous studies have shown that biodiversity loss can, in turn, adversely affect ecosystem functioning (Tilman 1999; Worm et al. 2006). Hence it is important to understand how diverse taxa respond to changes in reef environmental conditions.

Pronounced on-to-offshore environmental gradients, including gradients in salinity, depth, nutrients, sedimentation and pollution (Fabricius et al. 2005; Fox and Bellwood 2007; Cleary et al. 2005, 2008) determine community structure throughout the reef ecosystem. In addition to the above, community structure of coral reefs is also determined by storm damage, thermal stress, over-harvesting of grazers and predators, to mention just a few (Szmant 2002). All of these stressors have a distinct spatial component (Szmant 2002; Goatley and Bellwood 2013). High nutrient concentrations can positively affect the growth of phytoplankton, microalgae and bacterioplankton in the water column, and filter feeders and algae in the benthic realm; when severe, high nutrient concentrations can cause a pronounced reduction of the euphotic zone (Aerts et al. 1997; Szmant 2002). The interaction between coral and algal cover is counteracted by the intensity of herbivory (Hughes et al. 2007; Burkepile and Hay 2008). Sediments bound within the turf algal cover suppress fish herbivory, and in reefs with lower herbivore biomass and altered

sediment fluxes, the development of longer algal turfs may become more come (Goatley and Bellwood 2013). This can lead to smothering and reductions in recruitment of corals and other sedentary reef organisms (Erftemeijer et al. 2012).

Coral reefs are among the most productive and diverse ecosystems on earth. In addition to corals, coral reefs support a diverse array of other taxa including sponges, fishes, crustaceans, foraminifera, archaea and bacteria. Around 800 species of reef building corals can be found on coral reefs throughout the world (Burke et al. 2011). Corals are colonial organisms composed of several polyps attached to a limestone skeleton and living in close relationship with symbiotic algae (*zooxanthellae*). This association makes them the most important reef-builders by depositing calcium carbonate to build their skeletons (Sheppard et al. 1988). They help to provide the fundamental three dimensional structure, which largely contributes to reef complexity and diversity (Perry et al. 2012). Moreover, through their colonial structures, coral reefs alter the energy and circulation protecting near-shore environments, which act as nurseries and shelter for other taxa (Buddemeier et al. 2004).

Marine sponges are abundant and conspicuous components of coral reefs. They are sedentary benthic organisms, which are usually attached to rocky substrates and feed by filtering organic particles from the water. Due to their high diversity and biomass, these are ecologically important in reef environments (Diaz and Rützler 2001). Their significance in benthic environments derives from the important functional roles they play: e.g., carbonate framework bioerosion (solid reef carbonate transformation into fine sediment), carbonate framework consolidation, stabilization and regeneration, bentho-pelagic coupling (sponges feed on pelagic ultraplankton and provide food for other pelagic organisms, e.g., fishes); habitat provision for numerous reef species; water column composition modification (through filtration and secondary metabolite emanation); and nutrient cycling including primary production and nitrification through complex microbial symbioses (Diaz and Rützler 2001; Bell 2008).

Foraminifera are small benthic invertebrates (protists) living in the topmost layer (0.5 cm) of sediment. Larger Benthic Foraminifera (LBF) are an important component of tropical shallow marine ecosystems, including coral reefs (Hohenegger 2006; Renema and Troelstra 2001). Similarly to zooxanthellate corals, LBF house photosymbionts in carbonate tests. Important differences are that LBF house a wider variety of symbionts, including diatoms, diniflagellates, rhodophytes, and chlorophytes, whereas cyanobacteria are present in many host as well (see review of Lee 2006). Like corals, foraminifera prosper in oligotrophic warm waters and host a distinct symbiont community (Oliveira-

Silva et al. 2012). LBF are sensitive to changes in water quality in coral reefs, but this has been documented largely by studying assemblage composition (Renema and Troelstra 2001; Uthicke and Nobes 2008; Schueth and Frank 2008). Unveiling the underlying physiology of this response is only recently starting, using both in situ and laboratory experimental approaches (e.g. Ziegler and Uthicke 2011; Nobes et al. 2008; Reymond et al. 2011). Due to their limited life span and reproductive cycle (Frontalini and Coccioni 2011), foraminifera respond faster than macrofauna to shifting environmental conditions (Hallock et al. 2003; Bouchet et al. 2012).

Bacteria and archaea are abundant members of the vast marine microbial community and are important players in processes such as the geochemical cycling of carbon, nitrogen and sulphur, transformation and degradation of nutrients and organic matter derived from both surface ocean production and terrestrial runoff (Lee et al. 2001; Webster et al. 2004). For oligotrophic coral reefs, this cycling activity is of crucial importance in order to degrade organic matter and maintain high levels of primary production (Schöttner et al. 2011). Coral reef carbonate sands, due to their complex surface structure and highly porous matrix, present a high abundance of prokaryotes (Wild et al. 2006). Coral reef sponges, in turn, have also been shown to harbour exceptional microbial densities, which can make up from 35 to 40% of sponge biomass (Vacelet 1975; Hentschel et al. 2002, 2012; Taylor et al. 2007).

Following the large ecological changes on Caribbean reefs and the realization that benthic community structure is to a great extent determined by the interplay between corals, algae, and herbivorous fishes, more interest was paid to these groups as well (e.g., Bellwood et al. 2004; Hughes et al. 2010; Roff and Mumby 2012). The conservation of coral reefs also tends to rely on indicators from these taxa. Yet, there has been little attention paid to other ecological groups inhabiting these same reefs (Plaisance et al. 2011; Hoeksema 2012b). It is unclear, however, to what extent variation in the composition of corals, for example, reflects that of other less studied taxa. The few studies that have assessed a diverse set of taxa have so far yielded mixed results. Karakassis et al. (2006), for example, found significant congruence between the community structure of macrofauna and megafauna, megafauna and fish and fish and microzooplankton. Beger et al. (2007) found significant congruence between fishes and corals and between corals and mollusks in Indo-Pacific coral reefs. In contrast, Sutcliffe et al. (2012), in a study of seabed assemblages, found that none of the taxonomic groups studied was a good surrogate for the others. In Moorea, a diverse set of taxa differed strongly with respect to their spatial compositional variation (Adjeroud 1997). Environmental variables explained large amounts of variation in sponges and corals, intermediate amounts in mollusks and much less variation in macroalgae and

echinoderms (Adjeroud 1997). Cleary et al. (2005) showed that distance to shore, depth and exposure to oceanic currents were important explanatory environmental variables for corals, sponges, foraminifera and sea urchins. Likewise, Cleary et al. (2008) showed that abiotic variables (e.g., temperature, heavy metal concentrations in seawater), habitat structure variables (e.g., algal cover and sand cover) and spatial factors explained more than 50% of the variation in composition of corals, echinoderms and fishes in the Jakarta Bay and Pulau Seribu reef system.

In the present study, we assessed taxon composition in the Spermonde coral reef system. Five different ecologically important taxa in coral reefs systems, *i.e.*, corals, sponges, foraminifera, bacteria and archaea, were sampled. Important environmental parameters such as coral reef habitat structure and remotely sensed data were used in this study to explain spatial variation in the composition of all taxa sampled. The remotely sensing data focused on four of the most important threats to coastal coral reefs: eutrophication (chlorophyll-a concentrations), bleaching (surface temperature), sedimentation (remote sensing reflectance at 645 nm) and runoff (colored dissolved organic matter index).

Our main goal was to relate variation in taxon composition sampled at different depths (3 and 12 m) to local substrate, the distance between sampling sites, and remotely sensed environmental parameters and to assess if different taxa respond similarly to these environmental and spatial variables.

3.3. Material and Methods

3.3.1. Study site

The Spermonde Archipelago is situated adjacent to the city of Makassar capital of the Indonesian province of South Sulawesi and home to more than two million inhabitants (Renema 2010). This reef system consists of 160 cay crowned reefs dispersed over 40 km of continental shelf (Renema and Troelstra 2001; Cleary et al. 2005). It lies on a carbonate shelf, which increases in depth with distance from the coast (Renema and Troelstra 2001; de Voogd et al. 2006; Hoeksema 2012a) (Figure 3.3.1). The westernmost islands lie on a rim beyond which shelf depth abruptly drops to depths exceeding 800 meters in the Makassar Strait (Moll 1983). Its proximity to Makassar, leaves these coral reefs exposed to many anthropogenic disturbances including river discharge

(sedimentation, agricultural runoff, eutrophication), oil spills, destructive fisheries, tourism and coral mining (Hoeksema 2004; de Voogd and Cleary 2007).

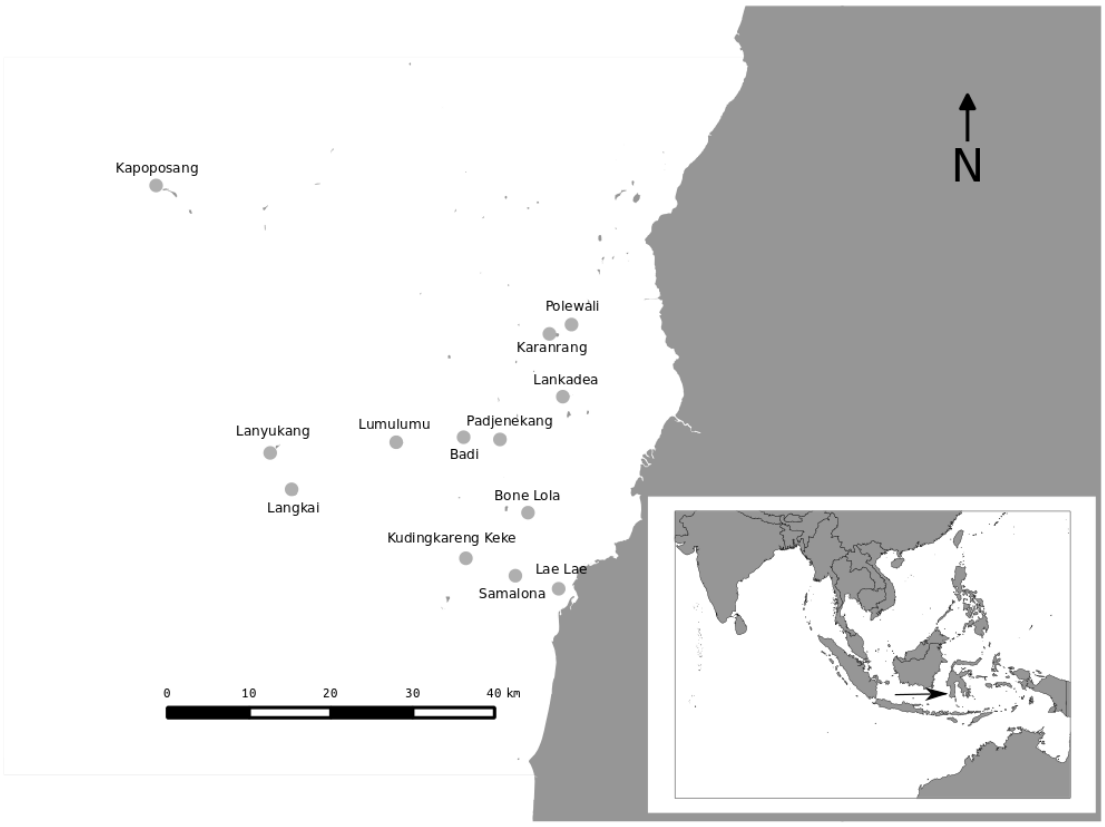

Figure 3.3.1 - Map of the study area showing the sampling sites.

Previous studies (Cleary et al. 2005; Cleary and Renema 2007; Hoeksema 2012a) showed that the Spermonde is subject to strong in-to-offshore gradients in both biotic and abiotic factors (e.g., salinity, depth, oceanic currents, pollution). Inshore reefs are also subject to more anthropogenic perturbations and environmental factors (e.g., river runoff) related to their proximity to the coast. The influence of these disturbances over the Spermonde Archipelago depends on factors such as distance from the coast, shelf depth, and season. During the wet season (Northwest monsoon from December to May), river runoff is stronger due to increased rainfall and the resultant plume of turbid water can reach a greater distance to the coast. During the dry season (Southeast monsoon from June to November) the easterlies are weakened by the high mountains of Sulawesi thus strongly reducing their impact and reducing cross shelf mixing when compared to the Northwest monsoon (Wijsman-Best et al. 1981).

3.3.2. Data collection

Research for the present study was carried out from the 8th to the 25th of August, 2010. A total of fourteen sites were surveyed using SCUBA (Figure 3.3.1). We sampled the northwest side of the reefs Lae Lae, Samalona, Kudingkareng Keke, Lumulumu, Bone Lola, Lankadea, Padjenekang, Polewali, Karanrang and Kapoposang, the west side of Langkai and Lanyukan reefs and the southwest side of Badi reef. These reefs were chosen because they are dispersed along an in-to-offshore gradient and are thus subjected to different environmental influences.

3.3.3. Corals

Coral genera were visually identified during intercept transect surveys (English et al. 1997). Photos of unrecognized genera were taken for closer examination using Veron 2000. In each site, we surveyed transects at two different depths: at the beginning of the reef slope (3 m) and at the maximum depth of coral cover (12 m, with exception of the reefs Karanrang (11 m), Lae Lae (9 m) and Polewali (10 m); for the sake of simplicity 12 m will be used throughout the text when referring to the deeper transects).

3.3.4. Habitat structure

Substrate cover was assessed using the 'life-form line intercept' method for surveys (Edinger and Risk 2000). For the purposes of this study, we assessed four distinct forms of substrate. These included the cover of live corals, dead corals, rubble and sand. The line intercept transect data was analyzed in order to calculate the percent cover of each of the previously mentioned life forms surveyed.

3.3.5. Sponges

Sponge species and their abundance were noted in $1m^2$ quadrats laid at each consecutive 1 m section along a 30 m transect line. Smaller (cryptic, boring and thinly encrusting < 4 cm) specimens were excluded from this study). Species were visually identified in the field, and fragments of unrecognized species were collected for closer examination. Voucher specimens are preserved in 70% ethanol and housed at the sponge collections Naturalis Biodiversity Center.

3.3.6. Foraminifera

Foraminifera were collected every three meters of depth in a transect starting at the reef base to the reef crest. However, for the purposes of this study only transects sampled at 3±1 m and 12±1 m were taken in consideration. On the reef slope a sample of the reef substratum was collected to a depth in the sediment/rubble that no larger benthic forams were observed. Afterwards the samples were washed to remove the foraminifera from the larger and heavier parts of coral rubble, dried, and further processed in the laboratory. Foraminifera larger than 500 µm were subjected to further study. Only samples with more than approximately 200 individuals of LBF were sorted out at and identified at species level using a stereomicroscope.

3.3.7. Microbes

In each site, sediment and sponge samples (when present) were collected. Sediment samples were taken using the mini core method. Mini-cores, consisting of the top 5 cm of sediment, were collected using a plastic disposable syringe from which the end had been cut in order to facilitate sampling (Capone et al. 1992). Twenty one fragments of *Stylissa massa* were collected, stored in 96% EtOH (Cleary et al. 2013a) and kept at temperatures lower than 4ºC immediately after collection. Once in the laboratory, samples were stored at -20 ºC until DNA extraction.

3.3.8. Environmental variables - Satellite data

Spermonde coral reef waters are characterized as Case II (*i.e.,* waters with optical properties that are typically controlled by three independent components: phytoplankton (including its main light harvesting pigment-clorophyll-a) and their associated debris; dissolved organic matters of terrigenous origin, known variously as yellow substance, Gelbstoff, Gilvin or CDOM; and mineral particles and various suspended sediments). Furthermore, the contribution of all these constituents for the optical water leaving signal of these waters demands a careful analysis in order to infer the weight of each one of them in the final magnitude of the signal and thus avoid the overestimation of phytoplankton concentrations (Richardson and LeDrew 2006).

In the present study, environmental variables including near-surface chlorophyll-a concentration (Chlor_a), sea surface temperature (SST), remote sensing reflectance at 645 nm (Rrs_645) and colored dissolved organic matter index (CDOM_index) were assessed for the study region using ocean color satellite imagery. Colored dissolved

organic matter index was used as a tracer of riverine inputs (Fichot 2013) and Rrs_645 as a proxy for total suspended sediments derived from land based erosion (Miller and McKee, 2004; Chen et al. 2007). CDOM is largely composed of humic and fulvic substances resulting either from decaying plant material brought by land run-off in areas with high vegetation productivity or originating from mangroves and seagrasses (Carder et al. 1999 in Martin 2004; Richardson and LeDrew 2006). MODIS band 1 (645 nm) with 250 m pixel resolution has been shown to perform well in coastal turbid waters (Franz et al. 2006; Chen et al. 2007) and Miller and Mckee (2004) showed a significant and robust linear relationship (r^2=0.89) between Rrs_645 and total suspended matter concentration. These components often lead to turbid waters, which can be highly reflective at the NIR bands producing considerable errors in the assessment of the derived products (Wang and Shi 2007). In the present study, we employed a SWIR atmospheric correction for deriving chlorophyll (Chlor_a) (Wang et al. 2007). The agreement between in situ chlorophyll values and MODIS in complex turbid waters is significantly improved when the SWIR algorithm is used (Franz et al. 2006). This satellite image treatment has been used in coastal waters with success in several studies (Chen et al. 2007; Lahet and Stramski 2010; Zhang et al. 2010).

Aqua Moderate Resolution Imaging Spectroradiometer (MODIS-Aqua) Level 1A LAC (1 km resolution) data were obtained from the NASA Goddard Space Flight Center through the Ocean Color web site (http://oceancolor. gsfc.nasa.gov/cgi/) and processed to Level 3 format using NASA's SeaWiFS Data Analysis System (SeaDAS version 7.0) software. The atmospheric correction used over the highly reflective coastal waters of the Spermonde Archipelago was made using the short wave infrared (SWIR) correction algorithm (Gordon and Wang 1994; Wang and Shi 2007).

Due to the satellites incapacity to measure ambient temperature at depth, the temperature values used for the analyses at 12 m and 3 m were the same – *i.e.*, the “skin” sea temperature. Our results should thus be interpreted as how the skin surface temperature influences variation in taxon composition at 3 m and 12 m depth. However, the main goal of this study was to use the remote sensed variables as proxies of environmental patterns of variation and not to quantitatively estimate the parameters in Case II waters. Since the accuracy of satellite data (compared to in situ data) tends to be higher with long-term averaging (Patt et al. 2003), time series of monthly mean data were generated. Mean values were generated for the previously mentioned satellite-derived parameters for the years 2008, 2009 and 2010. In order to avoid months with high cloud cover, only the months of June, July and August were analyzed in this study.

3.3.9. DNA extraction

We isolated PCR-ready genomic DNA from sediment and sponge samples using the FastDNA® SPIN Kit following the manufacturer's instructions. This is an extraction method frequently used for this purpose (Urakawa et al. 2010; Costa et al. 2013; Cleary et al. 2013a). Briefly, we prepared sediment samples by centrifuging each one for 30 min at 4400 rpm at 4 ºC and the sponge samples by cutting each fragment into small pieces. 500 mg of sediment and sponge were transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. The microbial cell lysis was performed in the FastPrep® Instrument (Q Biogene) for 80 seconds at the recommended speed. The extracted DNA was eluted into DNase/Pyrogen-Free Water to a final volume of 50 µl and stored at -20°C until use.

3.3.10. PCR amplification

Archaeal and bacterial 16S rRNA gene fragments were amplified for DGGE using a nested PCR assay (two consecutive amplification reactions). For the archaeal 16S rRNA gene amplification, the first PCR amplification was performed using DNA with archaea specific primers ARC344f-mod and Arch958R-mod (Pires et al. 2012). After a denaturation step at 94°C during 5 min, 30 thermal cycles of 1 min at 94°C, 1 min at 56°C and 1 min at 72°C were carried out followed by an extension step at 72°C for 7 min (Pires et al. 2012). The second PCR amplification was carried out using a dilution (1:25) of the amplicons from the first PCR with DGGE archaea specific primers 524F-10 and Arch958R-mod (GC) (Pires et al. 2012). After a denaturation step at 94°C during 5 min, 35 thermal cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C were carried out followed by an extension step at 72°C for 7 min. For the bacterial 16S rRNA gene amplification the first PCR amplification was performed from DNA using the F-27 and R-1494 primers (Gomes et al. 2008). After a denaturation step at 94°C for 5 min, 25 thermal cycles of 45 sec at 94°C, 45 sec at 56°C and 1:30 min at 72°C were carried out followed by an extension step at 72°C for 10 min. The second PCR amplification was carried out using a dilution (1:25) of the amplicons of the first PCR with DGGE primers F984-GC and R-1378 (Gomes et al. 2008). After a denaturation step at 94°C during 4 min, 25 thermal cycles of 1 min at 95°C, 1 min at 53°C and 1.30 min at 72°C were carried out followed by an extension step at 72°C for 7 min.

3.3.11. DGGE of 16S rRNA gene fragments

For DGGE analysis, we used the DCode Universal Mutation Detection System (Bio-Rad, Paris, France). A double gradient polyacrylamide gel (10–6% acrylamide) with a 50–58% denaturing gradient was loaded with PCR product (7µl for *Stylissa massa* sponge samples and 4µl for sediment samples) together with 5µl of DGGE loading buffer to the denaturing gradient gel electrophoresis (DGGE). The run of loaded gels was carried out at constant temperature (58ºC) and voltage (160 V) during 16 hours in a 1× Tris-acetate-EDTA buffer.

The DGGE gels were coloured using the silver staining method (Heuer et al. 2001), scanned and analysed with the BioNumerics Version 6.6 program (Applied Maths). The gel image was corrected for background noise (disk size=10%) and densitometric curves were extracted using an averaging thickness of 15 pts. The densitometric profiles were filtered using an arithmetic averaging and a least square filtering cut-off below 1.00%. DNA bands were automatically selected with band search parameters set at: 1.0% minimum profiling and a shoulder sensitivity of 2%. Based on the densiometric curves, a binary numerical band matching matrix with the numerical intensities of the bands was created and exported.

3.3.12. Cloning

The DGGE of archaea from *S. massa* only revealed a single band. We, therefore, decided to clone the PCR product. We added 0.25 µl of 10 mM dATP, and 0.5 unit of Taq polymerase to a composite of 5 archaeal *S. massa* PCR products. The resultant reaction was incubated at 70ºC for 20 min. These DNA fragments were purified with Gene clean II kit (MPbio) and subsequently cloned into the pGEM®-T Easy vector (Promega Corp. Madison, WI) according to the instructions provided by the supplier. The ligated plasmids were transformed into competent cells (*Escherichia coli* JM109; Promega) and plated on LB medium containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), isopropyl-β-D-thiogalactopyranoside (IPTG), and 100 µg/ml ampicillin. Plasmid DNA was isolated from white colonies, purified and sequenced in GATC Biotec (http://www.gatc-biotech.com). The obtained sequence data were compared with different sequences available in the GenBank database using blast-n search (http://www.ncbi.nlm.nih.gov/BLAST/). Partial 16S rRNA gene sequences generated in this study can be downloaded from the NCBI SRA: Accession number not yet available.

3.3.13. Analyses

All environmental data (including satellite and substrate data), spatial and species-by-transect abundance matrices were imported into R (R Core Team 2013). We did not import the matrix of archaea from *S. massa* because there was only a single band. Sequence analysis using BLAST revealed that the dominant archaeal symbiont present in *S. massa* was very closely related (99% - 100%) to an uncultured archaeon previously isolated from *Phakellia fusca* hosts in the South China Sea (Han et al. 2012). Given that depth explained significant amounts of variation in composition for various taxa in previous studies (Cleary et al. 2005; Cleary and Renema 2007), we analysed 3 and 12 m transects separately here in order to assess how taxa responded to environmental and spatial variables at different depths. All taxon data matrices were $\log_{10}$ (x+1) transformed and distance matrices constructed using the Bray-Curtis index with the vegdist() function in the vegan package (Oksanen et al. 2009) in R. The Bray-Curtis index is one of the most frequently applied (dis)similarity indices used in ecology (Legendre and Gallagher 2001; Cleary 2003; Becking et al. 2006; de Voogd et al. 2009). The environmental data matrices were $\log_{10}$ (x+1) transformed and distance matrices constructed using the Euclidean index with the vegdist function in the vegan package. In order to assess to what degree the variation in composition of taxa could be explained by spatial and environmental variables, we used permutational regression (Legendre et al. 1994). First, triangular distance matrices were unfolded to vectors using a modified function in R of the dist2sym function found in Venables and Ripley (Venables and Ripley 2002). We used the forward.sel function of the packfor package (Jombart et al. 2009), which selects significant explanatory variables based on a forward selection procedure and uses a permutation test to infer significant associations between taxon composition and spatial and environmental variables. The forward selection test used was based on a novel forward selection procedure that corrects for the inflated Type I error and overestimation of explained variance associated with classical forward selection (Blanchet et al. 2008). For those taxa in which variation in composition was explained both by spatial and environmental variables, we used variance partitioning (Borcard and Legendre 2002) with the varpart function in vegan to partition the total variation in community composition into a purely environmental fraction, a spatially structured environmental fraction, and a purely spatial fraction. Taxon diversity was assessed with rarefied species richness (S) (Gotelli and Colwell 2001) and Shannon's (H') diversity index (Shannon and Weaver 1949) using the diversity and specnumber functions of the vegan package.

3.4. Results

With the exception of sponge bacteria, which were only sampled at 3 m, all the taxa were most diverse at 12 m depth (Table 3.4.1).

Table 3.4.1 - Shannon's (H') diversity index and rarefied species richness (S) for corals, sponges, forams, sediment bacteria, sediment archaea and sponge bacteria from Badi (Bad), Bone Lola (Bon), Kapoposang (Kap), Karanrang (Kar), Kudingkareng Keke (Kud), Lae Lae (Lae); Langkai (Lan), Lankadea (Lnk), Lanyukang (Lny), Lumulumu (Lum), Padjenekang (Paj), Polewali (Pol) and Samalona (Sam) at both depths (3 and 12 m).

		Corals		Sponges		Forams		Sediment Bacteria		Sediment Archaea		Sponge Bacteria	
Reefs	Depth	H	S	H	S	H	S	H	S	H	S	H	S
Bad	3	1.518	13	---	---	---	---	3.505	51	2.627	35	2.877	33
Bad	12	1.597	18	3.028	31	0.798	6	---	---	---	---	---	---
Bon	3	1.804	18	2.452	20	---	---	3.545	53	2.959	29	2.913	38
Bon	12	2.827	23	3.267	60	1.550	12	3.496	55	3.101	40	---	---
Kap	3	1.707	13	1.385	11	---	---	3.557	52	2.900	32	2.977	38
Kar	3	2.315	23	2.135	21	1.247	12	3.514	55	2.908	29	3.019	34
Kar	12	2.843	29	3.408	44	2.414	38	3.569	54	3.097	33	---	---
Kud	3	1.597	9	2.753	26	0.738	6	3.528	50	2.847	29	2.936	39
Kud	12	2.794	30	3.511	57	1.538	12	3.551	55	3.037	35	---	---
Lae	3	2.121	15	1.855	10	1.465	12	3.636	53	2.822	30	3.178	43
Lae	12	1.791	7	3.197	38	0.964	14	3.492	50	3.217	41	---	---
Lan	3	1.773	12	---	---	---	---	3.352	46	2.849	33	2.898	37
Lan	12	2.395	16	3.135	43	1.784	25	3.435	47	3.077	34	---	---
Lnk	3	2.659	24	---	---	---	---	3.391	48	3.120	41	3.035	38
Lnk	12	2.830	25	---	---	2.171	27	3.546	52	2.777	28	---	---
Lny	3	1.983	11	---	---	---	---	---	---	---	---	---	---
Lny	12	2.405	18	---	---	---	---	---	---	---	---	---	---
Lum	3	1.448	9	---	---	1.523	15	---	---	---	---	3.176	44
Lum	12	2.350	24	3.066	35	1.934	18	3.514	53	3.168	39	---	---
Paj	3	2.535	20	---	---	0.738	8	3.405	49	2.847	33	3.098	41
Paj	12	2.266	27	---	---	1.530	16	3.702	60	3.391	41	---	---
Pol	3	2.133	17	2.357	24	1.458	15	3.478	52	2.773	29	2.884	33
Pol	12	2.440	20	3.375	47	---	---	3.370	47	3.080	35	---	---
Sam	3	1.526	10	2.699	17	1.015	15	3.576	54	3.027	36	3.107	41
Sam	12	2.671	22	2.891	37	1.486	16	---	---	---	---	---	---

Of the taxa, foraminifera were the least diverse and sediment bacteria the most diverse. At 3 meters, species richness varied from 9 (Lumulumu) to 24 (Lankadea) for corals, 10 (Lae Lae) to 26 (Kudingkareng Keke) for sponges and 6 (Kudingkareng Keke) to 15 (Lumulumu, Polewali and Samalona) for forams. Band richnesss at 3 m varied from 46 (Langkai) to 55 (Karanrang) for sediment bacteria 29 (Bone Lola, Karanrang and Kudingkareng Keke) to 41 (Lankadea) for sediment archaea and 33 (Badi and Polewali) to 44 (Lumulumu) for sponge bacteria. At 12 meter, species richness varied from 7 (Lae Lae) to 30 (Kudingkareng Keke) for corals, 31 (Badi) to 60 (Bone Lola) for sponges and 6 (Badi) to 38 (Karanrang) for forams. Band richness at 12 m varied from 47 (Lankadea and

Polewali) to 60 (Padjenekang) for sediment bacteria and 28 (Lankadea) to 41 (Padjenekang) for sediment archaea.

Of the remotely sensed variables (Figure 3.4.1 and Table 3.4.2), CDOM was lowest at Kapoposang (2.295) and highest at Polewali (4.113). Chlor_a was lowest at Kudingkareng Keke (0.233 $mg.m^{-3}$) and highest at Lae Lae (0.634 $mg.m^{-3}$). SST and Rrs_645 were lowest at Langkai (29.259 deg.C; 0.000213 sr^{-1}) and highest at Lankadea (29.549 deg.C; 0.000893 sr^{-1}).

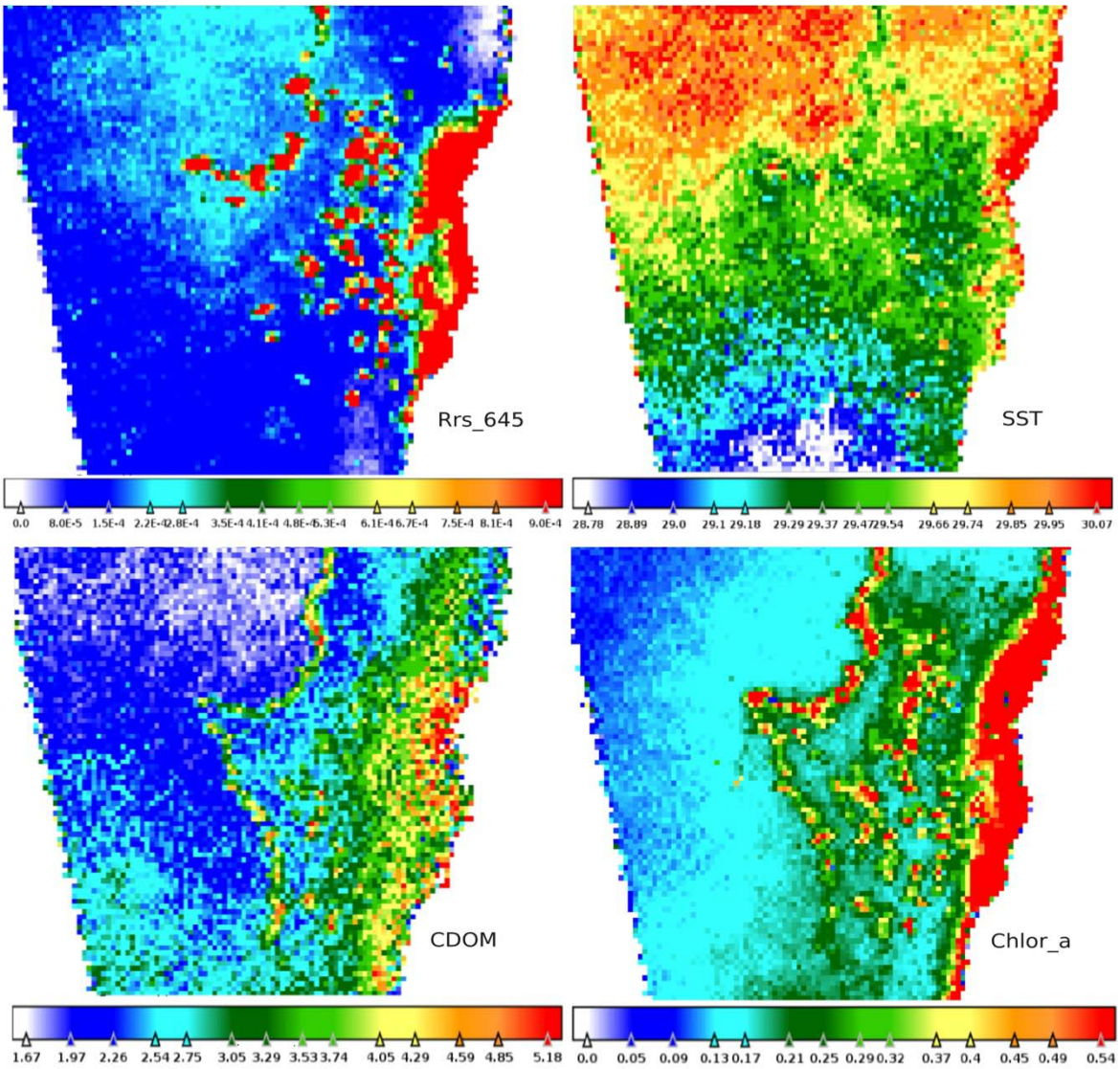

Figure 3.4.1 - Seasonal (June, July and August) mean of Rrs_645, SST, CDOM and Chlor_a values based on monthly mean data from 2008 to 2010 derived from the MODIS-Aqua sensor for the Spermonde Archipelago.

Table 3.4.2 - CDOM, Chlor_a, Rrs_645 and SST values 2010 derived from the MODIS-Aqua sensor from Badi (Bad), Bone Lola (bon), Kapoposang (Kap), Karanrang (Kar), Kudingkareng Keke (Kud), Lae Lae (Lae); Langkai (Lan), Lankadea (Lank), Lanyukang (Lny), Lumulumu (Lum), Padjenekang (Paj), Polewali (Pol) and Samalona (Sam).

Reefs	CDOM	Chlor_a	Rrs_645	SST
Bad	3.376	0.266	0.000626	29.424
Bon	3.805	0.468	0.000555	29.453
Kap	2.295	0.272	0.000644	29.534
Kar	4.081	0.366	0.000769	29.461
Kud	3.570	0.233	0.000299	29.317
Lae	3.791	0.634	0.000791	29.414
Lan	2.808	0.234	0.000213	29.259
Lnk	4.035	0.421	0.000893	29.549
Lny	2.748	0.245	0.000248	29.337
Lum	3.077	0.275	0.000343	29.433
Paj	3.706	0.281	0.000613	29.451
Pol	4.113	0.447	0.000748	29.511
Sam	3.846	0.318	0.000283	29.350

Live coral cover at 3 meters varied from 28.1% (Lanykang) to 83.27 % (Badi). Dead coral cover varied from 3.93 % (Kudingkaren Keke) to 34.43 % (Lae Lae). Rubble cover varied from 0.43 (Kapoposang) to 45.6 % (Kudingkaren Keke) and sand cover varied from 0 (Badi) to 88.4 % (Kapoposang). Live coral cover at 12 meters varied from 4.53 (Lae Lae) to 71.57 % (Lumulumu). Dead coral cover varied from 13.17 (Samalona) to 37.47% (Polewali). Rubble cover varied from 0 (Polewali) to 40.43% (Samalona) and sand cover varied from 0 (Badi) to 28.17% (Lae Lae).

Spatial and environmental variables explained up to 74% of variation in composition of all studied taxa. There were, however, marked differences among taxa/groups (Figure 3.4.2, Figure 3.4.3 and Table 3.4.3).

With the exception of forams and sponge bacteria, all taxa sampled at 3 m varied significantly with respect to the distance between sites (sponges: Adjusted R^2 = 0.17, F = 6.66, P = 0.02; corals: Adjusted R^2 = 0.05, F = 4.88, P = 0.026; sediment bacteria: Adjusted R^2 = 0.05, F = 4.13, P = 0.049; sediment archaea: Adjusted R^2 = 0.09, F = 6.15, P = 0.016) (Figure 3.4.2). The only taxon where variation in composition was significantly related to distance at 12 m depth was sponges (Adjusted R^2 = 0.067, F = 4.86, P = 0.03). All the six groups varied significantly with respect to one or more environmental variables but this varied strongly between depths (Table 3.4.3).

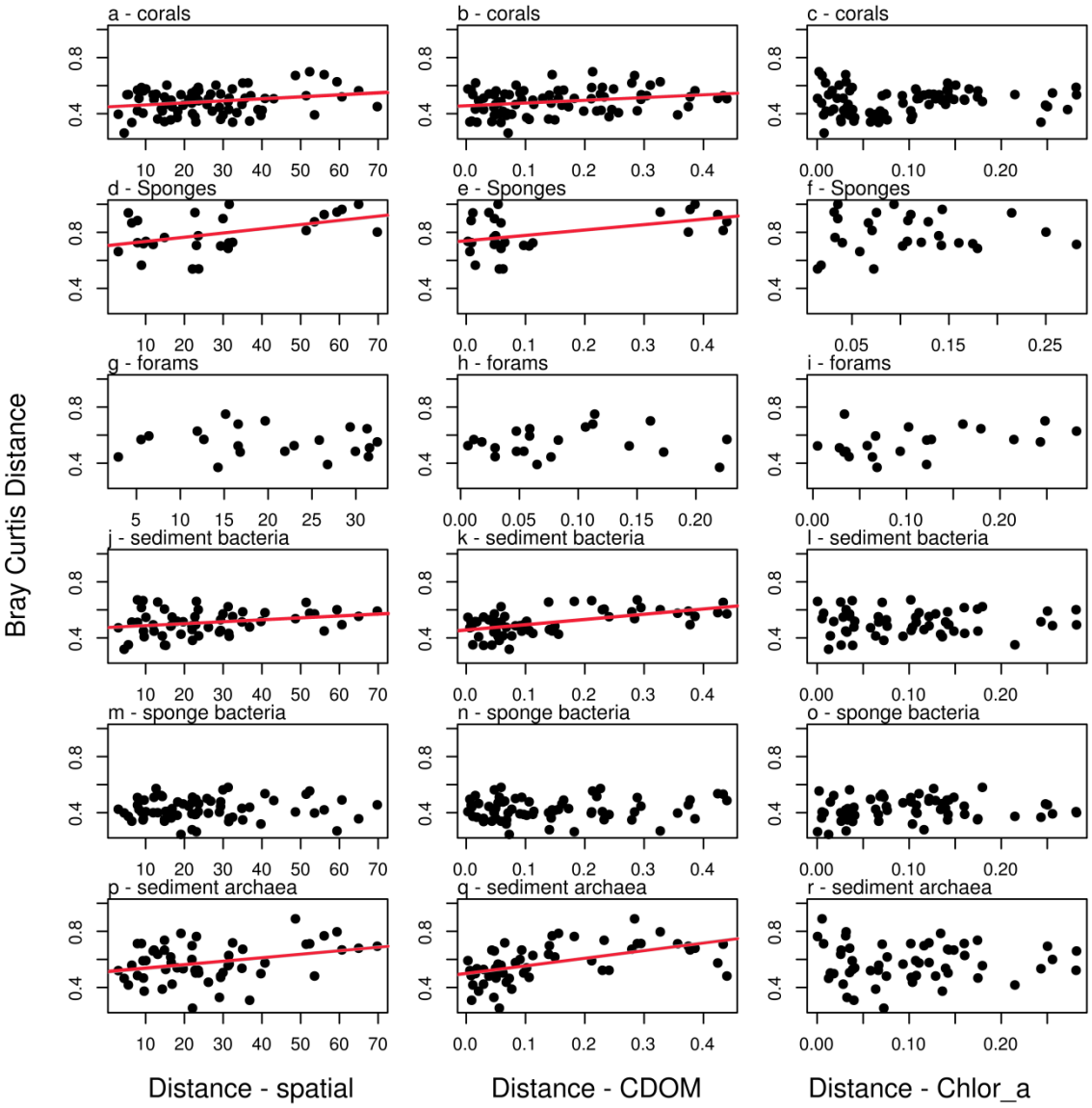

Figure 3.4.2 - Variation in the composition of corals (a, b, c), sponges (d, e, f), forams (g, h, i), sediment bacteria (j, k, l) and sediment archaea (m, n, o) at 3 m depth and as a function of distance between sites (a, d, g, j, m), colored dissolved organic matter index (CDOM) (b, e, h, k, n) and Chlor_a (c, f, i, l, o). Significant relationships are indicated by a red regression line.

Only forams showed no significant relationship to environmental variables at 3 m depth. Variation in the composition of shallow sediment bacteria and archaea sampled was primarily explained by CDOM.

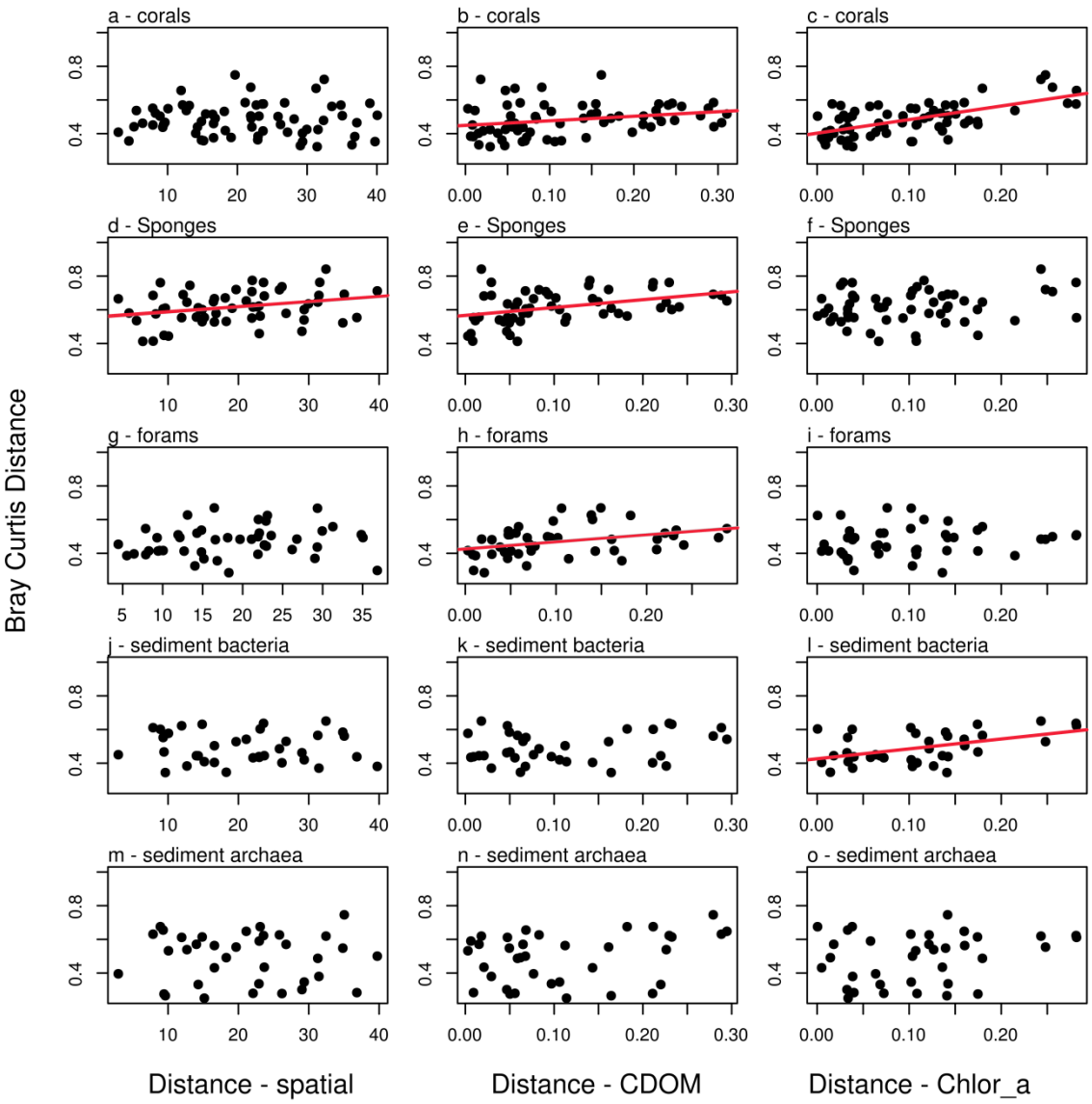

Figure 3.4.3 - Variation in the composition of corals (a, b, c), sponges (d, e, f), forams (g, h, i), sediment bacteria (j, k, l), sponge bacteria (m, n, o) and sediment archaea (p, q, r) at 12 m depth as a function of distance between sites (a, d, g, j, m), colored dissolved organic matter index (CDOM) (b, e, h, k, n) and Chlor_a (c, f, i, l, o). Significant relationships are indicated by a red regression line.

Variation in the composition of corals, in contrast, was primarily explained by SST and sand cover. Space (Adjusted R^2 = 0.173, F = 6.66, P = 0.02) and CDOM (Adjusted R^2 = 0.172, F = 6.59, P = 0.018) explained very similar amounts of variation in the composition of shallow water sponges, a reflection of the spatial structuring of CDOM.

Table 3.4.3 - Results of forward selection regression analysis for corals, sponges, forams, sediment bacteria, sediment archaea and sponge bacteria at 3 and 12 m depths.

Group	Depth	Expl. Var.	Adj. R^2	F	P
Corals	**3 m**	SST	0.130	12.53	0.001
		Sand cover	0.213	8.98	0.007
	12 m	Coral cover	0.540	77.32	0.001
		CDOM	0.646	20.17	0.001
		Sand cover	0.682	8.04	0.010
		Chlor_a	0.709	6.74	0.012
		Dead Coral	0.723	4.29	0.045
		Rrs_645	0.742	5.25	0.024
Sponges	**3 m**	Distance	0.173	6.66	0.017
	12 m	CDOM	0.135	9.45	0.005
		Distance	0.238	8.15	0.003
Forams	**3 m**	-	-	-	-
	12 m	CDOM	0.112	6.55	0.012
Sediment bacteria	**3 m**	CDOM	0.301	24.239	0.001
		Sand cover	0.359	5.846	0.014
	12 m	Coral cover	0.262	13.447	0.002
		SST	0.442	11.980	0.001
		Dead Coral	0.506	5.267	0.028
		Rubble	0.558	4.720	0.033
Sediment archaea	**3 m**	CDOM	0.274	21.366	0.001
		Dead Coral	0.353	7.463	0.012
	12 m	Dead Coral	0.213	10.496	0.005
		Coral cover	0.322	6.419	0.013
Sponge bacteria	**3 m**	SST	0.046	4.146	0.040
	12 m	-	-	-	-
			8.680		

CDOM also explained significant variation in the composition of deeper (12 m depth) forams. In contrast to shallow transects, substrate variables (live and dead coral cover) were the most important explanatory variables of variation in the composition of deeper sediment bacteria and archaea. A number of variables including live coral cover, CDOM, sand cover and Chlor_a explained almost 74% of the variation in deeper coral composition. When live coral cover was excluded, the most important explanatory variable of coral composition was Chlor_a, explaining more than 39% of the variation in composition.

Although four of the six studied groups sampled in shallow water varied significantly with space, none of the variation in composition was due to the purely spatial component (Figure 3.4.4). The spatially structured environmental component explained from 5% (corals and sediment bacteria and archaea) to 20% (sponges) of variation in composition.

The purely environmental component explained from 17% (corals) to 30% (sediment bacteria and archaea) of variation in composition.

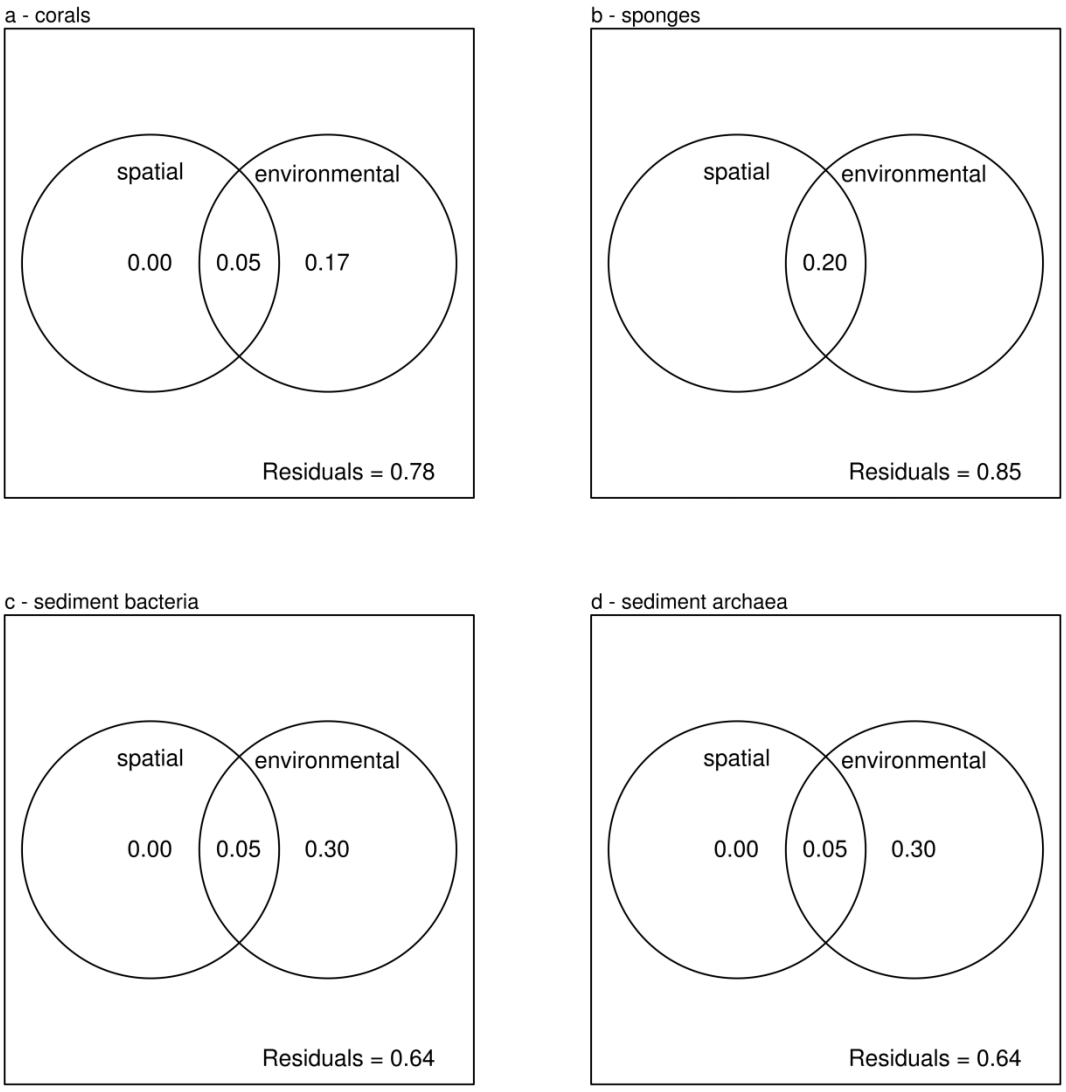

Figure 3.4.4 - Venn diagrams showing the amount of variation explained by purely spatial, purely environmental and spatially structured environmental (overlap) components for a) corals, b) sponges c) sediment bacteria and d) sediment archaea at 3 meters.

Of the deep water assemblages, only sponges showed a significant relationship between composition and depth (Figure 3.4.5). Here the purely spatial component explained 10% of variation in composition and the purely environmental component 17%.

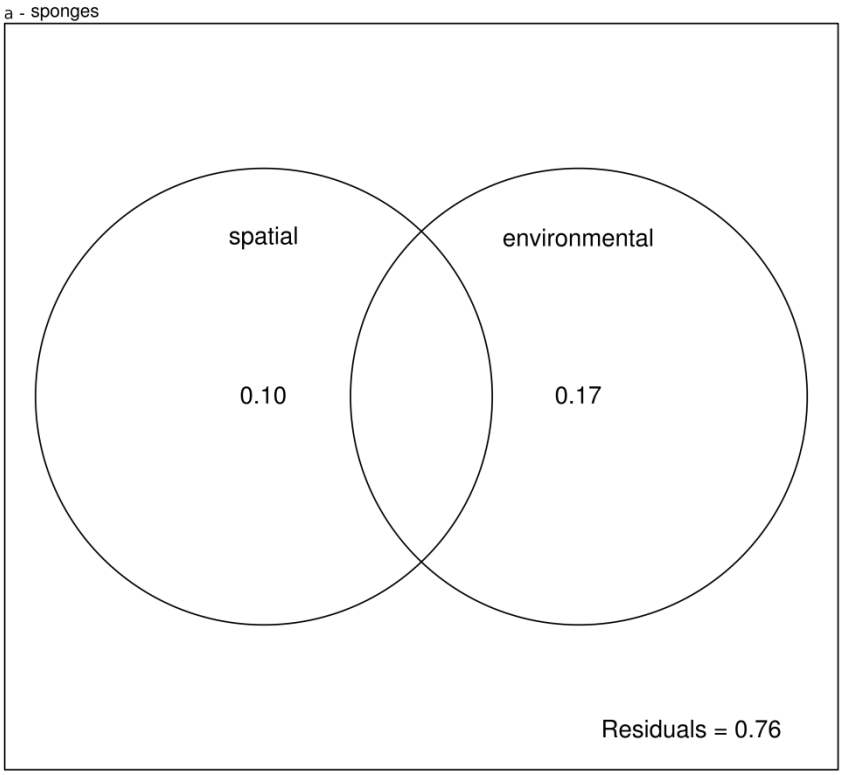

Figure 3.4.5 - Venn diagrams showing the amount of variation explained by purely spatial, purely environmental and spatially structured environmental (overlap) components for sponges at 12 meters.

3.5. Discussion

CDOM, Chlor_a and Rrs_645 showed an in-to-offshore gradient whereas SST varied along a North to South gradient. Gordon et al. (2012) reported a warmer Indonesian throughflow during the Southeast monsoon (boreal summer) in the years preceding our sampling. These years were used for the satellite data analysis (2008-2009). The Indonesian throughflow consists in a water mass fed essentially by waters from the North Pacific Thermocline, which flow through the Makassar Strait thereby enabling communication between two different oceans. This warmer water mass reaches the northern reefs of the Spermonde archipelago leading to higher SST values in these reefs. River discharge from the Jene Berang (that passes through the city of Makassar) and the

Maros river (in the north) strongly influence water transparency of the closest reefs through the input of terrigenous sand, silt and land based sources of pollution (Cleary et al. 2005). While the discharge of the Maros river is mainly derived from a carbonate rich drainage area, the discharge of the Jene Berang river, besides being larger and containing volcanically derived erosion products (Renema and Troelstra 2001), also includes land-based agricultural and urban run-off as well as effluents from the Makassar sewer system (Renema and Troelstra 2001; Becking et al. 2006). Additionally, the rivers located further north (e.g., Salo Lampe and Salo Puke) cross essentially rural areas. The increasing agriculture land use and deforestation led to high rates of soil erosion and sedimentation which, through river transportation, reach the marine environment and the coastal coral reefs.

The diversity indices varied considerable between depths. Interestingly, at 3 m, reefs close to Makassar city such as Samalona, Lae Lae, Kudingkareng Keke and Bone Lola had high diversities of sponges, sediment bacteria, sediment archaea and sponge bacteria. In contrast, the northeastern reefs including Lumulumu, Padjenekang, Karanrang and Polewali had high diversities of corals and forams. At 12 meters the diversity of sponge and sediment bacteria and archaea was high in Kudingkareng Keke and in northern reefs such as Padjanekang and Karanrang. For corals and forams Karanrang and Lankadea had the higher diversity followed by Bone Lola and Kudingkareng Keke for corals and Lumulumu and Langkai for forams. This reinforces the notion that a number of these taxa are unable to deal with high levels of pollution and eutrophication associated with coastal areas located next to mega cities like Makassar. These conditions adversely affect photosymbiont hosting organisms such as corals and forams and benefit taxa that rely on dissolved organic matter assimilation to fulfill an important part of their energetic needs, such as sponges, bacteria and archaea (de Goeij et al. 2008; Shank et al. 2010). The persistent exposure to land based perturbations also have led to the high values of sand, ruble and dead coral in inshore reefs (Lae Lae, Samalona and Polewali).

Our results showed that satellite derived environmental variables, in particular colored dissolved organic matter index (CDOM) and Chlor_a, explained significant amounts of variation in the composition of the studied taxa. As an important absorber of short wavelength in the visible light region, dissolved organic matter has been reported to protect marine organisms from ultraviolet (UV) radiation and shorter wavelengths of PAR (Shank et al. 2010; Kuwahara et al. 2010; Ayoub et al. 2012). High CDOM concentrations normally occur in reefs adjacent to intact shoreline, *i.e.,* close to high densities of mangroves, seagrasses and/or inputs from terrestrial vegetation through runoff. Some studies have shown higher attenuation of UV radiation in inshore reefs resulting in a

lowered susceptibility of inshore reef organisms to photic stress when compared to their offshore counterparts (Ayoub et al. 2009, 2012). Our results support studies that have shown dissolved organic matter to be important in structuring coastal communities (Arrigo al. 1996; Stedmon et al. 2000; Ayoub et al. 2009; McCarren et al. 2010; Grubisic et al. 2012; Baña et al. 2014). The present study also showed that the composition of prokaryote communities from shallow sediments was primarily related to CDOM. During the photo breakdown of CDOM, a series of photochemical reactions occur resulting in low molecular weight byproducts. These are liable to be assimilated by microbial communities enhancing their activity (Daniel et al. 2006; Shank et al. 2010). Also sponges, as filter feeders, rely on dissolved organic matter assimilation to fulfill their carbon needs (Yahel et al. 2003; de Goeij et al. 2008) and thus can also be affected by variation in CDOM composition and concentrations. Some authors considered dissolved organic matter as the primary source of carbon for sponges (Yahel et al. 2003; Hunting et al. 2010). Curiously, in contrast to sediment-dwelling bacteria and archaea in shallow waters, no variation in sponge bacterial composition was explained by CDOM. Enclosed in a very nutrient rich environment (Hentschel et al. 2012), sponge bacteria appear to be less sensitive to environmental fluctuations in nutrient concentrations. Nutrient availability inside sponges is also necessarily different when compared to nutrient availability in sediment; these differences can lead to the adoption of distinct nutritional strategies by the microbial community. The nutritional importance of CDOM for microbial communities can be higher in sediment where the competition for nutrients is greater and availability lower. Our results showed CDOM to be the main explanatory variable for sediment microbial communities at 3 m depths.

Chlorophyll-a concentrations are directly related to nutrient availability in surface waters. High nutrient levels differentially benefit algae and filter feeding benthos such as sponges as opposed to photosymbiont hosting organisms such as corals and forams (Mutti and Hallock 2003). Our results showed corals to respond significantly to chlorophyll-a concentrations as indicated by the significant relationship obtained between coral composition and Chlor_a. Spatial competitors (macroalgae) and the biomass of bioeroders (sponges, bivalves, etc.) are also known to be higher in areas of nutrient enrichment (Holmes 2000; Baird et al. 2013).

Variation in remote sensing reflectance at 645 nm (Rrs_645) only explained a significant amount of variation in the composition variation of corals at 12 m depth. Suspended sediments are key determinants of turbidity and consequentially of the amount of light available for photosynthetic organisms. As algal symbiont-bearing organisms, corals are sensitive to variation in the depth of the photic zone. High sedimentation rates can result

in a reduction of coral growth, recruitment, calcification and accretion rates (Rogers 1990). Coral species appear to respond differently to this type of environmental perturbation depending largely on their polyp size and sediment-shedding mechanisms (Bongaerts et al. 2012; Erftemeijer et al. 2012). Acropora corals, for example, have been shown to be highly sensitive to increased sedimentation. Massive corals, such as various Porites and Platygyra species, in contrast, tend to be much more tolerant (McClanahan and Obura 1997; Torres and Morelockl 2002).

Our results showed strong differences in the relationship between composition and environmental variables at different depths. Cleary et al. (2005) reported depth as the most important explanatory variable for sponges, corals and foraminifera followed by distance offshore. With respect to sponges, of the 144 species sampled during this study, 78 were observed in 3 m transects whereas 126 were observed in 12 m transects. Abundance of sponge individuals was also greater at 12 m (n=961) versus 3 m (n= 356). Sponge assemblages at 3 m depth were thus sparser and less diverse.

Environmental variables also vary with depth. As depth increases, light penetration, temperature and hydrodynamic energy are reduced (Cleary et al. 2005; Lesser et al. 2006), and the influence of environmental variables such as dissolved organic matter, suspended sediment and nutrient availability are altered. Reefs in turbid waters can experience a reduction in light penetration of about 70% in the first 2 m depth (Browne et al. 2010). Conversely, in reefs with low levels of turbidity similar reductions in light availability are only attained at 10 m (Browne et al. 2010). In the Spermonde archipelago, turbidity is similar at shallow and deeper depths at the inshore reefs, but higher at deeper depths in the midshore reefs. This suggests that complex interactions among environmental parameters can affect variation in composition of several taxa.

Substrate complexity can influence coral reef community composition (Lara and Gonzalez 1998; Nakamura and Sano 2005; Cleary et al. 2008). Here, substrate variables only explained variation in the composition of corals and sediment bacteria and archaea. The relationship between coral composition and coral and sand cover reflects the dominant coral groups in habitats with high coral versus high sand cover. In high coral cover environment, branching corals, particularly *Acropora spp.*, tend to dominate. In habitat with high sand cover, massive and encrusting corals belonging, e.g., to the genera *Porites* tend to be the dominant corals (Cleary et al. 2008; Cleary et al. 2013b). Habitats with a high sand cover are sometimes the result of previous environmental perturbations, such as, coral mining or land-based pollution (Cleary et al. 2006; Rachello-Dolmen and Cleary 2007). *Acropora* species, for example, have been shown to be particularly sensitive to

pollution (Bellwood and Hughes 2001; Torres and Morelockl 2002) and disappear from affected areas. In the Pulau Seribu reef system, inshore reefs that have been adversely impacted by their proximity to the city of Jakarta have very high sand cover (63%) compared to (22%) for reefs further offshore (Rachello-Dolmen and Cleary 2007; Cleary et al. 2008). *Acropora* species have also largely disappeared from these inshore reefs (Van der Meij et al. 2010). Once prevalent, sand can continue to exert a strong influence on coral composition. Algae, sediment and sand are noted poor habitats for settlement and survival, as opposed to coralline algae and dead coral substrate that are suitable for settlement (Harrington et al. 2004; Norström et al. 2007). According to Norström et al. (2007), the morphology of dead coral has a strong influence on the morphology of the settled coral larvae; branching dead coral appears to be a preferable settlement substrate for branching coral larvae, while the same holds for massive dead coral and massive coral larvae. Mergne and Scheer (1974; reviewed in Huston 1985) also reported a reduction in the number of coral species as sand and coral debris cover increased from 15 to 90%.

Interestingly, shallow and deeper bacteria and archaea appeared to respond differently to environmental conditions. In shallow transects variation in composition was primarily related to CDOM whereas in deeper transects variation in composition was primarily related to the local substrate. Differences in coral reef substrate were previously reported to alter the microbial community (Wild et al. 2005, 2006). The abundance of prokaryotes in carbonate reef sands, for example, was reported to be one order of magnitude higher than in silicate sands of a similar grain size spectrum. The complex surface structure and highly porous matrix of the carbonate reef sands enhances the surface area available for prokaryotes to penetrate (Wild et al. 2006; Schöttner et al. 2011). According to Wild et al. (2004), corals can exude around 5 liters of mucus per square meter of reef area per day. Most of the mucus is released in the seawater and sinks to the surrounding sand. In the sand, this mucus is consumed by the prokaryotic communities which act as nutrient recyclers. This is consistent with our results showing live and dead coral cover as the most important explanatory variables of variation in the composition of deeper sediment bacteria and archaea. The nutrient composition of sediment close to live corals and to dead coral is, thus, necessarily different and appears to be an important determinant of prokaryotic composition. The higher availability of land based organic matter in shallow waters may diminish the substrate importance in structuring prokaryotic composition. Due to their proximity to river runoff, the inshore shallow reefs are likely to be dominated by land-derived silicate sands (Schöttner et al. 2011).

The lack of purely spatial structuring in the present study contradicts previous studies of the area (Cleary and Renema 2007; Becking et al. 2006). Importantly, in the present study

we sampled both on-to-offshore and along shore at similar spatial scales. This sampling strategy thus appears to have negated any purely spatial structuring. Although our results indicate the prevalence of environment as opposed to purely spatial phenomena in structuring marine assemblages, it is important to note that spatial phenomena may still play a role in structuring these assemblages at smaller or larger scales than assessed in this study.

3.6. Conclusions

The large amount of total variation explained by satellite derived environmental factors supports remote sensing as an important tool for studying coral reef taxa. Our results also reveal marked differences in the relationship between composition and environment within taxa from different depths and importantly between taxa. This implies that different approaches in terms of multi-taxon reef habitat management should be taken into consideration when designing effective conservation strategies.

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3.8. References

Adjeroud M (1997) Factors influencing spatial patterns on coral reefs around Moorea, French Polynesia. *Marine Ecology Progress Series* 159: 105–119.

Aerts L, Vansoest R (1997) Quantification of sponge/coral interactions in a physically stressed reef community, Ne Colombia. *Marine Ecology Progress Series* 148: 125–134.

Arrigo KR, Brown CW (1996) Impact of chromophoric dissolved organic matter on UV inhibition of primary productivity in the sea. *Marine Ecology Progress Series*. 140: 207-216.

Ayoub L, Hallock P, Coble P (2009) Colored dissolved organic material increases resiliency of coral reefs by controlling exposure to UVR. *Proceedings 11th International Coral Reef Symposium* 1: 579-583.

Ayoub LM, Hallock P, Coble PG, Bell SS (2012) Maa-Like absorbing substances in Florida Keys phytoplankton vary with distance from shore and CDOM: implications for coral reefs. *Journal of Experimental Marine Biology and Ecology* 420: 91–98.

Baird AH, Pratchett MS, Hoey AS, Herdiana Y & Campbell SJ (2013) *Acanthaster planci* is a major cause of coral mortality in Indonesia. *Coral Reefs* 1-10.

Baña Z, Ayo B, Marrasé C, Gasol JM & Iriberri J (2014) Changes in bacterial metabolism as a response to dissolved organic matter modification during protozoan grazing in coastal Cantabrian and Mediterranean waters. *Environmental Microbiology 16*: 498-511. doi:10.1111/1462-2920.12274.

Becking L, Cleary DFR, De Voogd N, Renema W, De Beer M, Van Soest R, Hoeksema B (2006) Beta diversity of tropical marine benthic assemblages in the Spermonde Archipelago, Indonesia. *Marine Ecology-An Evolutionary Perspective* 27: 76–88.

Beger M, Mckenna SA, Possingham HP (2007) Effectiveness of surrogate taxa in the design of coral reef reserve systems in the Indo - Pacific. *Conservation Biology* 21: 1584–1593.

Bell JJ (2008) The functional roles of marine sponges. *Estuarine, Coastal and Shelf Science* 79: 341–353.

Bellwood DR, Hughes TP, Folke C & Nyström M (2004) Confronting the coral reef crisis. *Nature* 429: 827-833.

Bellwood D, Hughes T (2001) Regional-scale assembly rules and biodiversity of coral reefs. *Science* 292: 1532–1534.

Blanchet FG, Legendre P, Borcard D (2008) Forward selection of explanatory variables. *Ecology* 89: 2623–2632.

Bongaerts P, Hoeksema BW, Hay KB, Hoegh-Guldberg O (2012) Mushroom corals overcome live burial through pulsed inflation. *Coral Reefs* 31: 399.

Borcard D, Legendre P (2002) All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling* 153: 51–68.

Bouchet VMP, Alve E, Rygg B, Telford RJ (2012) Benthic foraminifera provide a promising tool for ecological quality assessment of marine waters. *Ecological Indicators* 23: 66–75.

Browne NK, Smithers SG, Perry CT (2010) Geomorphology and community structure of Middle Reef, Central Great Barrier Reef, Australia: An inner-shelf turbid zone reef subject to episodic mortality events. *Coral Reefs* 29: 683–689.

Bryant D, Burke L, Mcmanus J, Spalding M et al. (1998) Reefs at risk: A map-based indicator of threats to the world's coral reefs. World Resources Institute Washington, DC.

Buddemeier RW, Kleypas JA, Aronson RB (2004) Coral reefs & global climate change: potential contributions of climate change to stresses on coral reef ecosystems. Pew Center on Global Climate Change.

Burke LM, Reytar K, Spalding M, Perry A et al. (2011) Reefs at risk revisited. World Resources Institute Washington, DC.

Burke LM, Selig E, Spalding M (2002) Reefs at risk in Southeast Asia. World Resources Institute Washington, DC.

Burkepile DE & Hay ME (2008) Herbivore species richness and feeding complementarity affect community structure and function on a coral reef. *Proceedings of the National Academy of Sciences*, *105*: 16201-16206.

Capone D, Dunham S, Horrigan S, Duguay L (1992) Microbial nitrogen transformations in unconsolidated coral reef sediments. *Marine Ecology Progress Series*. 80: 75–88.

Chen Z, Hu C, Muller-Karger F (2007) Monitoring turbidity in Tampa Bay using MODIS/Aqua 250-M imagery. *Remote Sensing of Environment* 109: 207–220.

Cleary DFR (2003) An examination of scale of assessment, logging and Enso-induced fires on butterfly diversity in Borneo. *Oecologia* 135: 313–321.

Cleary DFR, Becking L, De Voogd N, Renema W, De Beer M, Van Soest R, Hoeksema B (2005) Variation in the diversity and composition of benthic taxa as a function of distance offshore, depth and exposure in the Spermonde Archipelago, Indonesia. *Estuarine Coastal and Shelf Science* 65: 557–570.

Cleary DFR, Becking LE, de Voogd NJ, Pires AC, Polónia, AR, Egas, C, Gomes N (2013) Habitat-and host-related variation in sponge bacterial symbiont communities in Indonesian waters. *FEMS Microbiology Ecology* 85: 465-482.

Cleary DFR, De Vantier L, Giyanto Vail L, Manto P, De Voogd NJ, Rachello-Dolmen PG, Tuti Y, Budiyanto A, Wolstenholme J, Hoeksema BW, Suharsono (2008) Relating variation in species composition to environmental variables: a multi-taxon study in an Indonesian coral reef complex. *Aquatic Sciences* 70: 419–431.

Cleary DFR, Polónia, ARM, Renema W, Hoeksema BW,Wolstenholme J, Tuti Y, de Voogd NJ (2013b) Coral reefs next to a major conurbation: a study of temporal change (1985-2011) in coral cover and composition in the reefs of Jakarta Indonesia. *Marine Ecology Progress Series 501*: 89-98. doi: 10.3354/meps10678

Cleary DFR, Renema W (2007) Relating species traits of foraminifera to environmental variables in the Spermonde Archipelago, Indonesia. *Marine Ecology Progress Series* 334: 73–82.

Cleary DFR, Suharsono, Hoeksema BW (2006) Coral diversity across a disturbance gradient in the Pulau Seribu Reef Complex of Jakarta, Indonesia. *Biodiversity and Conservation* 15: 3653–3674.

Costa R, Keller-Costa T, Gomes NC, da Rocha UN, van Overbeek L & van Elsas JD (2013) Evidence for selective bacterial community structuring in the freshwater sponge *Ephydatia fluviatilis*. *Microbial Ecology* 65: 232–244.

Daniel C, Graneli W, Kritzberg E, Anesio A (2006) Stimulation of metazooplankton by photochemically modified dissolved organic matter. *Limnology and Oceanography* 51: 101–108.

De Bie T, De Meester L, Brendonck L, Martens K, Goddeeris B, Ercken D, Hampel H, Denys L, Vanhecke L, Van Der Gucht K, Van Wichelen J, Vyverman W, Declerck SAJ

(2012) Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters* 15: 740–747.

de Goeij JM, van Den Berg H, van Oostveen MM, Epping EHG, Van Duyl FC (2008) Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. *Marine Ecology Progress Series* 357: 139–151.

de Voogd N, Becking L, Cleary D (2009) Sponge community composition in the Derawan Islands, Ne Kalimantan, Indonesia. *Marine Ecology Progress Series* 396: 169–180.

de Voogd NJ, Cleary DFR (2007) Relating species traits to environmental variables in Indonesian coral reef sponge assemblages. *Marine and Freshwater Research* 58: 240–249.

de Voogd N, Cleary D, Hoeksema B, Noor A, van Soest R (2006) Sponge beta diversity in the Spermonde Archipelago, SW Sulawesi, Indonesia. *Marine Ecology Progress Series* 309: 131–142.

Diaz M, Rutzler K (2001) Sponges: An essential component of Caribbean Coral Reefs. *Bulletin of Marine Science* 69: 535–546.

Done T, Turak E, Wakeford M, De Vantier L, McDonald A & Fisk D (2007) Decadal changes in turbid-water coral communities at Pandora Reef: loss of resilience or too soon to tell? *Coral reefs 26*: 789-805.

Edinger EN, Risk, MJ (2000) Reef classification by coral morphology predicts coral reef conservation value. *Biological Conservation* 92: 1–13.

English SA, Baker V, Wilkinson CR (1997) Survey manual for tropical marine resources. Australian Institute of Marine Science Townsville.

Erftemeijer PLA, Riegl B, Hoeksema BW, Todd PA (2012) Environmental impacts of dredging and other sediment disturbances on corals: a review. *Marine Pollution Bulletin* 64: 1737-1765.

Fabricius K, De'ath G, McCook L, Turak E & Williams DM (2005) Changes in algal, coral and fish assemblages along water quality gradients on the inshore Great Barrier Reef. *Marine Pollution Bulletin 51*, 384-398.

Fichot CG, Kaiser K, Hooker SB, Amon RMW, Babin M, Belanger S, Walker SA, Benner R (2013) Pan-Arctic distributions of Continental runoff in the Arctic Ocean. Scientific Reports 3.

Fox RJ & Bellwood DR (2007) Quantifying herbivory across a coral reef depth gradient. *Marine Ecology Progress Series 339*: 49-59.

Franz BA, Werdell PJ, Meister G, Kwiatkowska EJ, Bailey SW, Ahmad Z, Mcclain CR (2006) MODIS land bands for ocean remote sensing applications. In: Proc. Ocean Optics XVIII, Montreal, Canada, Volume 10.

Frontalini F, Coccioni R (2011) Benthic foraminifera as bioindicators of pollution: A review of Italian Research over the last three decades. *Revue De Micropaléontologie* 54: 115–127.

Goatley CH & Bellwood DR (2013) Ecological Consequences of Sediment on High-Energy Coral Reefs. *PloS one 8*: e77737.

Gomes NCM Borges LR, Paranhos R, Pinto FN, Mendonca-Hagler LCS, Smalla, K (2008) Exploring the diversity of bacterial communities in sediments of urban mangrove forests. *FEMS Microbiology Ecology* 66: 96–109.

Gooday A, Levin L, Linke P, Heeger T (1992) The role of benthic foraminifera in deep-sea food webs and carbon cycling. In: Rowe, Gt And Pariente, V (Ed.), Deep-Sea Food Chains and The Global Carbon Cycle, NATO Advanced Science Institutes Series, Volume 360, pp. 63–91.

Gordon H, Wang M (1994) Retrieval of water-leaving radiance and aerosol optical-thickness over the oceans with SeaWIFS - A preliminary algorithm. *Applied Optics* 33: 443–452.

Gordon AL, Huber BA, Metzger EJ, Susanto RD, Hurlburt HE & Adi TR (2012) South China Sea throughflow impact on the Indonesian throughflow. *Geophysical Research Letters 39*: L11602. doi:10.1029/2012GL052021.

Gotelli NJ & Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology letters 4*: 379-391.

Graham NA, Wilson SK, Jennings S, Polunin NV, Bijoux JP & Robinson J (2006) Dynamic fragility of oceanic coral reef ecosystems. *Proceedings of the National Academy of Sciences* 103: 8425-8429.

Grubisic LM, Brutemark A, Weyhenmeyer GA, Wikner J, Båmstedt, U & Bertilsson S (2012) Effects of stratification depth and dissolved organic matter on brackish bacterioplankton communities. *Marine Ecology Progress Series* 453: 37-48.

Hallock P, Lidz BH, Cockey-Burkhard EM & Donnelly KB (2003) Foraminifera as bioindicators in coral reef assessment and monitoring: the FORAM index. In *Coastal Monitoring Through Partnerships.* Springer Netherlands, pp. 221-238.

Han M, Liu F, Zhang F, Li Z, Lin H (2012) Bacterial and archaeal symbionts in the South China Sea Sponge *Phakellia Fusca*: Community structure, relative abundance, and ammonia- oxidizing populations. *Marine Biotechnology* 14: 701–713.

Harrington L, Fabricius K, De'ath G, Negri A (2004) Recognition and selection of settlement substrata determine post-settlement survival in corals. *Ecology* 85: 3428–3437.

Hentschel U, Hopke J, Horn M, Friedrich A, Wagner M, Hacker J, Moore B.(2002) Molecular evidence for a uniform microbial community in sponges from different oceans. *Applied Environmental Microbiology* 68: 4431–4440.

Hentschel U, Piel J, Degnan SM, Taylor MW (2012) Genomic insights into the marine sponge microbiome. *Nature Reviews Microbiology* 10: 641–U75.

Heuer H, Wieland G, Schönfeld J, Schönwälder A, Gomes N, Smalla K (2001) Bacterial community profiling using DGGE or TGGE Analysis. *Environmental Molecular Microbiology: Protocols and Applications* 9: 177–190.

Hoeksema BW (2004) Biodiversity and the natural resource management of coral reefs in Southeast Asia. In: Visser L.E. (ed), Challenging Coasts: Transdisciplinary Excursions into Integrated Coastal Zone Development. Amsterdam University Press, pp 49-71.

Hoeksema BW (2012a) Distribution patterns of mushroom corals (Scleractinia: Fungiidae) across the Spermonde Shelf, South Sulawesi. *Raffles Bulletin of Zoology* 60: 183-212.

Hoeksema BW (2012b) Evolutionary trends in onshore-offshore distribution patterns of mushroom coral species (Scleractinia: Fungiidae). *Contributions to Zoology* 81: 199-221.

Hohenegger J (2006) The importance of symbiont-bearing benthic foraminifera for West Pacific carbonate beach environments. *Marine Micropaleontology* 61: 4-39.

Holmes K (2000) Effects of eutrophication on bioeroding sponge communities with the description of New West Indian Sponges, *Cliona Spp*. (*Porifera: Hadromerida: Clionidae*). *Invertebrate Biology* 119: 125–138.

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L et al B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Current Biology* 17: 360-365.

Hughes TP, Graham NA, Jackson JB, Mumby, PJ & Steneck RS (2010) Rising to the challenge of sustaining coral reef resilience. *Trends in Ecology & Evolution 25*: 633-642.

Hunting ER, De Goeij JM, Asselman M, Van Soest RWM, Van Der Geest HG (2010) Degradation of mangrove-derived organic matter in mangrove associated sponges. *Bulletin of Marine Science* 86: 871–877.

Huston M (1985) Patterns of species diversity on coral reefs. *Annual Review of Ecology and Systematics* 16: 149–177.

Jombart T, Dray S, Dufour AB (2009) Finding essential scales of spatial variation in ecological data: A multivariate approach. *Ecography* 32: 161–168.

Karakassis I, Machias A, Pitta P, Papadopoulou K, Smith C, Apostolaki E, Giannoulaki M, Koutsoubas D, Somarakis S (2006) Cross-community congruence of patterns in a marine ecosystem: Do the parts reflect the whole? *Marine Ecology Progress Series* 310: 47– 54.

Kuwahara VS, Nakajima R, Othman BHR, Kushairi MRM, Toda T (2010) Spatial variability of UVR attenuation and biooptical factors in shallow coral-reef waters of Malaysia. *Coral Reefs* 29: 693–704.

Lahet F, Stramski D (2010) MODIS Imagery of turbid plumes in San Diego Coastal waters during rainstorm events. *Remote Sensing of Environment* 114: 332–344.

Lara E, Gonzalez E (1998) The relationship between reef fish community structure and environmental variables in the Southern Mexican Caribbean. *Journal of Fish Biology* 53: 209–221.

Lee Y, Lee J, Lee H (2001) Microbial symbiosis in marine sponges. *Journal of Microbiology* 39: 254–264.

Lee JJ (2006) Algal symbiosis in larger foraminifera. *Symbiosis 42*: 63-75.

Legendre P, Gallagher E (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia* 129: 271–280.

Legendre P, Lapointe F, Casgrain P (1994) Modeling brain evolution from behavior - A permutational regression approach. *Evolution* 48: 1487–1499.

Lesser M (2006) Benthic-pelagic coupling on coral reefs: Feeding and growth of Caribbean sponges. *Journal of Experimental Marine Biology and Ecology* 328: 277–288.

McCarren J, Becker JW, Repeta DJ, Shi Y, Young CR, Malmstrom RR et al. (2010) Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proceedings of the National Academy of Sciences* 107: 16420-16427.

Mcclanahan T, Obura D (1997) Sedimentation effects on shallow coral communities in Kenya. *Journal of Experimental Marine Biology and Ecology* 209: 103–122.

McCook L (2001) Competition between corals and algal turfs along a gradient of terrestrial influence in the nearshore central Great Barrier Reef. *Coral Reefs 19*: 419-425.

Miller R, Mckee B (2004) Using MODIS Terra 250 m imagery to map concentrations of total suspended matter in coastal waters. *Remote Sensing of Environment* 93: 259– 266.

Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. *Ecological Economics* 29: 215–233.

Moll H (1983) Zonation and Diversity of Scleractinia on reefs off S.W. Sulawesi, Indonesia. Ph.D. Thesis, Leiden.

Mutti M, Hallock P (2003) Carbonate Systems along nutrient and temperature gradients: Some sedimentological and geochemical constraints. International Journal of Earth *Sciences* 92: 465–475.

Nakamura Y, Sano M (2005) Comparison of invertebrate abundance in a seagrass bed and adjacent coral and sand areas at Amitori Bay, Iriomote Island, Japan. *Fisheries Science* 71: 543–550.

Nobes K, Uthicke S & Henderson R (2008) Is light the limiting factor for the distribution of benthic symbiont bearing foraminifera on the Great Barrier Reef? *Journal of Experimental Marine Biology and Ecology 363*: 48-57.

Norstrom AV, Lokrantz J, Nystrom M, Yap HT (2007) Influence of dead coral substrate morphology an patterns of juvenile coral distribution. *Marine Biology* 150: 1145–1152.

Oksanen J, Kindt R, Legendre P, O'hara B, Simpson G, Solymos P, Stevens M, Wagner H (2009) Vegan: Community ecology package. R Package Version 1.15-2. 2009.

Oliveira-Silva P, Barbosa CF, De Almeida CM, Sicoli Seoane JC, Cordeiro RC, Turcq BJ, Soares-Gomes A (2012) Sedimentary geochemistry and foraminiferal assemblages in coral reef assessment of Abrolhos, Southwest Atlantic. *Marine Micropaleontology* 94-95: 14–24.

Patt F, Barnes R, Eplee Jr R, Franz B, Robinson W, Feldman G, Bailey S, Gales J, Werdell P, Wang M et al. (2003) Algorithm updates for the fourth SeaWIFS data reprocessing. *NASA Tech. Memo* 206892: 22–74.

Patton WK (1994) Distribution and ecology of animals associated with branching corals (*Acropora spp*.) from the Great Barrier Reef, Australia. *Bulletin of Marine Science* 55: 193–211.

Perry CT, Edinger EN, Kench PS, Murphy GN, Smithers SG, Steneck RS, Mumby PJ (2012) Estimating rates of biologically driven coral reef framework production and erosion: a new census-based carbonate budget methodology and applications to the reefs of Bonaire. *Coral Reefs* 31: 853-868.

Pires ACC, Cleary DFR, Almeida A, Cunha A, Dealtry S, Mendonca-Hagler LCS, Smalla K, Gomes NCM (2012) Denaturing gradient gel electrophoresis and barcoded pyrosequencing reveal unprecedented archaeal diversity in mangrove sediment and rhizosphere samples. *Applied and Environmental Microbiology* 78: 5520– 5528.

Plaisance L, Caley MJ, Brainard RE, Knowlton N (2011) The diversity of coral reefs: What are we missing? *PloS One* 6: e25026.

Pratchett MS, Munday PL & Wilson SK (2008). Effects of climate-induced coral bleaching on coral-reef fishes. Ecological and economic consequences. *Oceanography and Marine Biology: An Annual Review* 46: 251-296.

R Core Team (2013) R: A Language and Environment for Statistical Computing. Vienna, Austria. http://www.R-project.org.

Rachello-Dolmen PG, Cleary DFR (2007) Relating coral species traits to environmental conditions in the Jakarta Bay/Pulau Seribu Reef System, Indonesia. *Estuarine Coastal and Shelf Science* 73: 816–826.

Renema W, Troelstra S (2001) Larger foraminifera distribution on a mesotrophic carbonate shelf in SW Sulawesi (Indonesia). *Palaeogeography Palaeoclimatology Palaeoecology* 175: 125+.

Renema W (2010) Is increased calcarinid (foraminifera) abundance indicating a larger role for macro-algae in Indonesian Plio-Pleistocene coral reefs? *Coral reefs 29*: 165-173.

Reymond CE, Uthicke S & Pandolfi JM (2011) Inhibited growth in the photosymbiont-bearing foraminifer Marginopora vertebralis from the nearshore Great Barrier Reef, Australia. *Marine Ecology Progress Series 435*: 97-109.

Richardson LL, LeDrew EF (2006) Remote sensing of aquatic coastal ecosystem processes. *Science and Management Applications*: 9.

Roff G & Mumby PJ (2012) Global disparity in the resilience of coral reefs. *Trends in ecology & evolution 27*: 404-413.

Rogers CS (1990) Responses of coral reefs and reef organisms to sedimentation. *Marine Ecology Progress Series* 621: 185-202.

Schöttner S, Pfitzner B, Grünke S, Rasheed M, Wild C & Ramette A (2011) Drivers of bacterial diversity dynamics in permeable carbonate and silicate coral reef sands from the Red Sea. *Environmental microbiology 13*: 1815-1826.

Schueth JD & Frank TD (2008) Reef foraminifera as bioindicators of coral reef health: low isles reef, Northern Great Barrier Reef, Australia. *The Journal of Foraminiferal Research 38*1: 11-22.

Shank GC, Zepp RG, Vahatalo A, Lee R, Bartels E (2010) Photobleaching kinetics of chromophoric dissolved organic matter derived from mangrove leaf litter and floating sargassum colonies. *Marine Chemistry* 119: 162– 171.

Shannon CE, Weaver W (1949) The Mathematical Theory of Communication. University of Illinois Press, Urbana, IL: 125 pp.

Sheppard C, Wells SM & Jenkins MD (1988) *Coral reefs of the world. Vol. 2: Indian Ocean, Red Sea and gulf*. IUCN, Conservation Monitoring Centre.

Stedmon CA, Markager S & Kaas H (2000) Optical properties and signatures of chromophoric dissolved organic matter (CDOM) in Danish coastal waters. *Estuarine, Coastal and Shelf Science* 51: 267-278.

Sutcliffe PR, Pitcher CR, Caley MJ, Possingham HP (2012) Biological surrogacy in tropical seabed assemblages fails. *Ecological Applications* 22: 1762–1771.

Szmant AM (2002) Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries 25*: 743-766.

Taylor M, Radax R, Steger D & Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews* 71: 295–347.

Tilman D (1999) The ecological consequences of changes in biodiversity: A search for general principles. *Ecology* 80: 1455–1474.

Torres J, Morelock J (2002) Effect of Terrigenous Sediment Influx on Coral Cover and Linear Extension Rates of Three Caribbean Massive Coral Species. *Caribbean Journal of Science* 38: 222–229.

Urakawa H, Martens-Habbena W, Stahl DA (2010) High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method. *Applied Environmental Microbiology* 76: 2129–2135.

Uthicke S & Nobes K (2008) Benthic foraminifera as ecological indicators for water quality on the Great Barrier Reef. *Estuarine, Coastal and Shelf Science* 78: 763-773.

Vacelet J (1975) Étude en microscopie électronique de l'association entre bactéries et spongiaires du genre Verongia (Dictyoceratida). *Journal De Microscopie Et De Biologie Cellulaire* 23: 271–288.

Van der Meij SET, der Suharsono, Hoeksema BW (2010) Long-term changes in coral assemblages under natural and anthropogenic stress in Jakarta Bay (1920-2005). *Marine Pollution Bulletin* 60: 1442-1454.

Venables W, Ripley B (2002) Random and mixed effects. In: Modern applied statistics with S, Springer. 37, 271–300.

Wang M, Shi W (2007) The NIR-SWIR combined atmospheric correction approach for MODIS ocean color data processing. *Optics Express* 15: 15722–15733.

Wang M, Tang J, Shi W (2007) MODIS derived ocean color products along the China East Coastal Region. *Geophysical Research Letters* 34. doi:10.1029/2006GL028599.

Webster N, Negri A, Munro M, Battershill C (2004) Diverse microbial communities inhabit Antarctic sponges. *Environmental Microbiology* 6: 288–300.

Wijsman-Best Moll M & de Klerk LG. (1981) Present status of the coral reefs in the Spermonde Archipelago (South Sulawesi, Indonesia). Proc. 4th Int. *Coral Reef Symposium*, Manila 1: 263-267.

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed MY & Jørgensen BB (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature 428*: 66-70.

Wild C, Rasheed M, Jantzen C, Cook P, Struck U, Huettel M & Boetius A (2005) Benthic metabolism and degradation of natural particulate organic matter in carbonate and silicate reef sands of the northern Red Sea. *Marine Ecology Progress Series 298*, 69-78.

Wild C, Laforsch C, Huettel M (2006) Detection and enumeration of microbial cells in highly porous carbonate reef sands. *Marine and Freshwater Research* 57: 415–420.

Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Lotze H K, Micheli F, Palumbi SR, Sala E, Selkoe KA, Stachowicz JJ, Watson R (2006) Impacts of biodiversity loss on ocean ecosystem services. *Science* 314: 787– 790.

Yahel G, Sharp J, Marie D, Hase C, Genin A (2003) In situ feeding and element removal in the symbiont-bearing sponge Theonella Swinhoei: Bulk DOC is the major source for carbon. *Limnology and Oceanography* 48: 141–149.

Zhang M, Tang J, Dong Q, Song Q, Ding J (2010) Retrieval of total suspended matter concentration in the Yellow and East China Seas from MODIS Imagery. *Remote Sensing of Environment* 114: 392–403.

Ziegler M & Uthicke S (2011) Photosynthetic plasticity of endosymbionts in larger benthic coral reef Foraminifera. *Journal of Experimental Marine Biology and Ecology 407*: 70-80.

Chapter 4: Composition of Archaea in seawater, sediment and sponges in the Kepulauan Seribu reef system, Indonesia

Composition of Archaea in seawater, sediment and sponges in the Kepulauan Seribu reef system, Indonesia

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4.1. Abstract

Coral reefs are among the most diverse and productive ecosystems in the world. Most research has, however, focused on eukaryotes such as corals and fishes. Recently, there has been increasing interest in the composition of prokaryotes, particularly those inhabiting corals and sponges, but these have mainly focused on Bacteria. There have been very few studies of coral reef Archaea, despite the fact that Archaea have been shown to play crucial roles in nutrient dynamics, including nitrification and methanogenesis, of oligotrophic environments such as coral reefs. Here, we present the first study to assess Archaea in four different coral reef biotopes (seawater, sediment and two sponge species, *Stylissa massa* and *Xestospongia testudinaria*). The archaeal community of both sponge species and sediment was dominated by *Crenarchaeota*, while the seawater community was dominated by *Euryarchaeota*. The biotope explained more than 72% of the variation in archaeal composition. The number of OTUs was highest in sediment and seawater biotopes and substantially lower in both sponge hosts. No 'sponge-specific' archaeal OTUs were found, *i.e.*, OTUs found in both sponge species but absent from non-host biotopes. Despite both sponge species hosting phylogenetically distinct microbial assemblages, there were only minor differences in KEGG (Kyoto Encyclopedia of Genes and Genomes) functional pathways. In contrast, most functional pathways differed significantly between microbiomes from sponges and non-host biotopes including all energy metabolic pathways. With the exception of the methane and nitrogen metabolic pathway, all energy metabolic pathways were enriched in sponges when compared to non-host biotopes.

Keywords: *Thaumarchaeota*; *Crenarchaeota*; *Euryarchaeota*; KEGG; PICRUSt; LEFse

4.2. Introduction

Of the two known prokaryotic domains of life, Archaea is the least studied and understood. The archaeal domain was first described by Woese and Fox (1978). Since then it has undergone several taxonomic amendments. Several phyla have been proposed but a lack of consensus on some of the proposed phyla persists. At present, the domain Archaea consists of the following phyla: *Crenarchaeota*, *Thaumarchaeota*, *Euryarchaeota*, *Korarchaeota* and *Nanoarchaeota*. The *Korarchaeota* phylum was based on 16S rRNA gene sequence amplification of data from environmental sequence studies (Barnes et al. 1996; Elkins et al. 2008). Recently, Brochier-Armanet et al. (2008, 2011) and Spang et al. (2010) suggested that mesophilic *Crenarchaeota* differed from hyperthermophilic *Crenarchaeota* and proposed *Thaumarchaeota* (mesophilic *Crenarchaeota*) as a new phylum. Huber et al. (2002) proposed the establishment of *Nanoarchaeota* as a new phylum based on the low similarity of *Nanoarchaeum equitans* sequences with known organisms; however, some other studies have suggested that *Nanoarchaeota* is a fast-evolving lineage of the *Euryarchaeota* phylum related to *Thermococcales* (Brochier-Armanet et al. 2005). Other recently proposed taxa include the *Aigarchaeota* (Nunoura et al. 2011) and *Geoarchaeota* (Kozubal et al. 2012). With the proposal of *Thaumarchaeota* as a new phylum *Crenarchaeota* became restricted to a single class: *Thermoprotei*. This class is normally associated with extreme environments (e.g., hot and acidic environments) (Offre et al. 2013). *Thaumarchaeota*, however, is still considered a *Crenarchaeota* class in some rRNA gene databases.

At the time of their discovery, Archaea were thought to exclusively inhabit extreme environments (high temperature, salinity and/or pressure). Recently, culture independent methods have shown the domain to be much more widespread; it has been found in a variety of habitats (tropical and polar, terrestrial and aquatic, deep and shallow water; Webster et al. 2004; Wuchter et al. 2006; Siboni et al. 2008). Members of Archaea have also been found inhabiting the tissues of various sponge species (Preston et al. 1996; Webster et al. 2004), the mucous of scleractinian corals (Siboni et al. 2008), sediment (Dang et al. 2008; Cao et al. 2011) and the water column (Delong 1992; Wuchter et al. 2006).

Among the best known hosts of microbial communities (Turque et al. 2010; Webster and Taylor 2012), marine sponges, are also abundant and conspicuous components of coral reef systems and play important functional roles both in the benthos and water column. Sponges have been shown to affect water column composition (filtration, secondary

metabolite emanation and nutrient cycling including nitrification) (Wulff 2001; Bell 2008). Although sponges also feed on microbes (Hentschel et al. 2006; Lee et al. 2001), they harbour a remarkable variety of microbial endosymbionts within their tissues. Microbes provide their sponge host with nutrients, aid in metabolic waste processing and provide protection against ultraviolet light (Hentschel et al. 2002; Holmes and Blanch 2007; Webster and Taylor 2012). Sponges, in turn, offer a stable and nutrient-rich environment for microbes (Lee at al. 2001; Hentschel et al. 2012).

Sponges have also been shown to host distinct microbial communities when compared to other non-sponge hosts or non-host biotopes such as sediment or water (Jackson et al. 2012). Recent studies (Webster et al. 2010; Taylor et al. 2013), however, have shown that organisms previously thought to be found exclusively in sponges have also been found in non-sponge hosts or non-host environment. The extremely low concentrations of these organisms though in non-sponge samples had made them virtually impossible to detect using previous conventional molecular techniques, such as DGGE and clone libraries. It should be noted, however, that the presence of these organisms in non-sponge samples may also be the result of sponges releasing symbionts into the water column or sediment during spawning/injury events (Taylor et al. 2013).

The majority of studies have showed Bacteria to be more abundant than Archaea in marine sponges (Taylor et al. 2007; Sharp et al. 2007; Lee et al. 2011). Twenty six bacterial and two archaeal phyla were found in sponges from distinct locations around the world to date (Taylor et al. 2011). However, there are exceptions; the archaeon *Cenarchaeum symbiosum* dominates the *Axinella mexicana* microbial community representing more than 65% of all prokaryotic cells (Preston et al. 1996). This predominance suggests that Archaea play a major role in sponge metabolism. However, the exact roles of Archaea in sponges and in sediment and seawater remain largely unknown.

Despite this uncertainty, it is generally accepted that Archaea play an indispensable role in the transformation, degradation and recycling of nutrients and organic matter (Lee et al. 2001; Webster et al. 2004). Several studies (Wuchter et al. 2006; Cao et al. 2011) have suggested that Archaea and more specifically mesophilic *Crenarchaeota*, which use dissolved inorganic carbon as a carbon source (Prosser and Nicol 2008) may be similar to or even surpass Bacteria (β and γ-proteobacteria) as mediators of oceanic nitrification (Wuchter et al. 2006; Cao et al. 2011). Francis et al. (2005) reported a more widespread presence of ammonia-oxidizing Archaea (AOA) than ammonia-oxidizing Bacteria in both the water column (Black Sea and Monterey Bay) and sediment (San Francisco Bay, Bahía

del Tóbari). In sponges, a recent study (Radax et al. 2012) revealed the same pattern in four cold water sponges. Anaerobic Archaea belonging to the *Euryarchaeota* phylum also seem to be the only organisms capable of performing methanogenesis (Valentine 2007), which is the last step of carbon degradation and prevents the accumulation of organic compounds in the environment (Kendall et al. 2007)

Archaeal community composition and symbiont-sponge relationships appear to be host dependent; for example, coral hosts do not seem to establish specific associations with Archaea since most of the archaeal sequences found in corals are also present in the water column (Rosenberg et al. 2007). Sponges, in contrast, have been shown to host distinct microbial communities when compared to other non-sponge hosts or non-host biotopes (Jackson et al. 2007). Identifying the role and composition of Archaea and other microbes in different biotopes is thus essential in order to gain a better understanding of the coral reef ecosystem and the role of Archaea therein.

In the present study, we assessed the composition of Archaea in four biotopes, two non-host (sediment and seawater) and two host (the sponge species *Stylissa massa* and *Xestospongia testudinaria*) in four reef sites in the Kepulauan Seribu reef system, Indonesia. Our goals were to: 1. Assess to what extent sponges contain unique archaeal communities when compared to communities of Archaea in the surrounding environment (seawater and sediment); 2. Identify closely related organisms to abundant operational taxonomic units (OTUs) using BLAST search; 3. Construct a phylogeny of abundant OTUs in order to assess to what extent biotopes host phylogenetically distinct lineages; 4. Assess to what extent metabolic pathways differ between Archaea in different biotopes.

4.3. Material and methods

4.3.1. Study site

The Jakarta Bay and Kepulauan Seribu coral reef system, also known as Thousand Islands (hereafter referred to as JBTI), is located to the northwest of Jakarta in the Java Sea (Figure 4.3.1).

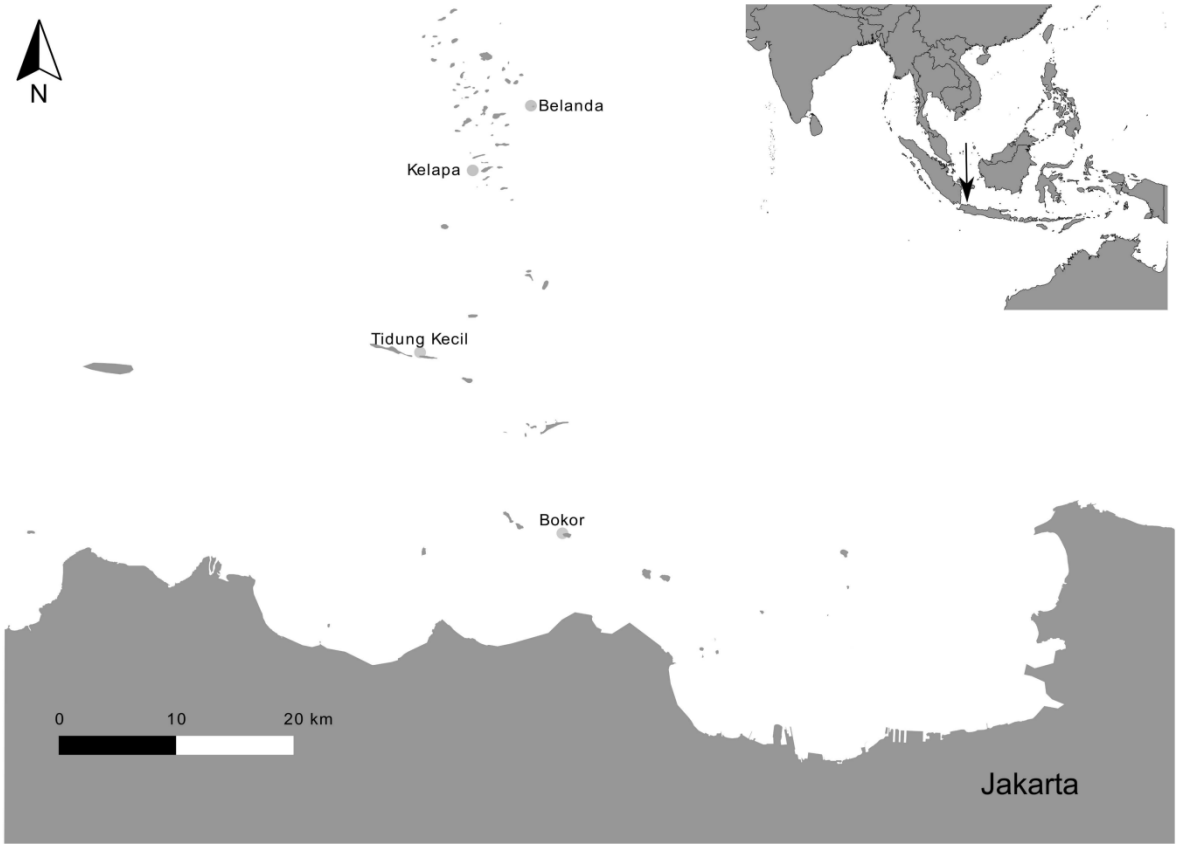

Figure 4.3.1 - Map of the study area (Jakarta Bay and Kepulauan Seribu coral reef system) showing the location of study sites sampled.

This reef system consists of 105 islands or cay-crowned reefs (Cleary et al. 2006) forming a coral island chain of about 80 km (Rachello-Dolmen and Cleary 2007). Thirteen rivers discharge into Jakarta Bay and represent important sources of organic and inorganic suspended matter (domestic sewage) as well as chemical pollutants and other substances (Rachello-Dolmen and Cleary 2007). Organic matter concentrations, however, decline strongly from in-to-offshore as do pollutant loads (Cleary et al. 2008).

4.3.2. Sampling

Four sites (Belanda, Pulau Kelapa, Tidung Kecil and Bokor) were surveyed using SCUBA between July 26th and the 10th of August 2011. At each site, samples were taken of sediment, seawater, and the sponges *Stylissa massa* and *Xestospongia testudinaria*. The sediment samples were taken using the mini core method. Mini-cores, consisting of the

top 5 cm of sediment, were collected using a plastic disposable syringe from which the end had been cut in order to facilitate sampling (Capone et al. 1992). The two sponges studied are common reef sponges in the Indonesian archipelago although they inhabit different habitats. *Stylissa massa* (Carter 1887) is a medium-sized orange colored sponge that mainly occurs in very shallow water (0.5-3 m) whereas the giant barrel sponge *Xestospongia testudinaria* (Lamarck 1815) grows mostly in deeper waters (3-50 m). Specimens were identified to species by NJ de Voogd. Cores of both sponge species were sampled including segments of surface and interior in order to sample, as much as possible, the whole bacterial community. The seawater samples were collected by filtering one liter (Sogin et al. 2006; Bowen et al. 2012) of seawater through a Millipore® White Isopore Membrane Filter (GTTP04700, 47 mm diameter, 0.22 µm pore size). All samples were stored in 96% EtOH (Previsic et al. 2009; Cleary et al. 2013) and kept at temperatures lower than 4ºC immediately after collection. Once in the laboratory, samples were stored at -20 ºC until DNA extraction.

4.3.3. DNA extraction and pyrosequencing

We isolated PCR-ready genomic DNA from seawater, sediment and sponge samples using the FastDNA® SPIN Kit following the manufacturer's instructions. This is an extraction method frequently used for this purpose (Urakawa et al. 2010; Costa et al. 2013; Cleary et al. 2013). Briefly, we prepared sediment samples by centrifuging each one for 30 min at 4400 rpm and 4 ºC; the membrane filter (seawater sample) and sponge samples were each cut into small pieces. The whole membrane filter and 500 mg of sediment and sponge were transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. The microbial cell lysis was performed in the FastPrep® Instrument (Q Biogene) for 80 seconds at the recommended speed. The extracted DNA was eluted into DNase/Pyrogen-Free Water to a final volume of 50 µl and stored at -20°C until use. Pyrosequencing and sequence analysis were performed using previously described methods (Pires et al. 2012; Cleary et al. 2013) with the exception of the pick OTUs step where we used the recently developed UPARSE clustering method and chimera check (Edgar 2013) and the most recent Greengenes database (http:// greengenes.secondgenome.com/downloads/database/13_5) for OTU picking and taxonomic assignment (see Appendix for a detailed description). In the most recent Greengenes release, the recently adopted phylum *Thaumarchaeota* is still considered a class of the *Crenarchaeota* phylum; in the present study we follow the Greengenes taxonomy. The sequences generated in this study can be downloaded from the NCBI SRA: Accession number SRP023167.

4.3.4. Identification of closely related organisms and phylogeny of abundant OTUs

Closely related organisms of numerically abundant OTUs (≥ 100 sequences) were identified using the NCBI Basic Local Alignment Search Tool (BLAST) command line 'blastn' tool with the -db argument set to nt (Zhang et al. 2000). BLAST was also used to obtain sequences for cultured Archaea, which were included in a bootstrap consensus phylogenetic tree based on 1000 replicate trees along with representative sequences belonging to all abundant OTUs; the tree was made using the Mega5 Program (http://www.megasoftware.net/; last checked 2012-11-20; (Tamura et al. 2011) with the Nearest-Neighbor-Interchange and Generalised Time-Reversible model (Tavaré 1986) with Gamma distributed and invariant sites.

4.3.5. Statistical analysis

A square matrix containing the presence and abundances of all OTUs per sample was imported into R (R Core Team 2013) using the read.table() function. Sequences not classified as Archaea (e.g., Bacteria or unclassified at the level of domain, 7865 sequences) and OTUs with < 5 sequences were removed prior to statistical analysis. The OTU abundance matrix was $\log_{10}(x + 1)$ transformed and a distance matrix constructed using the Bray-Curtis index with the vegdist() function in the vegan package (Oksanen et al. 2009) in R. The Bray-Curtis index is one of the most frequently applied (dis)similarity indices used in ecology (Legendre and Gallagher 2001; de Voogd et al. 2009). Variation in OTU composition among biotopes (*S. massa* and *X. testudinaria*, sediment and seawater) was assessed with Principal Coordinates Analysis (PCO) using the cmdscale() function in R with the Bray-Curtis distance matrix as input. We tested for significant variation in composition among biotopes using the adonis() function in vegan. In the adonis analysis, the Bray-Curtis distance matrix of species composition was the response variable with biotope as independent variable. The number of permutations was set at 999; all other arguments used the default values set in the function. Weighted averages scores were computed for OTUs on the first two PCO axes using the wascores() function in the vegan package.

4.3.6. Metagenome analysis

In the present study, we use PICRUSt (Langille et al. 2013) to predict the metagenome of each sample. PICRUSt is a bioinformatics tool that uses marker genes, in this case 16S

rRNA, to predict metagenome gene functional content. These predictions are precalculated for genes in databases including KEGG (Kyoto Encyclopedia of Genes and Genomes; Kanehisa and Goto 2000) and COG (Clusters of Orthologous Groups of proteins). In the present study we used the KEGG database. Output of PICRUST consists of a table of functional counts, *i.e*., KEGG Pathway counts by sample. Note that, because of functional overlap, some orthologs can be represented in multiple pathways. Since KOs can belong to several pathways, we used the categorize_by_function.py script in PICRUSt to collapse the PICRUSt predictions at the level of the individual pathways. This table was in turn used as input for LEfSe (Segata et al. 2011). Using LefSe, we tested data for statistical significance, biological consistency, and effect size relevance among biotopes.

4.4. Results

The sequencing effort yielded 50241 sequences, which were assigned to 4669 OTUs after quality control, OTU picking and removal of chimera. 2305 OTUs remained unidentified at the level of domain and 1428 OTUs were assigned to the Bacteria domain; these were, however, not included in the statistical analysis. The final dataset included 42313 sequences and 936 OTUs of which 146 OTUs remained unclassified at the phylum level.

All archaeal OTUs were assigned to 2 phyla, *Crenarchaeota* and *Euryarchaeota*. In addition to this, OTUs were assigned to 14 classes, 18 orders, 15 families, 12 genera and 3 species. Of these, the classes *Thermoplasmata* and *Thaumarchaeota*, the orders *E2* and *Cenarchaeales,* the families Marine group II and *Cenarchaeaceae*, the genera Cenarchaeum and *Nitrosopumilus* and the species *Cenarchaeum symbiosum* were the most abundant. Thirty three OTUs from 12356 sequence reads were identified from *X. testudinaria* hosts while 27 OTUs from 10580 sequence reads were identified from *S. massa* hosts. With respect to the non-host biotopes, 322 OTUs from 8570 sequence reads were identified from seawater samples while 724 OTUs from 10807 sequence reads were identified from sediment samples.

BLAST was used to find closely related organisms to all 37 abundant (≥ 100 sequences) OTUs (Table 4.4.1). The most abundant OTU overall was OTU-1, assigned to the species *Cenarchaeum symbiosum* and found predominantly in *S. massa* hosts and represented by 10473 sequences. This OTU was also the only dominant symbiont found in *S. massa*; the remaining 26 OTUs found in this sponge had less than 40 sequences each. OTU-1

was closely related to an organism previously isolated from *Phakellia fusca* hosts in the South China Sea.

Only *X. testudinaria* and sediment hosted biotope-specific abundant OTUs. Of the 9 abundant OTUs in *X. testudinaria*, 7 were host-specific (3, 17, 18, 16, 317, 594, 331); with the exception of OTU 18 (unclassified) all the remaining 8 were assigned to the *Cenarchaeaceae* family. Of these, 5 were assigned to the genus *Nitrosopumilus.* The most abundant OTU was OTU-2; assigned to the genus *Nitrosopumilus* and closely related to an organism isolated from *Xestospongia muta* hosts in Florida.

In the sediment biotope, the recorded number of OTUs (724) was almost twice as much as the sum of all OTUs from the remaining three biotopes (382). Of the 724 OTUs only 18 were considered abundant and only one of these was host-specific (OTU-28); this was assigned to the Marine Benthic Group B (MBGB) class. The most abundant OTU was OTU-7 assigned to the genus *Nitrosopumilus* and closely related to an organism isolated from sediment samples collected in Oujiang River, China.

In seawater samples, the most abundant OTU was OTU-4 assigned to Marine group II and closely related to an organism isolated from water samples collected in Arabian Sea. With the exception of OTU-11 (unclassified), all abundant OTUs in seawater were identified as belonging to the Marine group II family.

There were marked differences in the abundance of higher archaeal taxa among biotopes (Figure 4.4.1). The *Euryarchaeota* were more abundant in non-host biotopes than in sponge hosts. The abundance of *Euryarchaeota* in sponge hosts is largely due to the contribution of *S. massa* with four times more sequences than *X. testudinaria*; only 0.2 % of the *X. testudinaria* sequences were assigned to the *Euryarchaeota* phylum. In contrast, the *Crenarchaeota* were much more abundant in sponge hosts. The taxa MCG (Miscellaneous Crenarchaeotal Group; *Crenarchaeota*) and YLA114 (*Euryarchaeota*) were virtually all restricted to non-host biotopes.

4.4.1. Higher taxon abundance

Of the 15 families found in this study just two were detected (> 0.1%) in sponge hosts: *Cenarchaeaceae* (98% *S. massa* and 99.8% *X. testudinaria*) and Marine group II (1.95% *S. massa* and 0.2% *X. testudinaria*). The relative abundance of the most abundant OTU in each biotope was highest in *S. massa*, where more than 97% of sequences on average

belonged to a single OTU. For *X. testudinaria*, just over 69% of sequences on average belonged to a single OTU. Dominance was lowest for the sediment biotope where just over 14% of sequences belonged to a single OTU on average.

Table 4.4.1 - List of most abundant OTUs (≥ 100 sequences) including OTU-numbers; number of sequences (reads); biotope where the OTUs were found (Group); their taxonomic affiliation; GenBank GenInfo sequence identifiers (GI) of closely related organisms identified using BLAST and sequence identity (Sq ident) of these organisms with our representative OTU sequences.

OTU	Reads	Group	Phylum	Class	Order	Family	Genus	Species	GI	Sq ident
1	10,473	Sm	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Cenarchaeum*	*Symbiosum*	*340764424*	100
2	6,250	Xt	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691426*	99.3
3	2,228	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*445066213*	96.5
4	1,601	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*220685393*	100
5	1,659	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*125381583*	100
6	1,592	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*321159150*	100
7	1,961	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*529279729*	100
8	801	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*383933392*	100
9	881	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Cenarchaeum*	*Symbiosum*	*321159184*	99.8
10	636	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*310871774*	98.4
11	729	Wt	[b]							
12	360	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*220685221*	100
13	375	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*394999368*	100
15	188	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*39546718*	100
16	222	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*44506621l*	96.5
17	267	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691432*	96.8
18	261	Xt[a]	[b]							
19	195	Sd	*Euryarchaeota*	*Thermoplasmata*	*E2*	*CCA47*			*364530814*	99.8
20	139	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*145651460*	100
22	105	Sd	*Euryarchaeota*	*Thermoplasmata*	*E2*	*DHVEG-1*			*364527263*	99.8
24	202	Sd	*Crenarchaeota*	*MCG*					*145651451*	99.5
28	174	Sd[a]	*Crenarchaeota*	*MBGB*					*389591832*	100
29	107	Sd	*Euryarchaeota*	*Thermoplasmata*	*E2*				*408718821*	99.8
39	100	Sd	[c]						*223031543*	97.9
229	2589	Xt	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691431*	99.5
236	202	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*125381472*	99.8
246	589	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*383933255*	100
317	203	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691434*	99.0
331	173	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*445066213*	96.5
399	374	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*83416103*	100
594	197	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691432*	100
1,016	125	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*310871821*	100
1,519	273	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*548783414*	99.5
1,736	808	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*125381373*	100
2,921	377	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*529279806*	99.8
3,207	200	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*125381446*	100
4,233	641	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*529279817*	99.5

[a] Represent an OTU only present in a particular biotope

[b] Represent an OTU unclassified at the domain level (not included in the analysis)

[c] Represent an OTU unclassified at the phylum level

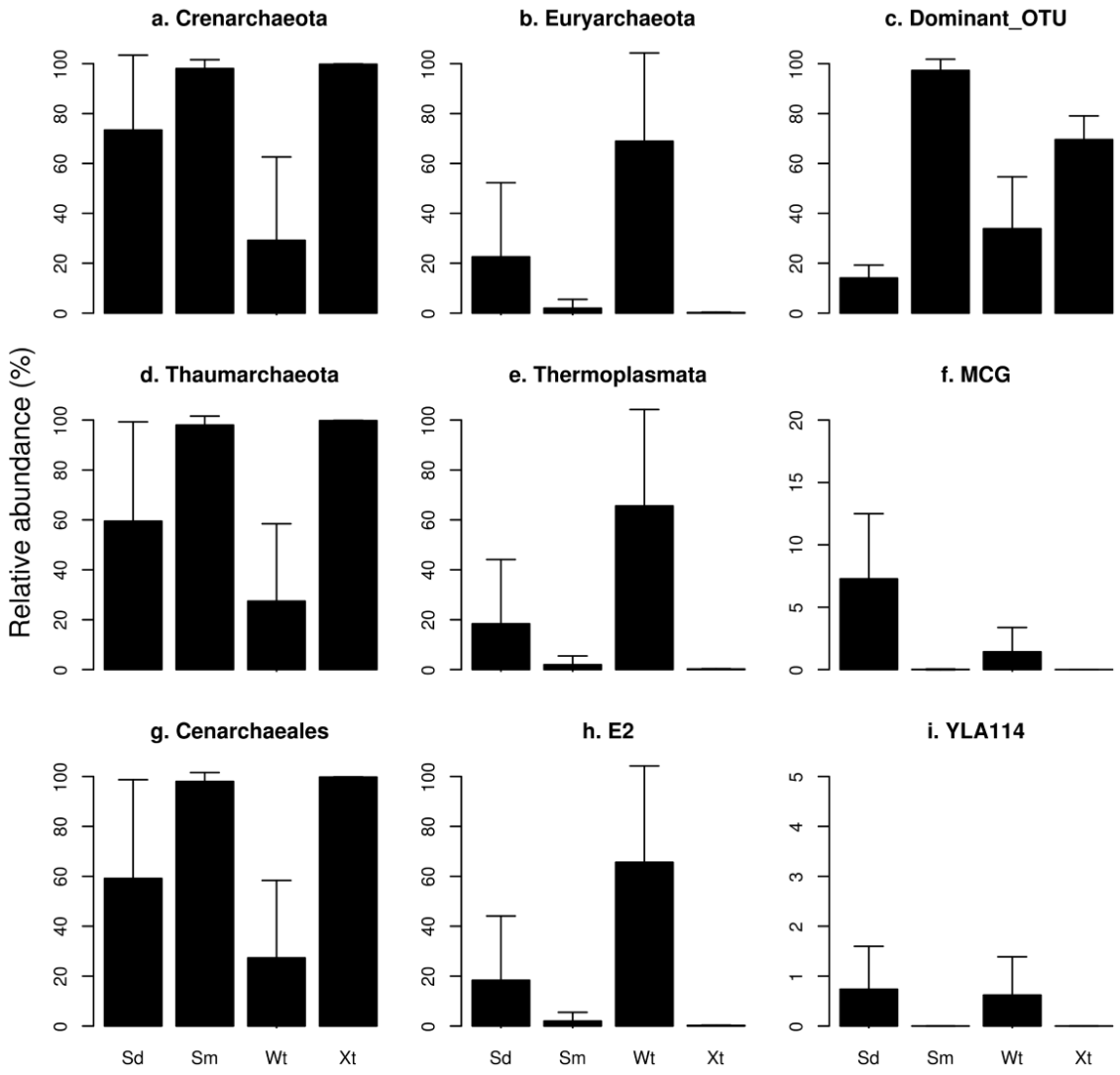

Figure 4.4.1 - Mean relative abundance of the most abundant archaeal phyla, classes, orders and the dominant OTUs for samples from seawater (Wt), sediment (Sd), *S. massa* (Sm) and *X. testudinaria* (Xt). Error bars represent a single standard deviation. The dominant OTU represents the mean abundance for the single most dominant OTU in each sample, thus not necessarily the same OTU.

4.4.2. Importance of biotope in structuring composition

There was a highly significant difference in archaeal composition among biotopes ($F_{3,12}$ = 10.39, $P < 0.001$, R^2 = 0.722). Variation among biotopes thus explained 72% of the variation in archaeal composition. A PCO ordination (Figure 4.4.2) of the first two axes shows four distinct clusters representing samples from the four biotopes.

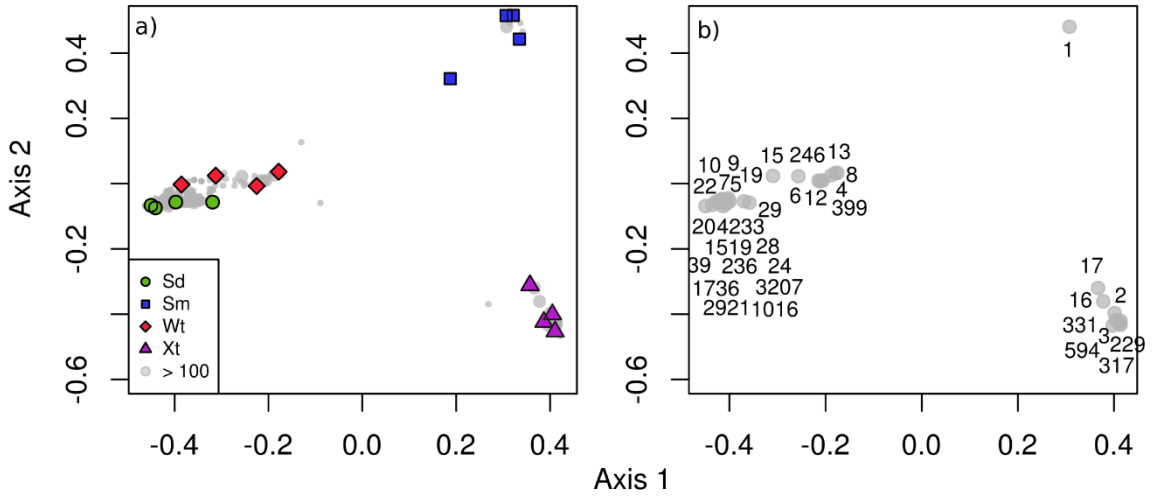

Figure 4.4.2 - Ordination showing the first two axes of the PCO analysis. a) Symbols represent samples from seawater (Wt), sediment and *S. massa* (Sm) and *X. testudinaria* (Xt). Very small circles represent OTUs < 100 sequence reads. b) Numbers represent abundant (≥100 sequence reads) OTUs referred to in Table 4.4.1.

Although forming distinct clusters, samples from sediment and seawater were closer to one another in the ordination than either to the sponge samples. Axis 1 of the PCO ordination separates samples from non-host biotopes and both sponge hosts. Axis 2 separates samples from *S. massa* and *X. testudinaria* hosts.

Less than 1% of OTUs were found in all four biotopes (6 of 936). Only 17.6% of the sponge OTUs were shared between *S. massa* and *X. testudinaria* hosts (9 of 51); however, these OTUs were also shared with seawater and sediment. No OTUs were found in both sponge hosts that were not present in either sediment or seawater. Only 14.9% of the OTUs (136 of 910) restricted to the non-host biotopes were shared between seawater and sediment and the majority (123 of 136) were not present in sponge hosts.

4.4.3. Phylogeny

In the phylogenetic tree (Figure 4.4.3) there were two main clusters, 1) a cluster consisting of OTUs belonging to the *Crenarchaeota* phylum and 2) a cluster consisting of OTUs belonging to the *Euryarchaeota* phylum.

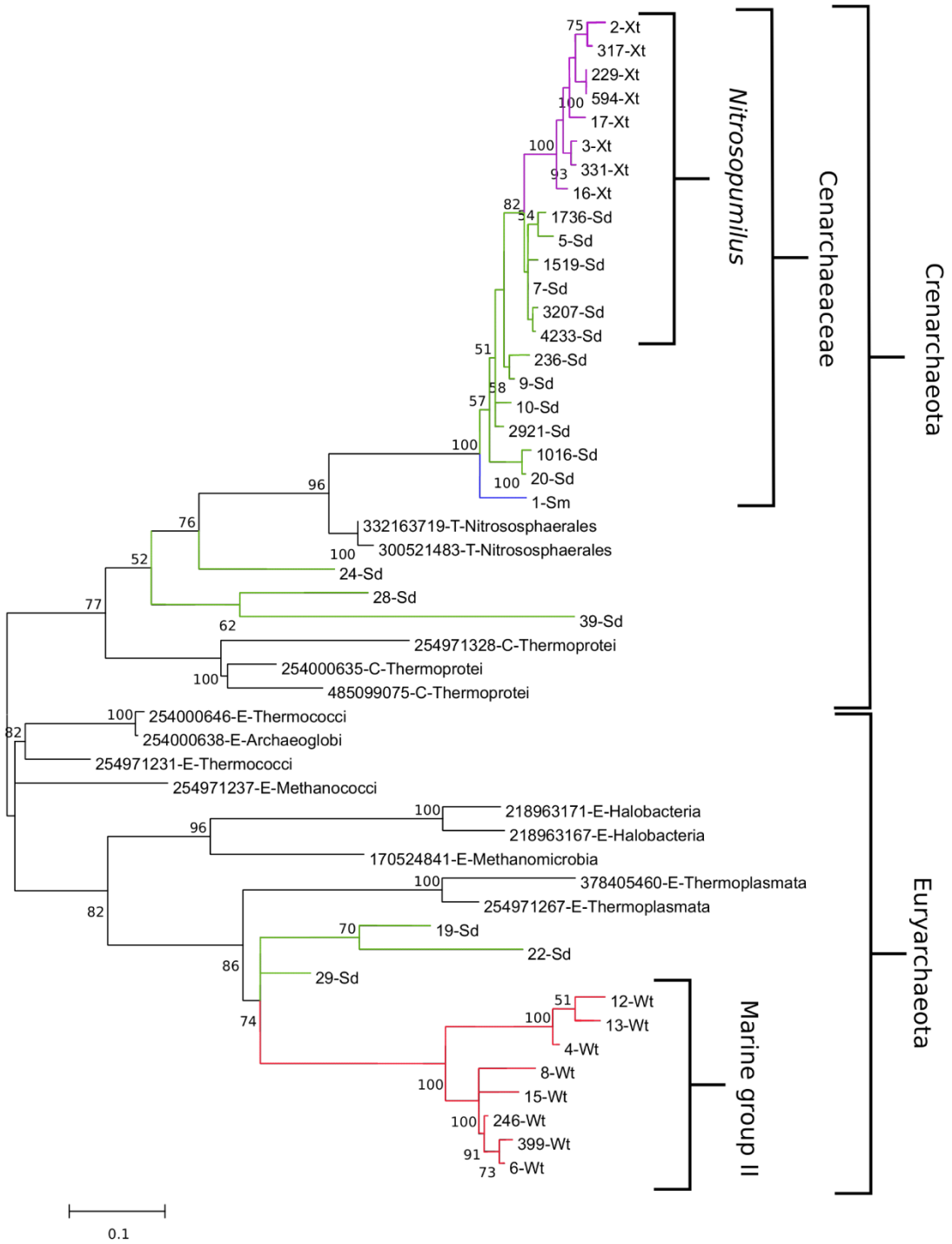

Figure 4.4.3 - Phylogenetic tree of the archaeal 16S rRNA gene sequences recovered from the studied biotopes (seawater, sediment, *S. massa* and *X. testudinaria*); built using the Mega5 program with the Nearest-Neighbor-Interchange and Generalised Time-Reversible model; bootstrap values lower than 50% were omitted. The number of each OTU is indicated as are GenBank GenInfo sequence identifiers of cultured archeal sequences. OTUs are assigned to the following clusters: Wt: mainly found in seawater biotope; Sd: found in sediment biotope; Sm: found in *S. massa* biotope; Xt: found in *X. testudinaria*.

Inside the main cluster of *Euryarchaeota*, the most abundant seawater OTUs belonging to Marine Group II formed a small distinct cluster. Inside the *Crenarchaeota* main cluster, *X. testudinaria*, *S. massa* and some sediment OTUs clustered together to form a cluster consisting of *Cenarchaeaceae* members. Some of the sediment and *X. testudinaria* OTUs present in the *Cenarchaeaceae* cluster grouped together to form a small cluster of members of the thaumarchaeon *Nitrosopumilus*, supported by high bootstrap value (82). OTUs found exclusively or predominantly in *X. testudinaria* formed a distinct and very well supported cluster (100).

4.4.4. Metagenome analysis

Using LEfSe, we identified significant differences between the different biotopes. Differences in the top level functional categories between biotopes included enrichment of the Cellular Processes in *X. testudinaria*; Environmental Information Processing in sediment; Genetic Information Processing and Human Diseases in *S. massa* and Organismal Systems in seawater. Differences in functional subcategories between biotopes included enrichment of the Amino acid metabolism and Metabolism of cofactors and vitamins in *S. massa*; Biosynthesis of other secondary metabolites, Energy metabolism and Metabolism of terpenoids and polyketides in *X. testudinaria*; Carbohydrate metabolism, Enzyme Families, Lipid metabolism and Xenobiotic biodegradation and metabolism in seawater and Glycan biosynthesis and metabolism in sediment. The relative abundance analysis of the functional individual pathways (Figure 4.4.4) gives some insight into the differences observed among biotopes showing which individual pathways generated the significant differences in the top level functional categories and subcategories.

Differences at this level included enrichment of the Citrate cycle (TCA cycle), Glycolysis/Gluconeogenesis, ABC Transporters, Pentose and glucuronate interconversions (Carbohydrate metabolism); Isoquinoline alkaloid biosynthesis, Methane metabolism (Energy metabolism), beta − Alanine metabolism (Metabolism of other amino acids), Aminobenzoate degradation, Caprolactam degradation, Chloroalkane and chloroalkene degradation, Nitrotoluene degradation (Xenobiotic biodegradation and metabolism) and Limonene and pinene degradation (Metabolism of terpenoids and polyketides) in non-host biotopes and enrichment of the Aminoacyl− tRNA biosynthesis (Genetic Information Processing/Translation), Phenylalanine, tyrosine and tryptophan biosynthesis, Valine, leucine and isoleucine biosynthesis, (Amino acid metabolism) Basal

transcription factors (Genetic Information Processing/ Transcription), Oxidative phosphorylation, Sulfur metabolism (Energy metabolism), Tetracycline biosynthesis (Metabolism of terpenoids and polyketides), Pantothenate and CoA biosynthesis, Porphyrin and chlorophyll metabolism, Ubiquinone and other terpenoid-quinone biosynthesis (Metabolism of cofactors and vitamins) and Atrazine degradation and Toluene degradation (xenobiotic biodegradation and metabolism) in sponge biotopes.

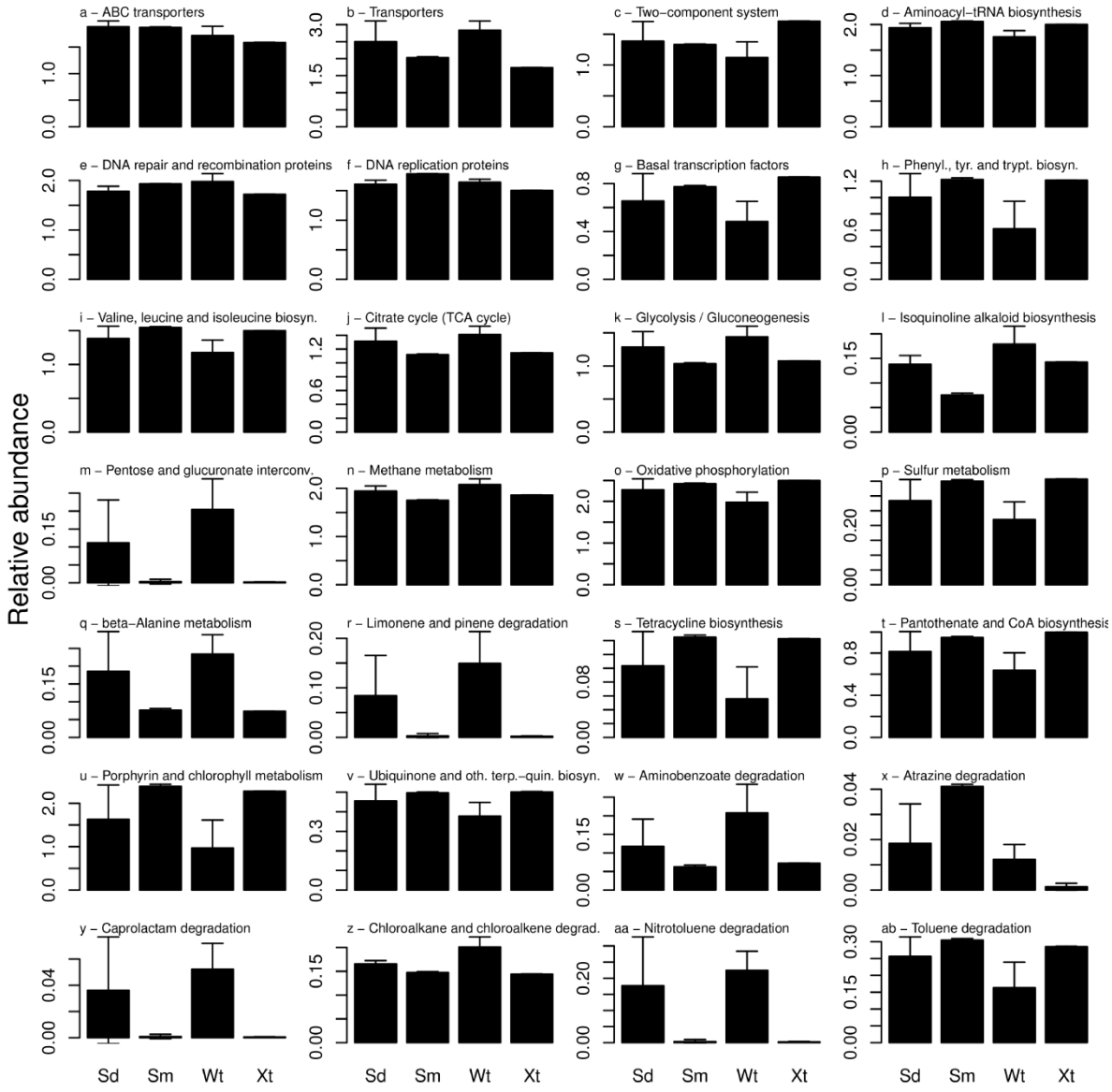

Figure 4.4.4 - Mean relative abundance of gene counts for selected functional individual pathways for samples from seawater (Wt), sediment (Sd), *S. massa* (Sm) and *X. testudinaria* (Xt). Error bars represent a single standard deviation. The individual pathways shown include the following KEGG categories a) ABC transporters, b) Transporters, c) Two-component system, d) Aminoacyl − tRNA biosynthesis, e) DNA

repair and recombination proteins, f) DNA replication proteins, g) Basal transcription factors, h) Phenylalanine, tyrosine and tryptophan biosynthesis, i) Valine, leucine and isoleucine biosynthesis, j) Citrate cycle (TCA cycle), k) Glycolysis/Gluconeogenesis, l) Isoquinoline alkaloid biosynthesis, m) Pentose and glucuronate interconversions, n) Methane metabolism, o) Oxidative phosphorylation, p) Sulfur metabolism, q) beta − Alanine metabolism, r) Limonene and pinene degradation, s) Tetracycline biosynthesis, t) Pantothenate and CoA biosynthesis, u) Porphyrin and chlorophyll metabolism, v) Ubiquinone and other terpenoid-quinone biosynthesis, w) Aminobenzoate degradation, x) Atrazine degradation, y) Caprolactam degradation, z) Chloroalkane and chloroalkene degradation, aa) Nitrotoluene degradation, ab) Toluene degradation.

4.5. Discussion

4.5.1. Higher taxon abundance

In line with previous studies (Lee et al. 2011; Pires et al. 2012) only two archaeal phyla (*Euryarchaeota*, *Crenarchaeota*) were detected in our samples. *Crenarchaeota* were the most abundant phylum in all biotopes except seawater. Several studies have found sponges to be exclusively associated with *Crenarchaeota* (Webster et al. 2004; Schmitt et al. 2008; Turque et al. 2010). Some other studies, however, observed *Euryarchaeota* in some sponges species albeit in low abundances (Holmes and Blanch 2007; Lee et al. 2011). In sponge samples most sequences belonged to the *Thaumarchaeota* class. Mesophilic *Crenarchaeota* (*Thaumarchaeota*) have been shown to be important players in geochemical cycles (Wuchter et al. 2006; Brochier-Armanet et al. 2008). Previous studies linked a high abundance of *Thaumarchaeota* to peaks in nitrification in the water column of the Dutch coastal North Sea and subsequent reduction of ammonia and accretion of nitrite concentrations (Wuchter et al. 2006; Pitcher et al. 2011); *Thaumarchaeota* may play a similar role in JBTI. The fact that the reefs of JBTI are subject to elevated levels of pollution (Rachello-Dolmen and Cleary 2007) means that organisms capable of nitrifying toxic ammonia (NH_3) to nitrate (NO_3^-) may play a crucial role in maintaining a healthy coral reef environment (Rusch et al. 2009; Bartlett 2013).

As expected, given the highly selective nature of sponges (Hentschel et al. 2006), the number of OTUs found in non-host biotopes was substantially higher than in sponge hosts. In contrast, Lee et al. (2011) found a less diverse archaeal community in Red Sea

seawater samples than in *X. testudinaria*. This difference in archaeal composition between seawater and *X. testudinaria* in both regions may reflect geographic differences. However, methodological differences (mainly in sample preservation before extraction and in the hypervariable region and universal primers selected) may also be at least partially responsible for the differences in archaeal composition between both studies.

Stylissa massa shared a higher percentage of OTUs with non-host biotopes than *X. testudinaria* (77.8% and 39.4% respectively). Among other things, this may indicate a higher permeability of *S. massa* to incorporate environmental archaeal OTUs or more pronounced antimicrobial activity by *X. testudinaria*. *Xestospongia* species have previously been shown to produce compounds with antimicrobial activity (Li et al. 2012) as has *S. massa* (Rohde et al. 2012). However, antimicrobial activity has been only demonstrated for bacteria and fungi (Zhou et al. 2010; Rohde et al. 2012); no antiarchaeal activity has been reported in sponges.

The majority of OTUs found in seawater samples were assigned to the phylum *Euryarchaeota* (68.7%). However, in contrast to Lee et al. (2011), where nearly all archaeal reads from seawater samples were classified as *Euryarchaeota*, in the present study, almost 30% of the seawater sequences were assigned to *Crenarchaeota* and of those, 94.6% belong to the class *Thaumarchaeota*. In line with our study, Qian et al. (2010) reported a dominance of *Euryarchaeota* in the upper layers (2 and 50 m) of Red Sea waters. Other studies (DeLong et al. 1994; Massana et al. 2000), however, found *Crenarchaeota* to be the dominant phylotype in seawater samples. Almost all *Euryarchaeota* classes have methanogenic taxa (Offre at al. 2013). In this study, besides the numerous *Thermoplasmata* class (196 OTUs 7846 sequences), two other archaeal taxonomic classes known as methanogens were found: *Methanobacteria* (17 OTUs, 62 sequences) and *Methanomicrobia* (5 OTUs, 12 sequences). The predominance of the *Thermoplasmata* class in seawater indicates that methanogenesis and methane oxidation are important processes in this biotope. The results of our PICRUSt and LEfSe analysis support this hypothesis and showed significant enrichment of the Methane metabolism pathway in seawater. The detection of methanogenic Archaea in oxic environments is not new. Despite being generally believed to be produced exclusively by strictly anaerobic Archaea, methane has been found to be supersaturated in oxygenated surface waters (Reeburgh 2007; Grossart et al. 2011). This phenomenon has been called the “ocean methane paradox” (Kiene 1991) and several explanations have emerged in the literature (Kiene 1991; Marty et al 1997; Grossart et al. 2011; Metcalf et al. 2012).

Marine group II (*Thermoplasmata/E2*) was the most abundant family (62.9% of all sequences) in seawater. Only 1.95, 0.21 and 0.44% of *S. massa*, *X. testudinaria* and sediment sequences on average were assigned to this family. Wemheuer et al. (2012) previously reported *Thermoplasmata* as the third most abundant archaeal class in seawater samples collected in the North Sea. DeLong and Pace (2001) noted that E2 was predominantly found in marine plankton and Holmes and Blanch (2007) reported the presence of Marine Group II in three sponges from the Timor Sea, Australia (*Axechina raspailioides*, *Reniochalina stalagmitis* and *Ptilocaulis* sp.). According to Baker et al. (2012) Marine Group II may also be active players in the recycling of organic carbon and nitrogen. One hundred and forty six OTUs remained unclassified at the phylum level. All of these potential novel taxa were found in sediment and/or seawater indicating that these are rich biotopes that require more intense research.

4.5.2. Importance of biotope in structuring composition

The PCO ordination and phylogenetic tree revealed marked compositional differences among the four biotopes. The four biotopes studied here thus host compositionally and phylogenetically distinct communities of Archaea. In contrast to our study, Lee et al. (2011) failed to distinguish differences in archaeal composition among sponge species, although they did find compositional differences between sponges and seawater.

4.5.3. Metagenome

The contribution of sponge symbionts to the health, performance and survival of their host are well known (Taylor et al. 2007; Freeman and Thacker 2011). According to Freeman and Thacker (2011) around 50% of sponge energy requirements are fulfilled by microbial processes. This is supported by our metagenomic results, which showed a significant enrichment of almost all functional individual pathways associated to the energy metabolism in *X. testudinaria*; namely Oxidative phosphorylation; Carbon fixation and Sulfur metabolism. Although sponges acquire part of their nutritional requirements through filter feeding, some species rely mainly on their microbial community to fulfill their energy and carbon needs. For example, *Aplysina cauliformis* and *Neopetrosia subtriangularis*, obtain about 77% of their carbon needs from their microbial cells as opposed to only 27% for *Niphates erecta* (Freeman and Thacker 2011). The higher abundance of enzymes encoding for carbon fixation in *X. testudinaria* indicates that this sponge species primarily relies on high microbe densities to acquire carbon. *S. massa*, in contrast, may not rely so

heavily on microbial symbiosis, and obtains a higher part of its carbon from the filtered particulate organic matter; although this remains to be demonstrated. In addition to providing supplemental nutrition, *X. testudinaria* archaeal symbionts may also play an important role in detoxifying sponge tissues and metabolizing toxic by-products such as hydrogen sulfide through sulfur oxidation, as has been shown by Hoffmann et al. (2009) and Radax et al. (2012) for sulphur-oxidising bacteria.

Nitrogen and methane metabolism were significantly enriched in seawater. 'Methane metabolism' encompasses methanogenesis and methane oxidation; normally in the archaeal domain both processes occur under strict anaerobic conditions (Offre et al. 2013). Based on this, one would expect that these processes to be much more prevalent in sediment than in oxic seawater. In the present study the significant enrichment of the Methane metabolism pathway together with the predominance of OTUs assigned to methanogenic classes in seawater biotopes can be the result of physical transport from anoxic sediment (Reeburgh 2007; Grossart et al. 2011) or attachment of methanogens to microanoxic environments present in the water column such as fecal pellets, photoautotrophs, or marine snow particles (Marty et al. 1997; Grossart et al. 2011). As happens in aerated soils (Angel et al. 2012), the methanogens present in JBTI seawater may also be able to survive under oxic conditions and become active only under favorable conditions. Moreover, *Thaumarchaeota* were recently associated with an aerobic process of methane production. *Nitrosopumilus maritimus* seems to be involved in methylphosphonic acid (MPn) biosynthesis which, in turn, is used by aerobic Bacteria as a source of phosphorus to produce methane (Metcalf et al. 2012). Although there were no *Nitrosopumilus* among the most abundant water OTUs, the number of *Nitrosopumilus* sequences in this biotope was not negligible (826 sequences).

The predominance of the archaeal ammonia monooxygenase subunit genes (AmoA) when compared to their bacterial counterparts in marine water and sediment biotopes is well known (Francis et al. 2005; Wuchter et al. 2006) and suggests an important role of Archaea in global nitrogen cycling. In the present study, the nitrogen metabolism pathway was significantly enriched in seawater, which is surprising due to the low abundance of Thaumarchaeota in water when compared to sponge biotopes. Some studies have found nitrogen metabolism related functions enriched in different sponge species when compared to seawater samples (Fan et al. 2012); however, these findings concern the entire prokaryotic community.

The sponge symbiotic relationship with photosynthetic microorganisms is well known (Wilkinson 1983; Taylor et al. 2007; Bell 2008; Erwin and Thacker 2008). This consortium

occurs more frequently in oligotrophic waters; sponges benefit from photosymbiotic derived nutrients while photosynthetic microbes benefit from metabolic end-products synthesized by sponges. Indeed, some sponge species obtain almost 50% of their nutritional requirements from their photosynthetic symbionts (Wilkinson 1983; Freeman and Thacker 2011). Cyanobacteria have been the photosymbiont more commonly reported in sponge hosts (Erwin and Thacker 2008; Erwin et al. 2011). Here, sponge archaeal communities were enriched in the function of Porphyrin and chlorophyll metabolism associated with photosynthesis. Some Archaea are able to convert light into chemical energy via ATP synthesis (Hohmann-Marriot and Blankenship 2011). These Archaea can be especially important to *S. massa* which, living in shallow water, may suffer from exposure to air during low tide and concomitant elevated UV exposure. The association with these symbionts may enable *S. massa* to deal with these stresses.

The higher expression of Citrate cycle (TCA cycle) and Glycolysis/Gluconeogenesis pathways in archaeons living in non-host biotopes appears to indicate that aerobic respiration is the primary carbon assimilation and energy generation process for these organisms. TCA Cycle components were previously identified in *C. Symbiosum* (Hallam et al. 2006), an archaeon also present in JBTI sediment samples

Nutrient availability inside sponges is necessarily different when compared to nutrient availability in seawater or sediment; these differences can lead to the adoption of distinct nutritional strategies by the archaeal community, as shown by the higher relative abundance of transporters in non-host biotopes. These transporters are involved in nutrient acquisition being responsible for the transport of sugars, lipid, proteins, nitrogen and others substrates across microbial membranes. The high expression of these transporters can be more important in water or sediment biotopes where the competition for nutrients is greater and its availability lower. These transporters can confer an important competitive advantage maximizing the archaeon nutrient uptake ability. Fan et al. (2012) recently showed ABC transporters to be more abundant in sponge symbionts when compared to planktonic microbes, however their study included both archaeal and bacterial microbiomes. In the present study, ABC transporters were most abundant in the sediment biotope as was the top level functional category 'Environmental Information Processing'. ABC transporters were also more abundant in *S. massa* than seawater or *X. testudinaria*. The higher expression of ABC transporters in *S. massa* suggests that this sponge is a relatively nutrient poor environment when compared to *X. testudinaria*.

Xenobiotic biodegradation and metabolism was one of the functional subcategories significantly enriched in seawater biotopes. Some of the functional individual pathways

with a high relative abundance in this biotope were: aminobenzoate, caprolactam, chloroalkane and chloroalkene and nitrotoluene degradation. However, some functional individual pathways of this functional subcategory were found in high relative abundance in sponge biotopes and particularly in *S.massa*, namely Atrazine and Toluene degradation. These organic compounds are used in a vast number of industries (metal, paint, textile, wood and chemical) and also in agriculture (herbicides) and are considered important environmental contaminants. These enter the aquatic environment largely through agricultural and industrial runoff (Murdock et al. 2013). These findings suggest that communities of Archaea may be relevant Xenobiotic degraders and act as bioremediators in polluted environments. The high relative abundance of enzymes involved in xenobiotic degradation in *S. massa* when compared to *X. testudinaria* may be the result of both species occupying distinct habitats; the shallow distribution of *S. massa* may make it more subject to anthropogenic pollutants than *X. testudinaria*. With the degradation of these xenobiotic compounds, archaeal symbionts obtain carbon, nitrogen and energy while promote the removal of toxic compounds from the sponge host tissue. Proteins thought to be involved in the degradation of aromatic compounds were previously found in the sponge species *Cymbastela coralliophila*, *Rhopaloeides odorabile*, and *Cymbastela concentrica* (Fan et al. 2012). Despite not being a xenobiotic, limonene may also be considered an environmental contaminant. Limonene degradation was enriched in non-host biotopes. Limonene is a monoterpene produced both biogenically and anthropogenically; it is used industrially in metal, electronic, printing and paint industries as a substitute for other solvents (chlorinated or hydrocarbons). In aquatic environments, this compound presents high acute toxicity to some aquatic organisms (fish and daphnia) and may bioaccumulate (Filipsson et al. 1998).

Sponge symbionts have been shown to produce antibiotic compounds with a protective function against potential sponge pathogens and competitors (Taylor et al. 2007; Flemer et al. 2012). Here, sponge archaeal communities were enriched in the function of Tetracycline biosynthesis; an antibiotic with antibacterial activity toward pathogenic microorganisms. Additionally, *S. massa* symbionts showed high expression of Human Diseases and more specifically infectious diseases subcategories (bacterial, viral, and parasitic) and significant enrichment in Amoebiasis and Tuberculosis individual pathways. Several studies have linked sponge-isolated compounds with the treatment of human diseases (Sipkema et al. 2005). It has been assumed that these compounds are synthesized not by the sponge itself but by their symbionts (Taylor et al. 2007; Flemer et al. 2012). Several studies have reported marine sponge secondary metabolites with

antibacterial activity against *Mycobacterium tuberculosis* (Quideau et al. 2002; Copp and Pearce 2007) and anti-amoebic properties against amoebic parasites (Lakshmi et al. 2009). These results suggest that *S. massa* is a potential source of secondary metabolites with activities against vectors of human diseases.

Stylissa massa symbionts were also significantly enriched with pathways associated with amino acid metabolism (biosynthesis of valine, isoleucine, leucine, phenylalanine, tyrosine and tryptophan) and metabolism of cofactors and vitamins. Besides being building blocks for proteins, amino acids are also considered precursors for the production of secondary metabolites (Demain 1998; Bromke 2013). For example, leucine seems to induce bacitracin synthetase while tryptophan induces the dimethylallyltryptophan production in ergot alkaloid biosynthesis (Haavik and Froyshov; Krupinski et al. reviewed in Demain 1998). This can be a justification for the higher expression of genes encoding the biosynthesis of amino acids in sponge biotopes where secondary metabolite production is higher. Pathways associated with the biosynthesis of other secondary metabolites were significantly enriched in *X. testudinaria*. *S. massa* seems also to rely more on their archaeal symbionts for the acquisition of very important compounds as vitamins and cofactors than on their filter-feeding activity. For example, vitamin B12, that needs to be acquired by sponges, is also an important cofactor in the Wood–Ljungdahl pathway, which is a mechanism used by sulfate reducers and methanogens to convert carbon compounds to organic carbon (Seravalli et al. 2002; Siegl et al. 2011).

Our study is the first to assess archaeal composition in different sponge hosts, seawater and sediment in a coral reef environment. As such, we provide novel insights into the distribution of Archaea. OTU composition differed significantly among biotopes and there were marked differences in the number of OTUs found in each biotope. The sediment biotope in particular harboured the greatest number of OTUs and a phylogenetically diverse archaeal community. Our phylogenetic tree, furthermore, provides evidence that sponges host phylogenetically distinct archaeal assemblages. The abundant OTUs from *X. testudinaria* formed a distinct and well supported cluster in our phylogenetic tree. Several significant differences were observed in functional pathways between archaeal communities of both sponge species and between non-host archaeal communities. The major differences in functional pathways were, however, between sponge and non-host biotopes. The results of our PICRUSt and LEfSe analysis suggested that phylogenetically distinct archaeal communities tend to perform specific roles in biotopes occupying the same physical environment. Our results also suggested different nutritional strategies in non-host and sponge Archaea and a clear interdependence between sponge hosts and archaeal symbionts in terms of nutrient acquisition. With the exception of the methane and

nitrogen metabolic pathways, all energy metabolic pathways were enriched in sponges when compared to non-host biotopes. This indicates the importance of non-host and sponge biotopes in structuring archaeal community composition. It also suggests that Archaea from non-host and sponge biotopes may play complementary roles in important ecosystem functions such as nutrient cycling. Further studies are needed to assess the importance of sponge and other archaeal communities in nutrient dynamics and other ecosystem functions in coral reef environments.

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4.7. References

Angel R, Claus P & Conrad R (2012) Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *ISME J* 6: 847–862. doi: 10.1038/ismej.2011.141.

Baker BJ, Lesniewski RA, Dick GJ (2012) Genome-enabled transcriptomics reveals archaeal populations that drive nitrification in a deep-sea hydrothermal plume. *ISME J* 6:2269–2279. doi:10.1038/ismej.2012.64.

Barns SM, Delwiche CF, Palmer JD, Pace NR (1996) Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *P Natl Acad Sci USA* 93:9188–9193. doi: 10.1073/pnas.93.17.9188.

Bartlett T (2013) Small scale experimental systems for coral research: Considerations, planning, and recommendations. NOAA Technical Memorandum NOS NCCOS 165 and CRCP 18. Charleston, South Carolina, pp 68.

Bell J (2008) The functional roles of marine sponges. *Estuar Coast Shelf S* 79:341–353. doi: 10.1016/j.ecss.2008.05.002.

Bowen JL, Morrison HG, Hobbie JE & Sogin ML (2012) Salt marsh sediment diversity: a test of the variability of the rare biosphere among environmental replicates. *ISME J* 6: 2014–2023. doi: 10.1038/ismej.2012.47.

Brochier-Armanet C, Gribaldo S, Zivanovic Y, Confalonieri F, Forterre P (2005) *Nanoarchaea*: representatives of a novel archaeal phylum or a fast-evolving euryarchaeal lineage related to *Thermococcales*? *Genome Biol* 6:R42. doi: 10.1186/gb-2005-6-5-r42.

Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P (2008) Mesophilic *Crenarchaeota*: proposal for a third archaeal phylum, the *Thaumarchaeota*. *Nat Rev Microbiol* 6: 245–252. doi: 10.1038/nrmicro1852.

Brochier-Armanet C, Gribaldo S, Forterre P (2011) Spotlight on the *Thaumarchaeota*. *ISME J*. 6:227-230. doi: 10.1038/ismej.2011.145.

Bromke MA (2013) Amino acid biosynthesis pathways in diatoms. *Metabolites* 3: 294–311.

Cao H, Li M, Hong Y, Gu J (2011) Diversity and abundance of ammonia-oxidizing Archaea and bacteria in polluted mangrove sediment. *Syst Appl Microbiol* 34:513–523. doi: 10.1016/j.syapm.2010.11.023.

Capone D, Dunham S, Horrigan S, Duguay L (1992) Microbial nitrogen transformations in unconsolidated coral reef sediments. *Mar Eco Prog Ser* 80: 75–88. doi: 10.3354/meps080075.

Cleary DFR, Becking LE, Voogd NJ, Pires AC, Polonia AR, Egas C, Gomes NCM (2013) Habitat and host-related variation in sponge bacterial symbionts communities in Indonesian waters. *FEMS Microbiol Ecol*. doi:10.1111/1574-6941.12135.

Cleary DFR, De Vantier L, Vail L, Manto P, de Voogd NJ, Rachello-Dolmen PG, Tuti Y, Budiyanto A, Wolstenholme J, Hoeksema BW et al. (2008) Relating variation in species composition to environmental variables: a multi-taxon study in an Indonesian coral reef complex. *Aquat Sci* 70:419–431. doi: 10.1007/s00027-008-8077-2.

Cleary DFR, Suharsono, Hoeksema B (2006) Coral diversity across a disturbance gradient in the Pulau Seribu reef complex off Jakarta, Indonesia. *Biodivers Conserv* 15:3653–3674. doi: 10.1007/s10531-004-4692-y.

Copp BR & Pearce AN (2007) Natural product growth inhibitors of *Mycobacterium tuberculosis*. *Nat Prod Rep* 24: 278–297. doi: 10.1039/b513520f.

Costa R, Keller-Costa T, Gomes NC, da Rocha UN, van Overbeek L & van Elsas JD (2013) Evidence for selective bacterial community structuring in the freshwater sponge *Ephydatia fluviatilis*. *Microb Ecol* 65: 232–244. doi: 10.1007/s00248-012-0102-2.

Dang H, Zhang X, Sun J, Li T, Zhang Z, Yang G (2008) Diversity and spatial distribution of sediment ammonia-oxidizing *Crenarchaeota* in response to estuarine and environmental gradients in the Changjiang Estuary and East China Sea. *Microbiology*+ 154:2084–2095. doi: 10.1099/mic.0.2007/013581-0.

de Voogd N, Becking L, Cleary D (2009) Sponge community composition in the Derawan Islands, ne Kalimantan, Indonesia. *Mar Ecol Prog Ser* 396:169–180. doi: 10.3354/meps08349.

DeLong E (1992) Archaea in coastal marine environments. *P Natl Acad Sci USA* 89:5685–5689. doi: 10.1073/pnas.89.12.5685.

DeLong E, Pace N (2001) Environmental diversity of Bacteria and Archaea. *Syst Biol* 50:470–478. doi: 10.1080/106351501750435040.

DeLong E, Wu K, Prézelin B, Jovine R et al. (1994) High abundance of Archaea in Antarctic marine picoplankton. *Nature* 371: 695–697. doi: 10.1038/371695a0.

Demain AL (1998) Induction of microbial secondary metabolism. *Int Microbiol* 259–264.

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10: 996+. doi: 10.1038/NMETH.2604.

Elkins JG, Podar M, Graham DE, Makarova KS,Wolf Y, Randau L, Hedlund BP, Brochier-Armanet C, Kunin V, Anderson I et al. (2008) A Korarchaeal genome reveals insights into the evolution of the Archaea. *P Natl Acad Sci USA* 105: 8102–8107. doi: 10.1073/pnas.0801980105.

Erwin PM & Thacker RW (2008) Cryptic diversity of the symbiotic cyanobacterium *Synechococcus spongiarum* among sponge hosts. *Mol Ecol* 17: 2937–2947. doi: 10.1111/j.1365-294X.2008.03808.x.

Erwin P, Olson J & Thacker R (2011). Phylogenetic diversity, host-specificity and community profiling of sponge-associated bacteria in the northern gulf of Mexico. *PloS One* 6: e26806. doi: 10.1371/journal.pone.0026806.

Fan L, Reynolds D, Liu M, Stark M, Kjelleberg S, Webster NS & Thomas T (2012) Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. *P Natl Acad Sci USA* 109: E1878–E1887. doi: 10.1073/pnas.1203287109.

Filipsson AF, Bard J & Karlsson S (1998) Concise international chemical assessment document 5: Limonene. World Health Organization. Geneva 5: 1–36.

Flemer B, Kennedy J, Margassery LM, Morrissey JP, O'Gara F & Dobson ADW (2012) Diversity and antimicrobial activities of microbes from two Irish marine sponges, *Suberites carnosus* and *Leucosolenia sp*. *J Appl Microbiol* 112: 289–301. doi: 10.1111/j.1365-2672.2011.05211.x.

Francis C, Roberts K, Beman J, Santoro A, Oakley B (2005) Ubiquity and diversity of ammonia oxidizing Archaea in water columns and sediments of the ocean. *P Natl Acad Sci USA* 102:14683–14688. doi: 10.1073/pnas.0506625102.

Freeman CJ & Thacker RW (2011) Complex interactions between marine sponges and their symbiotic microbial communities. *Limnol Oceanogr* 56: 1577–1586. doi: 10.4319/lo.2011.56.5.1577.

Grossart HP, Frindte K, Dziallas C, EckertW& Tang KW (2011) Microbial methane production in oxygenated water column of an oligotrophic lake. *P Natl Acad Sci USA* 108: 19657–19661. doi: 10.1073/pnas.1110716108.

Hallam S, Mincer T, Schleper C, Preston C, Roberts K, Richardson P & DeLong E (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. *PloS One Biology* 4: 520–536.

Hentschel U, Hopke J, Horn M, Friedrich A, Wagner M, Hacker J, Moore B (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microb 68:4431–4440. doi: 10.1128/AEM.68.9.4431-4440.2002.

Hentschel U, Usher K, Taylor M (2006) Marine sponges as microbial fermenters. *FEMS Microbiol Ecol* 55:167–177. doi: 10.1111/j.1574-6941.2005.00046.x.

Hentschel U, Piel J, Degnan SM, Taylor MW (2012) Genomic insights into the marine sponge microbiome. *Nat Rev Microbiol* 10: 641-654. doi:10.1038/nrmicro2839.

Hoffmann F, Radax R, Woebken D, Holtappels M, Lavik G, Rapp HT, Schlaeppy ML, Schleper C & Kuypers MMM (2009) Complex nitrogen cycling in the sponge *Geodia barretti*. *Environ Microbiol* 11: 2228–2243. doi: 10.1111/j.1462-2920.2009.01944.x.

Hohmann-Marriott MF & Blankenship RE (2011) Evolution of Photosynthesis In Merchant, SS and Briggs, WR and Ort, D, editor, *Annu Rev Plant Biol* 62: 515–548. doi: 10.1146/annurev-arplant-042110-103811.

Holmes B, Blanch H (2007) Genus-specific associations of marine sponges with Group I *Crenarchaeotes*. *Mar Biol* 150:759–772. doi: 10.1007/s00227-006-0361-x.

Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* 417:63–67. doi: 10.1038/417063a.

Jackson SA, Kennedy J, Morrissey JP, OGara F, Dobson AD (2012) Pyrosequencing reveals diverse and distinct sponge-specific microbial communities in sponges from a single geographical location in Irish waters. *Microb Ecol* 64:105–116. doi: 10.1007/s00248-011-0002-x.

Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30. doi: 10.1093/nar/28.1.27.

Kendall M, Wardlaw G, Tang C, Bonin A, Liu Y, Valentine D (2007) Diversity of Archaea in marine sediments from Skan Bay, Alaska, including cultivated methanogens, and description of *Methanogenium boonei sp*. nov. *Appl Environ Microb* 73:407–414. doi: 10.1128/AEM.01154-06.

Kiene RP (1991) In microbial production and consumption of greenhouse gases: Methane, nitrogen oxides, halomethanes. American Society for Microbiology: Washington DC, pp 111–146.

Kozubal MA, Romine M, deM Jennings R, Jay ZJ, Tringe SG, Rusch DB, Beam JP, McCue LA, Inskeep WP (2012) *Geoarchaeota*: a new candidate phylum in the Archaea from high-temperature acidic iron mats in Yellowstone National Park. *ISME J* 7:622201. doi: 10.1038/ismej.2012.132.

Lakshmi V, Saxena A, Mishra SK, Mishra M, Srivastava S & Ghoshal S (2009) Antiamebic activity of marine sponge *Haliclona exigua* (Krikpatrick). Bangladesh J of Pharmacology 4: 55–59. doi: 10.3329/bjp.v4i1.1083.

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, Knight R, Beiko RG & Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* 31: 814+. doi: 10.1038/nbt.2676.

Lee O, Wang Y, Yang J, Lafi F, Al-Suwailem A, Qian P (2011) Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. *ISME J* 5:650–664. doi: 10.1038/ismej.2010.165.

Lee Y, Lee J, Lee H (2001) Microbial symbiosis in marine sponges. *J Microbiol* 39:254–264.

Legendre P, Gallagher E (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia* 129:271–280. doi: 10.1007/s004420100716.

Li D, Xu Y, Shao C, Yang R, Zheng C, Chen Y, Fu X, Qian P, She Z, Voogd N et al. (2012) Antibacterial bisabolane-type sesquiterpenoids from the sponge-derived fungus *Aspergillus sp*. *Mar Drugs* 10:234–241. doi: 10.3390/md10010234.

Marty D, Nival P & Yoon W (1997). *Methanoarchaea* associated with sinking particles and zooplankton collected in the Northeastern tropical Atlantic. Oceanol Acta 20: 863–869.

Massana R, DeLong E, C. PA (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. *Appl Environ Microb* 66:1777–1787. doi: 10.1128/AEM.66.5.1777-1787.2000.

Metcalf WW, Griffin BM, Cicchillo RM, Gao J, Janga SC, Cooke HA, Circello BT, Evans BS, Martens-Habbena W, Stahl DA & van der Donk WA (2012) Synthesis of Methylphosphonic Acid by Marine Microbes: A Source for Methane in the Aerobic Ocean. *Science* 337: 1104–1107. doi: 10.1126/science.1219875.

Murdock JN, Shields J FD & Lizotte J RE (2013) Periphyton responses to nutrient and atrazine mixtures introduced through agricultural runoff. *Ecotoxicology* 22: 215–230. doi: 10.1007/s10646-012-1018-9.

Nakisah MA, Muryany MYI, Fatimah H, Fadilah RN, Zalilawati MR, Khamsah S & Habsah M (2012) Anti-amoebic properties of a Malaysian marine sponge *Aaptos sp* on

Acanthamoeba castellanii. *World J Microb Biot* 28: 1237–1244. doi: 10.1007/s11274-011-0927-8.

Nunoura T, Takaki Y, Kakuta J, Nishi S, Sugahara J, Kazama H, Chee GJ, Hattori M, Kanai A, Atomi H et al. (2011) Insights into the evolution of Archaea and eukaryotic protein modifier systems revealed by the genome of a novel archaeal group. *Nucleic Acids Res* 39:3204–3223. doi: 10.1093/nar/gkq1228.

Offre P, Spang A, Schleper C (2013) Archaea in biogeochemical cycles. *Annu Rev Microbiol* 67. doi: 10.1146/annurev-micro-092412-155614.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson G, Solymos P, Stevens M, Wagner H (2009) vegan: Community ecology package. R package version 1.15-2. 2009.

Pires A, Cleary D, Almeida A, Cunha Â, Dealtry S, Mendonça-Hagler L, Smalla K, Gomes N (2012) Denaturing gradient gel electrophoresis and barcoded pyrosequencing reveal unprecedented archaeal diversity in mangrove sediment and rhizosphere samples. *Appl Environ Microb* 78: 5520–5528. doi: 10.1128/AEM.00386-12.

Pitcher A, Villanueva L, Hopmans E, Schouten S, Reichart G, Damsté J (2011) Niche segregation of ammonia-oxidizing Archaea and anammox Bacteria in the Arabian Sea oxygen minimum zone. *ISME J* 5:1896–1904. doi: 10.1038/ismej.2011.60.

Preston C, Wu K, Molinski T, DeLong E (1996) A psychrophilic Crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. *P Natl Acad Sci USA* 93: 6241–6246. doi: 10.1073/pnas.93.13.6241.

Previsic A, Walton C, Kucinic M, Mitrikeski PT & Kerovec M (2009) Pleistocene divergence of Dinaric Drusus endemics (*Trichoptera*, *Limnephilidae*) in multiple microrefugia within the Balkan Peninsula. *Mol Ecol* 18: 634–647. doi: 10.1111/j.1365-294X.2008.04046.x.

Prosser JI, Nicol GW (2008) Relative contributions of Archaea and Bacteria to aerobic ammonia oxidation in the environment. *Environ Microbiol* 10: 2931–2941. doi: 10.1111/j.1462-2920.2008.01775.x.

Qian P, Wang Y, Lee O, Lau S, Yang J, Lafi F, Al-Suwailem A, Wong T (2010) Vertical stratification of microbial communities in the Red Sea revealed by 16s rDNA pyrosequencing. *ISME J* 5: 507–518. doi: 10.1038/ismej.2010.112.

Quideau S, Lebon M & Lamidey A (2002) Enantiospecific synthesis of the antituberculosis marine sponge metabolite (+)-puupehenone. The arenol oxidative activation route. *Org Lett* 4: 3975–3978. doi: 10.1021/ol026855t.

Rachello-Dolmen P, Cleary D (2007) Relating coral species traits to environmental conditions in the Jakarta Bay/Pulau Seribu reef system, Indonesia. *Estuar Coast Shelf S* 73: 816–826. doi: 10.1016/j.ecss.2007.03.017.

Radax R, Rattei T, Lanzen A, Bayer C, Rapp HT, Urich T & Schleper C (2012) Metatranscriptomics of the marine sponge *Geodia barretti*: tackling phylogeny and function of its microbial community. *Environ Microbiol* 14: 1308–1324. doi: 10.1111/j.1462-2920.2012.02714.x .

R Core Team (2013) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available from http://www.R-project.org/.

Reeburgh WS (2007) Oceanic methane biogeochemistry. *Chem Rev* 107: 486–513. doi: 10.1021/cr050362v.

Rohde S, Gochfeld D, Ankisetty S, Avula B, Schupp P, Slattery M (2012) Spatial variability in secondary metabolites of the indo-pacific sponge *Stylissa massa*. *J Chem Ecol* 1–13. doi: 10.1007/s10886-012-0124-8.

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* 5: 355–362. doi: 10.1038/nrmicro1635.

Rusch A, Hannides A, Gaidos E (2009) Diverse communities of active bacteria and Archaea along oxygen gradients in coral reef sediments. *Coral Reefs* 28: 15–26. doi: 10.1007/s00338-008-0427-y.

Schmitt S, Angermeier H, Schiller R, Lindquist N, Hentschel U (2008) Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. *Appl Environ MicroB* 74:7694–7708. doi: 10.1128/AEM.00878-08.

Segata N, Izard J,Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. *Genome Biol* 12: R60. doi: 10.1186/gb-2011-12-6-r60.

Seravalli J, Kumar M & Ragsdale S (2002) Rapid kinetic studies of acetyl-CoA synthesis: Evidence supporting the catalytic intermediacy of a paramagnetic NiFeC species in the autotrophic Wood-Ljungdahl pathway. *Biochemistry* 41: 1807–1819. doi: 10.1021/bi011687i.

Sharp KH, Eam B, Faulkner DJ & Haygood MG (2007) Vertical transmission of diverse microbes in the tropical sponge *Corticium sp*. *Appl Environ Microb* 73: 622–629. doi: 10.1128/AEM.01493-06.

Siboni N, Ben-Dov E, Sivan A, Kushmaro A (2008) Global distribution and diversity of coral associated Archaea and their possible role in the coral holobiont nitrogen cycle. *Environ Microbiol* 10: 2979–2990. doi: 10.1111/j.1462-2920.2008.01718.x.

Siegl A, Kamke J, Hochmuth T, Piel J, Richter M, Liang C, Dandekar T & Hentschel U (2011) Single-cell genomics reveals the lifestyle of *Poribacteria*, a candidate phylum symbiotically associated with marine sponges. *ISME J* 5: 61–70. doi: 10.1038/ismej.2010.95.

Sipkema D, Franssen M, Osinga R, Tramper J & Wijffels R (2005) Marine sponges as pharmacy. *Mar Biotechnol* 7: 142–162. doi: 10.1007/s10126-004-0405-5.

Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM & Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored “rare biosphere”. *P Natl Acad Sci USA* 103: 12115–12120. doi: 10.1073/pnas.0605127103.

Spang A, Hatzenpichler R, Brochier-Armanet C, Rattei T, Tischler P, Spieck E, Streit W, Stahl DA, Wagner M, Schleper C (2010) Distinct gene set in two different lineages of ammonia-oxidizing Archaea supports the phylum *Thaumarchaeota*. *Trends Microbiol* 18: 331–340. doi: 10.1016/j.tim.2010.06.003.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) Mega5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739. doi: 10.1093/molbev/msr121.

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. *Lect. Math. Life Sci* 17: 57–86.

Taylor MW, Hill RT & Hentschel U (2011) Meeting Report: 1st International Symposium on Sponge Microbiology. *Mar Biotechnol* 13: 1057–1061. doi: 10.1007/s10126-011-9397-0.

Taylor MW, Tsai P, Simister RL, Deines P, Botte E, Ericson G, Schmitt S, Webster NS (2013) Sponge-specific Bacteria are widespread (but rare) in diverse marine environments. *ISME J* 7:438–443. doi: 10.1038/ismej.2012.111.

Taylor M, Radax R, Steger D & Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol Mol Biol R* 71: 295–347. doi: 10.1128/MMBR.00040-06.

Turque A, Batista D, Silveira C, Cardoso A, Vieira R, Moraes F, Clementino M, Albano R, Paranhos R, Martins O et al. (2010) Environmental shaping of sponge associated archaeal communities. *PLoS One* 5: e15774. doi: 10.1371/journal.pone.0015774.

Urakawa H, Martens-Habbena W, Stahl DA (2010) High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method. Appl *Environ Microb* 76: 2129–2135. doi: 10.1128/AEM.02692-09.

Valentine DL (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nat Rev Microbiol* 5: 316–323. doi: 10.1038/nrmicro1619.

Webster NS, Taylor MW (2012) Marine sponges and their microbial symbionts: love and other relationships. Environ Microbiol 14:335–346. doi: 10.1111/j.1462-2920.2011.02460.x.

Webster N, Negri A, Munro M, Battershill C (2004) Diverse microbial communities inhabit Antarctic sponges. *Environ Microbiol* 6:288–300. doi: 10.1111/j.1462-2920.2004.00570.x.

Webster N, Taylor M, Behnam F, Lucker S, Rattei T, Whalan S, Horn M, Wagner M (2010) Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. *Environ Microbiol* 12: 2070–2082. doi: 10.1111/j.1462-2920.2009.02065.x.

Wemheuer B, Wemheuer F, Daniel R (2012) RNA-based assessment of diversity and composition of active archaeal communities in the German Bight. *Archaea-An International Microbiological Journal*. doi: 10.1155/2012/695826.

Wilkinson C (1983) Net Primary Productivity in Coral-Reef Sponges. *Science* 219: 410–412. doi: 10.1126/science.219.4583.410.

Wuchter C, Abbas B, Coolen M, Herfort L, Van Bleijswijk J, Timmers P, Strous M, Teira E, Herndl G, Middelburg J et al. (2006) Archaeal nitrification in the ocean. P Natl Acad Sci USA 103: 12317–12322. doi: 10.1073/pnas.0600756103.

Wulff J (2001) Assessing and monitoring coral reef sponges: Why and how? *B Mar Sci* 69: 831–846.

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7:203–214. doi: 10.1089/10665270050081478.

Zhou X, Xu T, Yang X, Huang R, Yang B, Tang L, Liu Y (2010) Chemical and biological aspects of marine sponges of the genus *Xestospongia*. *Chem Biodivers* 7: 2201–2227. doi:10.1002/cbdv.201000024.

Chapter 5: The putative functional ecology and distribution of archaeal communities in an Indonesian coral reef environment

The putative functional ecology and distribution of archaeal communities in an Indonesian coral reef environment

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5.1. Abstract

Archaea play crucial roles in a number of key ecological processes including nitrification and methanogenesis. However, their roles in coral reef environments are poorly understood. Here, archaeal communities isolated from six distinct biotopes, namely, sediment, seawater and four different sponge species *Stylissa massa*, *Stylissa carteri*, *Xestospongia testudinaria* and *Hyrtios erectus* from the Spermonde Archipelago, SW Sulawesi, Indonesia, were investigated. Archaeal communities from sediment and sponges were dominated by *Crenarchaeota* while the seawater community was dominated by *Euryarchaeota*. The biotope explained 73% of the variation in archaeal composition, with clear separation between microbial assemblages from *X. testudinaria* and *H. erectus*. In contrast, communities from seawater and both *Stylissa* species shared most abundant OTUs with the exception of a single dominant OTU specifically enriched in both *Stylissa* species. This OTU was closely related to *Cenarchaeum symbiosum*, a thaumarchaeon previously isolated from a number of sponge species in the order *Halichondrida.* The *in silico* prediction of functional gene content in archaeal assemblages also revealed significant differences between biotopes. Different ammonia assimilation strategies were exhibited by the archaeal communities: *X. testudinaria*, *H. erectus* and sediment archaeal communities were enriched for glutamate dehydrogenase with mixed specificity ($NAD(P)^+$) pathways while archaeal planktonic and *Stylissa* communities were enriched for specific glutamate dehydrogenase (NAD^+ or $NADP^+$) and glutamate synthase pathways. Importantly, our results indicate that archaeal communities in different biotopes have distinct ecophysiological roles.

Keywords: Archaea; Coral reef; Sponge Metabolomics; Nitrogen; Glutamate

5.2. Introduction

Archaea domain, composed of five phyla (*Crenarchaeota*, *Thaumarchaeota*, *Euryarchaeota*, *Korarchaeota* and *Nanoarchaeota*) can colonize a wide range of environmental conditions (pH, salinity, temperature) and can be present in almost all ecosystems (Hoppert 2013). In tropical marine environments, mesophilic *Crenarchaeota* (*Thaumarchaeota*) and *Euryarchaeota* are the most frequently phyla (Polónia et al. 2013; Pires et al. 2012; Yin et al. 2013). *Thaumarchaeota* are the most ubiquitous archaeal phylum (Offre et al. 2013) and can be abundant in aerobic terrestrial and marine environments (soil, sediment, seawater, hot springs, hydrothermal vents, marine sponges; Dang et al. 2013; Tourna et al. 2011; Reigstad et al. 2008; Wang et al. 2009; Polónia et al. 2013) whereas *Euryarchaeota* are found predominantly in seawater and sediment (Yan et al. 2006; Wemheuer et al. 2012). In addition to being abundant members of the vast marine microbial community, Archaea are also important players in processes such as the geochemical cycling of carbon, nitrogen and sulphur (Lee et al. 2001; Webster et al. 2004). For oligotrophic coral reefs, this cycling activity, and particularly the nitrogen cycle, is of crucial importance in order to degrade organic matter and maintain high levels of primary production (Schöttner et al. 2011). The importance of nitrogen for organisms and ecosystems is critical; nitrogen is an essential component of proteins, nucleic acids and cell wall constituents and limits marine ecosystem primary productivity (Francis et al. 2007; Francis et al. 2005). Despite the increasing number of archaeal studies, the geochemical cycling of nitrogen is still less understood in Archaea than in Bacteria. Archaea have been shown to be involved in nitrification, denitrification and nitrogen fixation (Offre et al. 2013). Denitrification has only been detected in halophilic (e.g., *Haloferax denitrificans*) or extreme thermophilic (e.g., *Pyrobaculum aerophilum*) Archaea and very few of which are cultivable (Cabello et al. 2004; Shapleigh 2006; Offre et al. 2013). The first observation of nitrification in Archaea was reported by Könneke et al. (2005). These authors reported the isolation of a chemolithoautotrophic archaeote which aerobically oxidized ammonia to nitrite (*Nitrosopumilus maritimus*). This finding was totally unexpected given that until then lithotrophic bacteria were thought to be virtually the only microbes involved in nitrification (Offre et al. 2013). Currently, Archaea are thought to play a critical role as ammonia oxidizers. Due to their low tolerance to high NH_3 concentrations, ammonia-oxidizing Archaea (AOA) have been suggested to outcompete their bacterial counterparts in ocean waters (Francis et al. 2005; Radax et al. 2012; Offre et al. 2013).

Few studies have analyzed archaeal composition or putative functions in coral reef biotopes. In the present study we assessed the composition of Archaea in six biotopes

including four host (*Stylissa massa, Stylissa carteri, Xestospongia testudinaria and Hyrtios erectus*) and two non-host (seawater and sediment) biotopes. Marine sponges are abundant and ecologically important components of coral reefs (Diaz & Rützler 2001) and have been shown to harbour exceptionally high microbial densities, which can make up from 35 to 40% of sponge biomass (Hentschel et al. 2002, 2012; Taylor et al. 2007). Sponge prokaryotic diversity is, in most cases, dominated by bacterial species (Sharp et al. 2007; Taylor et al. 2007; Lee et al. 2011; Fan et al. 2012). In some sponge species, however, Archaea are the dominant group. The microbial communities of *Axinella mexicana* and *Inflatella pellicula*, for example, are dominated by Archaea (Preston 1996; Jackson et al. 2013). In this study, our main goals were to: assess to what extent: 1. Archaeal communities in sponges differ from those in the surrounding non-host environment (seawater and sediment); 2. Biotopes host phylogenetically and functionally distinct lineages and 3. Communities from different biotopes are differentially enriched for genes involved in the nitrogen metabolism.

5.3. Material and methods

5.3.1. Study site

All sampling took place in the Spermonde Archipelago, South Sulawesi, Indonesia. This Archipelago consists of 160 fringing, barrier and patch reefs (Voogd et al. 2006; Figure 5.3.1) situated adjacent to the city of Makassar. Its proximity to a city of more than 2 million inhabitants (Renema 2010) leaves these coral reefs exposed to anthropogenic disturbances including river discharge (sedimentation, agricultural runoff), oil spills, destructive fisheries, tourism and coral mining (de Voogd & Cleary 2007).

5.3.1. Sampling

Four sponge species, sediment and seawater were collected in different reef sites surveyed using SCUBA in August 2012. The reefs Lae Lae, Samalona, Kudingkareng Keke, Bone Baku and Langkai were sampled. At each site, one sample of each biotope was taken, namely of sediment, seawater, and the sponges *Stylissa carteri* and *Stylissa massa* (order *Halichondrida:* family *Dictyonellidae*), *Xestospongia testudinaria* (order *Haplosclerida:* family *Petrosiidae*) and *Hyrtios erectus* (order *Dictyoceratida:* family *Thorectidae*).

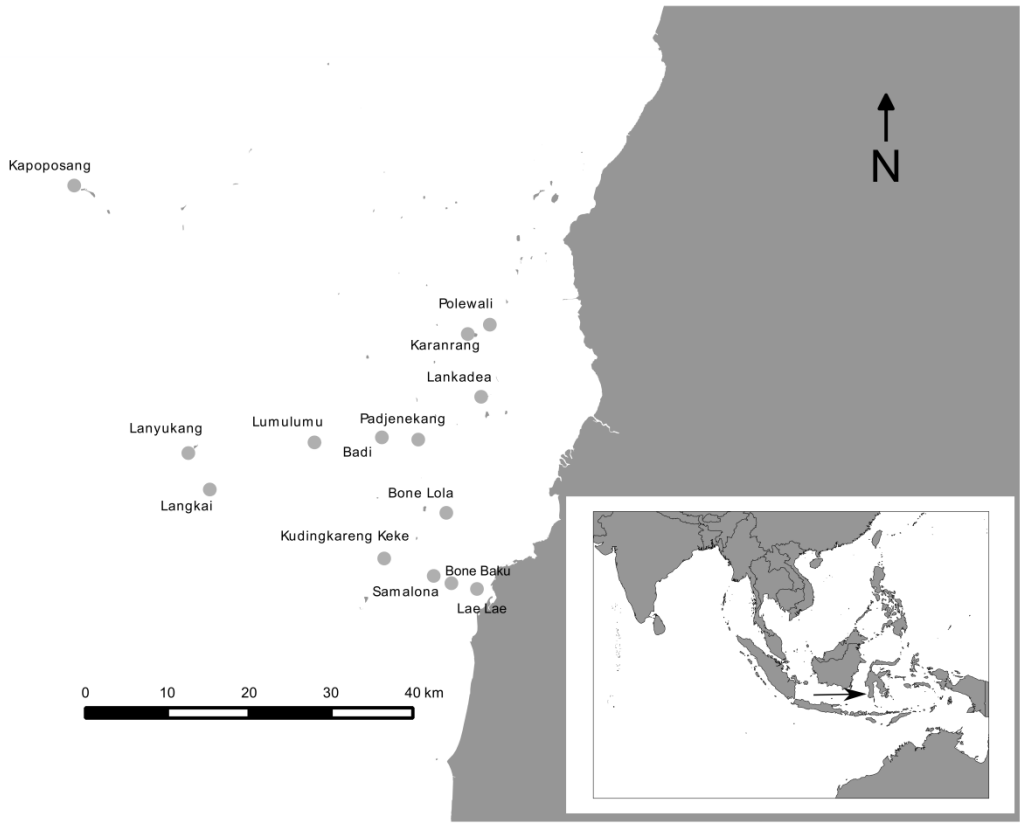

Figure 5.3.1 - Map of the study area (Spermonde Coral Reef System) showing the location of study sites sampled

The sediment samples were taken using the mini core method. Mini-cores, consisting of the top 5 cm of sediment, were collected using a plastic disposable syringe from which the end had been cut in order to facilitate sampling (Capone et al. 1992). Cores of all sponge species were sampled including segments of surface and interior in order to sample, as much as possible, the whole archaeal community (Pires et al. 2012; Polónia et al. 2013). The seawater samples were collected by filtering one liter (Sogin et al. 2006; Bowen et al. 2012) of seawater through a Millipore® White Isopore Membrane Filter (0.22 µm pore size). All samples were stored in 96% EtOH (Previsic et al. 2009; Cleary et al. 2013) and kept at temperatures lower than 4 ºC immediately after collection. Once in the laboratory, samples were stored at -20 ºC until DNA extraction.

5.3.2. DNA extraction and pyrosequencing

We isolated PCR-ready genomic DNA from seawater, sediment and sponge samples using the FastDNA® SPIN Kit (MPbiomedicals) following the manufacturer's instructions. This is an extraction method frequently used for this purpose (Urakawa et al. 2010; Costa et al. 2013; Cleary et al. 2013; Polónia et al. 2013). Briefly, the whole membrane filter and

500 mg of sediment and sponge were transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. The microbial cell lysis was performed in the FastPrep® Instrument (Q Biogene) for 80 seconds at speed 6.0. The extracted DNA was eluted into DNase/Pyrogen-Free Water to a final volume of 50 µl and stored at -20°C until use. Pyrosequencing and sequence analysis were performed using previously described methods (Pires et al. 2011; Cleary et al. 2013; Polónia et al. 2013 - see Appendix for a detailed description). In the most recent Greengenes release, the recently adopted phylum *Thaumarchaeota* is still considered a class of the *Crenarchaeota* phylum; in the present study we follow the Greengenes taxonomy. The sequences generated in this study can be downloaded from the NCBI SRA: Accession number not yet available.

5.3.3. BLAST, Phylogenetic and In silico Metagenome analysis

Briefly, sequence identifiers of closely related taxa of numerically dominant OTUs (≥ 100 sequences) were downloaded using the NCBI Basic Local Alignment Search Tool (BLAST) command line 'blastn' tool with the -db argument set to nt (Zhang et al. 2000). A phylogenetic tree including all dominant OTUs (≥ 100 sequences) was constructed using the Mega5 program (http://www.megasoftware.net/; last checked 2012/11/20; Tamura et al. 2011). To predict the metagenome of each sample we used PICRUSt (Langille et al. 2013). PICRUSt is a bioinformatics tool that uses marker genes, in this case 16S rRNA, to predict metagenome gene functional content. A detailed description of these methods has been published previously (Langille et al. 2013; Cleary et al. 2013; Polónia et al. 2013) and can be found in the supplementary materials and methods (see Appendix for a detailed description). In the present study we used the KEGG database and focused on KOs in the nitrogen energy metabolism pathway. We used R to generate bargraphs showing the percentage of total genes for each sample. In addition to this, we use the metagenome_contributions.py script to assess the relative contribution of selected orders. The metagenome_contributions.py script partitions functional contributions to function, OTU and sample. Results of this analysis are presented using barplots for each biotope.

5.3.4. Statistical analysis

A square matrix containing the presence and abundance of all OTUs per sample was imported into R (R Core Team 2013) using the read.table() function. Sequences not classified as Archaea (e.g., Bacteria) were removed prior to statistical analysis. The OTU abundance matrix was $\log_{10}(x+1)$ transformed (in order to normalise the distribution of the

data) and a distance matrix constructed using the Bray-Curtis index with the vegdist() function in the vegan package (Oksanen et al. 2009) in R. The Bray-Curtis index is one of the most frequently applied (dis)similarity indices used in ecology (Legendre & Gallagher 2001; Cleary 2003). Variation in archaeal composition among biotopes (sediment, seawater, *S. massa*, *S. carteri*, *X. testudinaria* and *H. erectus*) was assessed with Principal Coordinates Analysis (PCO) using the cmdscale() function in R with the Bray-Curtis distance matrix as input. Variation among biotopes was tested for significance using the adonis() function in vegan. In the adonis analysis, the Bray-Curtis distance matrix of species composition was the response variable with biotope as independent variable. The number of permutations was set at 999; all other arguments used the default values set in the function. Weighted averages scores were computed for OTUs on the first two PCO axes using the wascores() function in the vegan package.

5.4. Results

The sequencing effort yielded 95007 sequences, which were assigned to 617 OTUs after quality control, OTU picking, removal of chimera and removal of OTUs not assigned to the domain Archaea. All archaeal OTUs were assigned to 3 phyla, *Crenarchaeota* (63510 sequences), *Euryarchaeota* (31369 sequences) and [*Parvarchaeota*] (19 sequences). In addition to this, OTUs were assigned to 13 classes and 17 orders. Of these, the classes *Thaumarchaeota* (61984 sequences) and *Thermoplasmata* (30930 sequences), the orders *Cenarchaeales* (61615 sequences) and E2 (30927 sequences) were the most abundant.

5.4.1. Higher taxon abundance

There were marked differences in the abundance of higher archaeal taxa among biotopes (Figure 5.4.1). The *Euryarchaeota* achieved their greatest abundance in seawater where they comprised more than 98% on average of all sequences; *Euryarchaeota* were also abundant in both *Stylissa* hosts. In all other biotopes, more than 60% of sequences belonged to the *Crenarchaeota*. There was also a marked difference in dominance between host and non-host biotopes. In non-host biotopes, the single most dominant OTU made up less than 28% of all sequences.

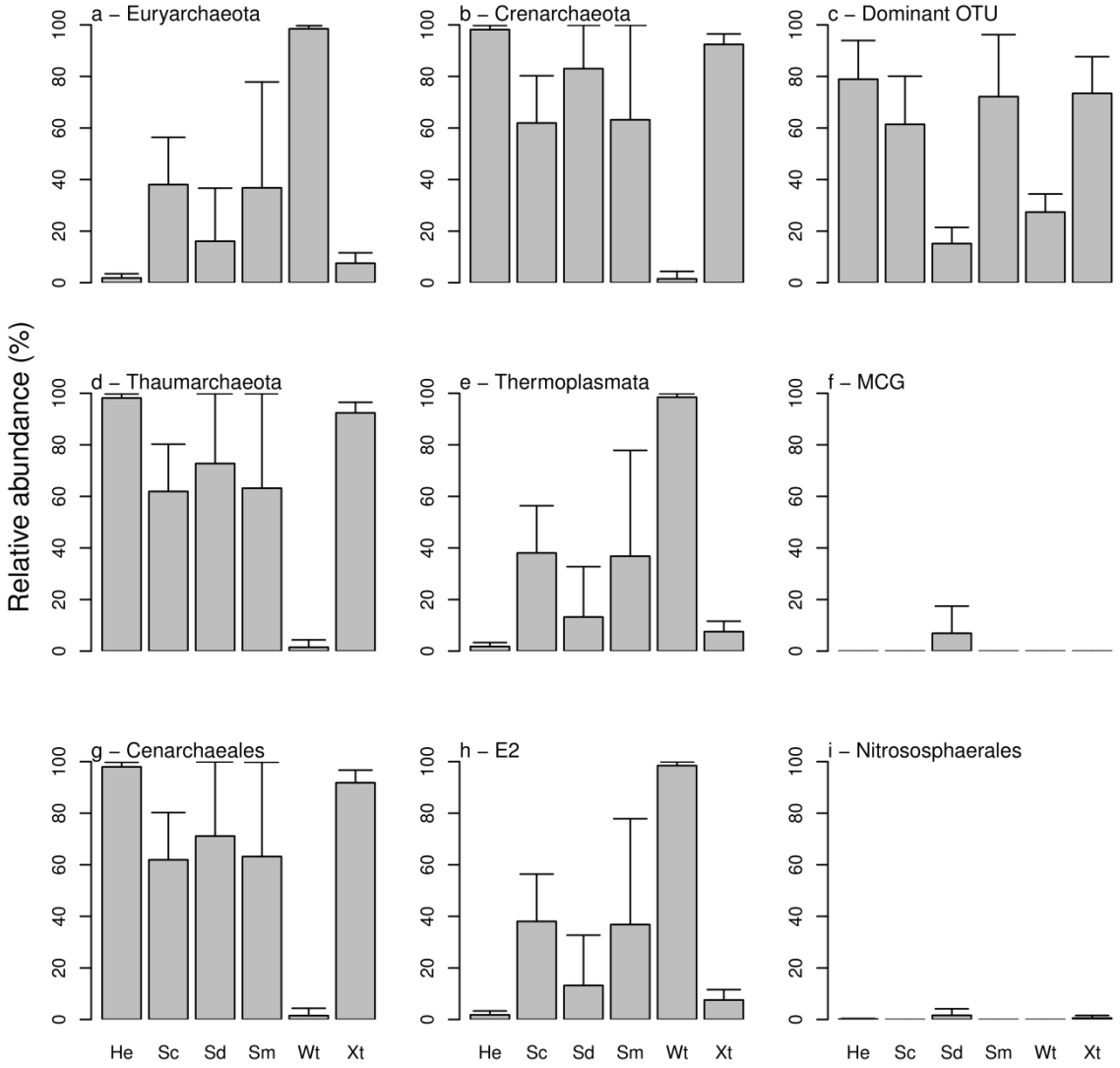

Figure 5.4.1 - Mean relative abundance of the most abundant archaeal phyla, classes, orders and families and the abundant OTUs for samples from seawater (Wt), sediment (Sd), *S. massa* (Sm), *S. carteri* (Sc), *X. testudinaria* (Xt) and *H. erectus* (He). Error bars represent a single standard deviation. The abundant OTU represents the mean abundance for the single most abundant OTU in each sample, thus not necessarily the same OTU.

In host biotopes, in contrast, the single most dominant OTU made up more than 60%, on average, of all sequences. The third most abundant class (Miscellaneous Crenarchaeotal Group; MCG) was virtually restricted to the sediment biotope and the third most abundant order (*Nitrososphaerales*) was found in sediment, *X. testudinaria* and *H. erectus*.

5.4.2. OTU composition analysis

BLAST was used to find closely related organisms to the most abundant (≥ 100 sequences) OTUs (Table 5.4.1).

Table 5.4.1 - List of most abundant OTUs (≥ 100 sequences) including OTU-numbers; number of sequences (reads); biotope where the OTUs were found (Group); their taxonomic affiliation, GenBank GenInfo sequence identifiers (GI) of closely related organisms identified using BLAST; sequence identity (Sq ident) of these organisms with our representative OTU sequences; isolation source (Source) of closely related organisms identified using BLAST and location where the isolation source was sampled (Location).

OTU	Reads	Group	Phylum	Class	Order	Family	Genus	Species	GI	Seq.ident
1	21591	St	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Cenarchaeum*	*symbiosum*	15778681	100
2	10746	He	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	155733561	99.07
3	10438	Xt	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	162312072	99.07
4	7962	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	445069696	100
5	3134	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	383933392	100
6	6022	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	383933301	100
7	4918	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	83416104	100
8	650	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	383470793	100
9	501	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	39546629	100
10	520	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	321159153	100
11	613	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	321159111	99.77
12	553	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	253756926	100
13	2007	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*	Unclassified	529279729	100
14	338	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	145651460	100
15	296	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Nitrososphaerales*	*Nitrososphaeraceae*	*Candidatus Nitrososphaera*	Unclassified	145654125	99.77
16	289	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group III	Unclassified	Unclassified	394999409	100
17	273	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	321158907	100
18	158	Sd	*Crenarchaeota*	*MCG*	Unclassified	Unclassified	Unclassified	Unclassified	374432603	99.77
19	194	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	310871774	98.37
20	182	Sd	*Euryarchaeota*	*Thermoplasmata*	E2	DHVEG-1	Unclassified	Unclassified	364527263	99.77
21	134	Sd	*Crenarchaeota*	*MCG*	Unclassified	Unclassified	Unclassified	Unclassified	42601811	100
22	116	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	83416103	100
23	249	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	125381541	99.77
24	226	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	193891139	100
26	125	Sd	*Crenarchaeota*	*MCG*	pGrfC26	Unclassified	Unclassified	Unclassified	374432521	100
27	318	SW	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	220684723	100
30	181	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	125381580	99.3
33	106	Sd	*Crenarchaeota*	*MBGB*	Unclassified	Unclassified	Unclassified	Unclassified	507105568	100
34	101	Sd	*Crenarchaeota*	*MCG*	Unclassified	Unclassified	Unclassified	Unclassified	364527383	100
38	6471	SH	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	529279754	98.6
41	483	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Cenarchaeum*	*symbiosum*	125381472	99.77
43	368	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	310871821	100
47	126	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	265262728	100
77	2006	Xt	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	305691434	99.01
84	1590	He	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	155733561	99.53
91	2099	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	383933309	100
102	155	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	321159158	100
118	1379	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	220685426	100
127	167	Sd	*Euryarchaeota*	*Thermoplasmata*	E2	DHVEG-1	Unclassified	Unclassified	364529015	99.77
150	737	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*	Unclassified	125381590	100
155	126	SW	*Euryarchaeota*	*Thermoplasmata*	E2	Marine group II	Unclassified	Unclassified	394999422	100
220	244	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*	Unclassified	548783387	100
246	195	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	310871877	100
349	425	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	108947531	100
424	598	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	529279806	99.77
432	155	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	Unclassified	Unclassified	265262760	99.77
542	382	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Cenarchaeum*	*symbiosum*	265262744	99.07

St: represent an OTU only present in *Stylissa* biotopes;
SW: represent an OTU predominantly present in *Stylissa* and seawater biotopes;
Sd: represent an OTU predominantly present in sediment biotope;
He: represent an OTU predominantly present in *H. erectus* biotope;
SH: represent an OTU predominantly present in sediment and *H. erectus* biotopes.

The most abundant OTU overall was OTU-1, assigned to the genus *Cenarchaeum* and found exclusively in *S. massa* and *S. carteri* hosts and represented by 21591 sequences. OTU-1 was closely related to an organism previously isolated from *Axinella damicornis* hosts in the Spanish Mediterranean coast; *Axinella sp* in California and *Phakellia fusca* in South China Sea.

5.4.1. Importance of biotope in structuring composition

There was a highly significant difference in archaeal composition among biotopes ($F_{5,18}$ = 9.75, P < 0.001, R^2 = 0.730). Variation among biotopes thus explained 73% of the variation in archaeal composition. A PCO ordination (Figure 5.4.2) of the first two axes shows four distinct clusters representing samples from the six biotopes. One cluster consists of samples from seawater and both *Stylissa* hosts with other clusters consisting of samples from sediment, *X. testudinaria* and *H. erectus*. The main axis of variation separates OTUs found predominantly in seawater and both *Stylissa* hosts from OTUs found predominantly in sediment.

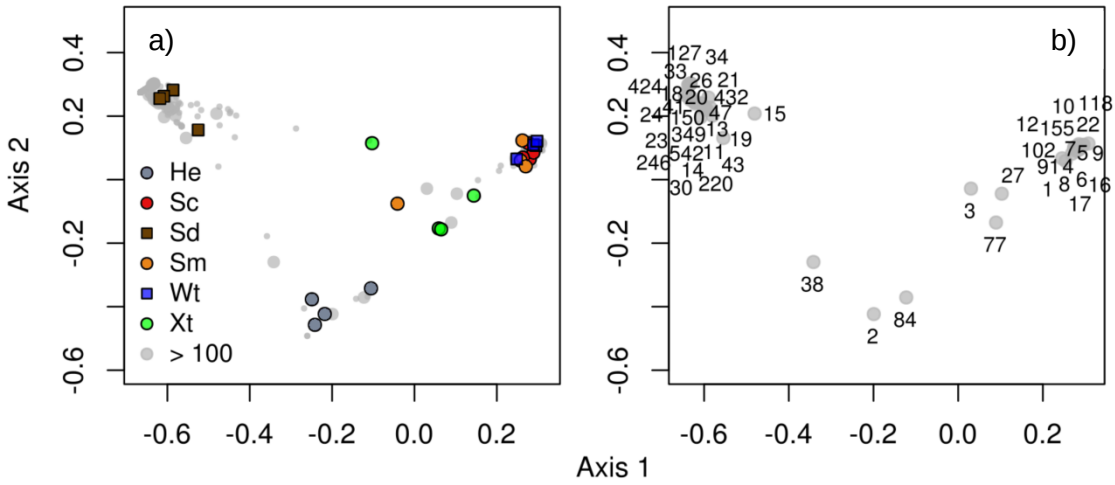

Figure 5.4.2 - Ordination showing the first two axes of the PCO analysis. a) Symbols represent biotopes for seawater (Wt), sediment (Sd), *S. massa* (Sm), *S. carteri* (Sc), X. *testudinaria* (Xt) and *H. erectus* (He). Very small circles represent OTUs < 100 sequence reads. b) Numbers represent abundant (≥100 sequence reads) OTUs.

5.4.2. Phylogeny

In the phylogenetic tree (Figure 5.4.3) there were two main clusters, 1) a cluster consisting of OTUs belonging to the *Crenarchaeota* phylum and 2) a cluster consisting of OTUs belonging to the *Euryarchaeota* phylum. Inside the main cluster of *Euryarchaeota*, the

most abundant OTUs found in seawater and *Stylissa* hosts, all belonging to Marine Group II, formed a distinct cluster.

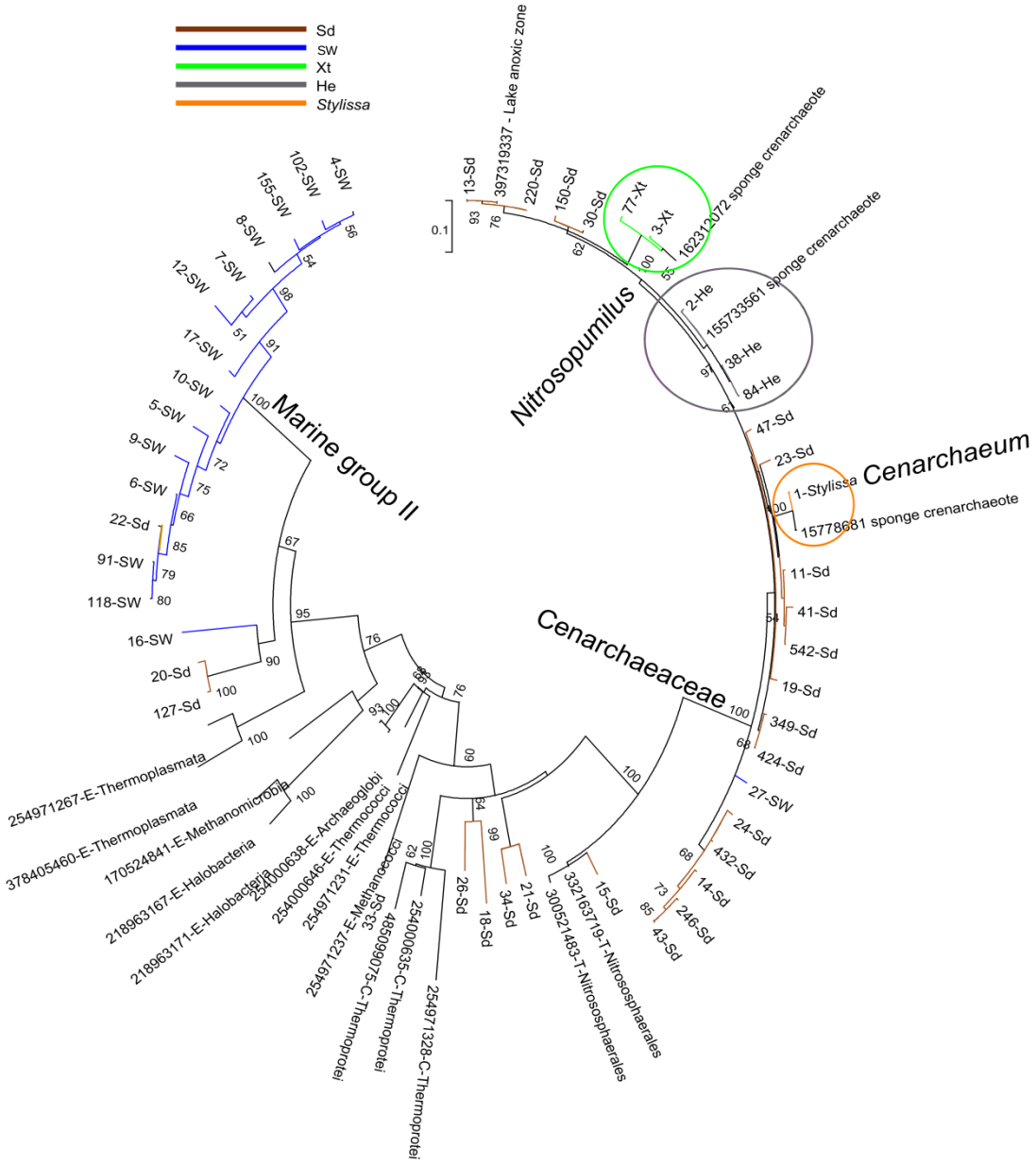

Figure 5.4.3 - Phylogenetic tree of the archaeal 16S rRNA gene sequences recovered from the studied biotopes (seawater, sediment and *S. massa*, *S. carteri*, *X. testudinaria* and *H. erectus*); built using the Mega5 program with the Nearest-Neighbor-Interchange and Generalised Time-Reversible model; bootstrap values lower than 50% were omitted. The number of each OTU is indicated as are GenBank GenInfo sequence identifiers of cultured archeal sequences. Classes of Archaea are indicated. OTUs are assigned to the following clusters: Sd: mainly found in sediment biotope; SW: mainly found in Sponges belonging to the genus *Stylissa* and seawater biotopes; Xt: found in *X. testudinaria*; He: mainly found in *H. erectus and Stylissa*: found in *S. massa* and *S. carteri*.

Inside the *Crenarchaeota* main cluster, OTUs found in *X. testudinaria* and *H. erectus* formed two distinct and well supported clusters that clustered together with OTUs found in sediment and assigned to the genus *Nitrosopumilus*. Abundant OTUs found mainly in sediment represented a phylogenetically diverse community with representatives in both of the main phyla identified in this study.

5.4.3. In silico Metagenome analysis

Only 20 of the 54 KEGG orthologs (KOs) involved in the nitrogen metabolism pathways were detected. The most abundant of these are presented in Figure 5.4.4. Almost all of these KOs are, however, shared with other pathways. For example, the majority of the detected KOs also participate in the amino acid metabolism.

Particularly intriguing was the absence of genes for ammonia oxidation (amoA) in a dataset with a high number of sequences assigned to known ammonia oxidising Archaea (e.g., 3041 *Nitrosopumilus* sequences). Since PICRUSt predicts the functional potential of microbial communities from their phylogeny, the presence of *Nitrosopumilus* sequences in the analysed dataset should result in at least some counts in the KOs for ammonia monooxygenase A (K10944). The reason for this absence was due to a discrepancy with respect to the presence/absence of this KO in the genome database. In particular, the KEGG entry for *Nitrosopumilus* showed K10944 (methane/ammonia monoxogenase subunit A), but in the cached IMG table, the same KO with the same genome accession (*Nitrosopumilus maritimus* SCM, NC_010085) was absent (personal communication, Jesse Zaneveld). This is something that will be addressed in the future, but given the relative abundance of known ammonia-oxidizing taxa (e.g., *Nitrosopumilus*), the gene count for K10944 would have been highest in sediment, intermediate in sponges and virtually absent in seawater.

The relative abundance of those KOs that were present revealed several differences among biotopes. *Stylissa carteri*, for example, was enriched with respect to glutamate dehydrogenase (K00260) and ferredoxin–nitrite reductase (K00366). Both of these were either absent or much less prevalent in other biotopes including *S. massa. X. testudinaria* and *H. erectus,* had very low relative abundances of almost all KOs with the exception of glutamate dehydrogenase ($NAD(P)^+$; K00261) and glutamine synthetase (K01915); the latter of which was present in all biotopes. Seawater was enriched with respect to

glutamate dehydrogenase ($NADP^+$; K00262), carbamate kinase (K00926) and glutamate synthase (NADPH/NADH; K00266), while nitrate reductase beta subunit (K00371) was enriched in sediment.

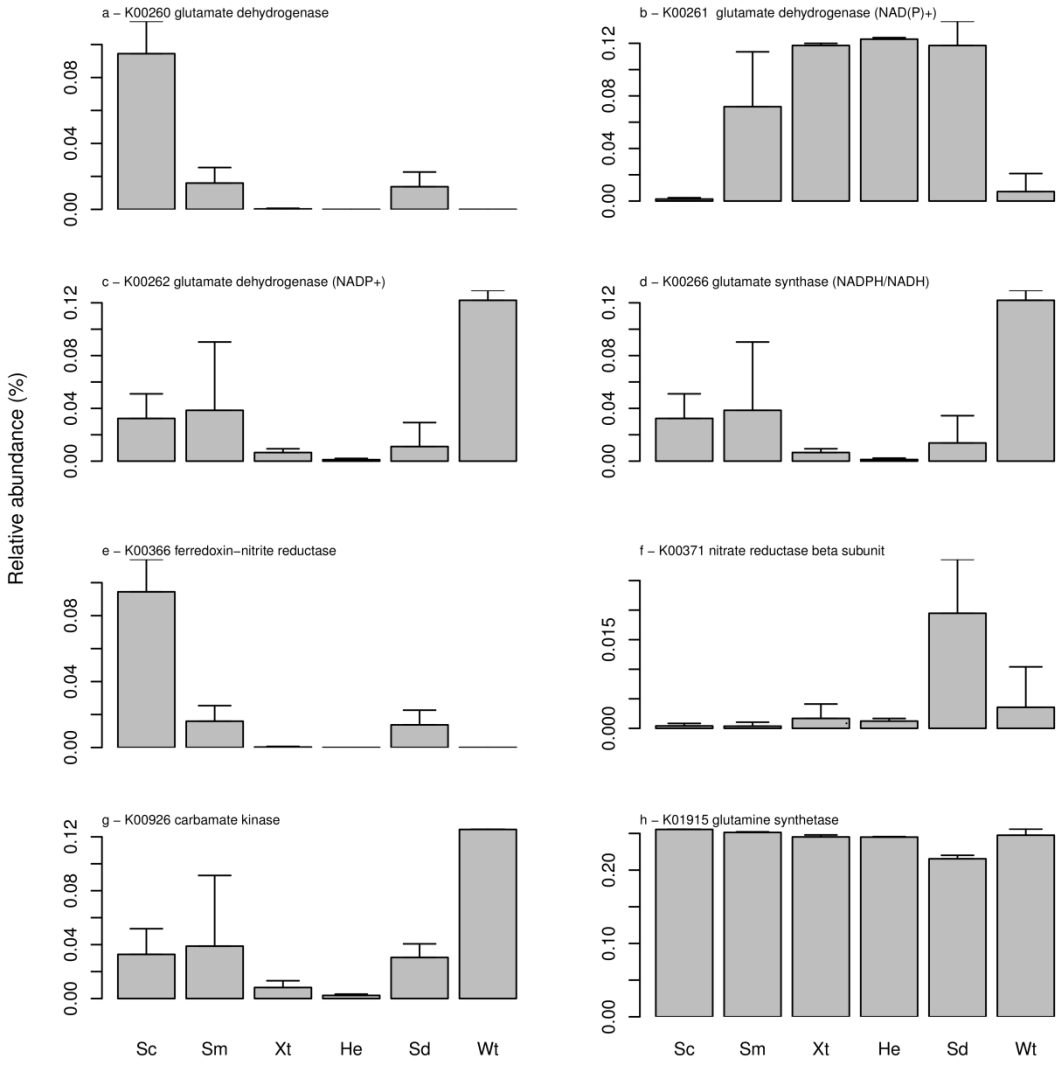

Figure 5.4.4 - Mean relative abundance of KEGG genes involved in the Nitrogen metabolism pathways for samples from seawater (Wt), sediment (Sd), *S. massa* (Sm), *S. carteri* (Sc), *X. testudinaria* (Xt) and *H. erectus* (He). Error bars represent a single standard deviation. The KEGG genes shown include the following: a) K00260 glutamate dehydrogenase; b) K00261 glutamate dehydrogenase ($NAD(P)^+$); c) K00262 glutamate dehydrogenase ($NADP^+$); d) K00266 glutamate synthase (NADPH/NADH); e) K00366 ferredoxin−nitrite reductase; f) K00371 nitrate reductase beta subunit; g) K00926 carbamate kinase; h) K01915 glutamine synthetase.

OTUs belonging to the *Cenarchaeales* order were the only ones contributing to the abundance of the glutamate dehydrogenase (K00260), glutamate dehydrogenase

($NAD(P)^+$; K00261), ferredoxin-nitrite reductase (K00366), and nitr ate reductase beta subunit (K00371) enzymes (Figure 5.4.5). In contrast, OTUs belonging to the E2 order were the only ones contributing to the abundance of glutamate dehydrogenase ($NADP^+$, K00262) and glutamate synthase (NADPH/NADH, K00266). OTUs belonging to the E2 order were also major contributors to the abundance of carbamate kinase (K00926). For glutamine synthetase (K01915), both OTUs orders, *Cenarchaeales* and E2, had similar contributions; with OTUs belonging to the *Cenarchaeales* order being the major contributors to the presence of this enzyme in sponge and sediment biotopes and OTUs belonging to the E2 order the major contributors to the abundance of this enzyme in the seawater biotope.

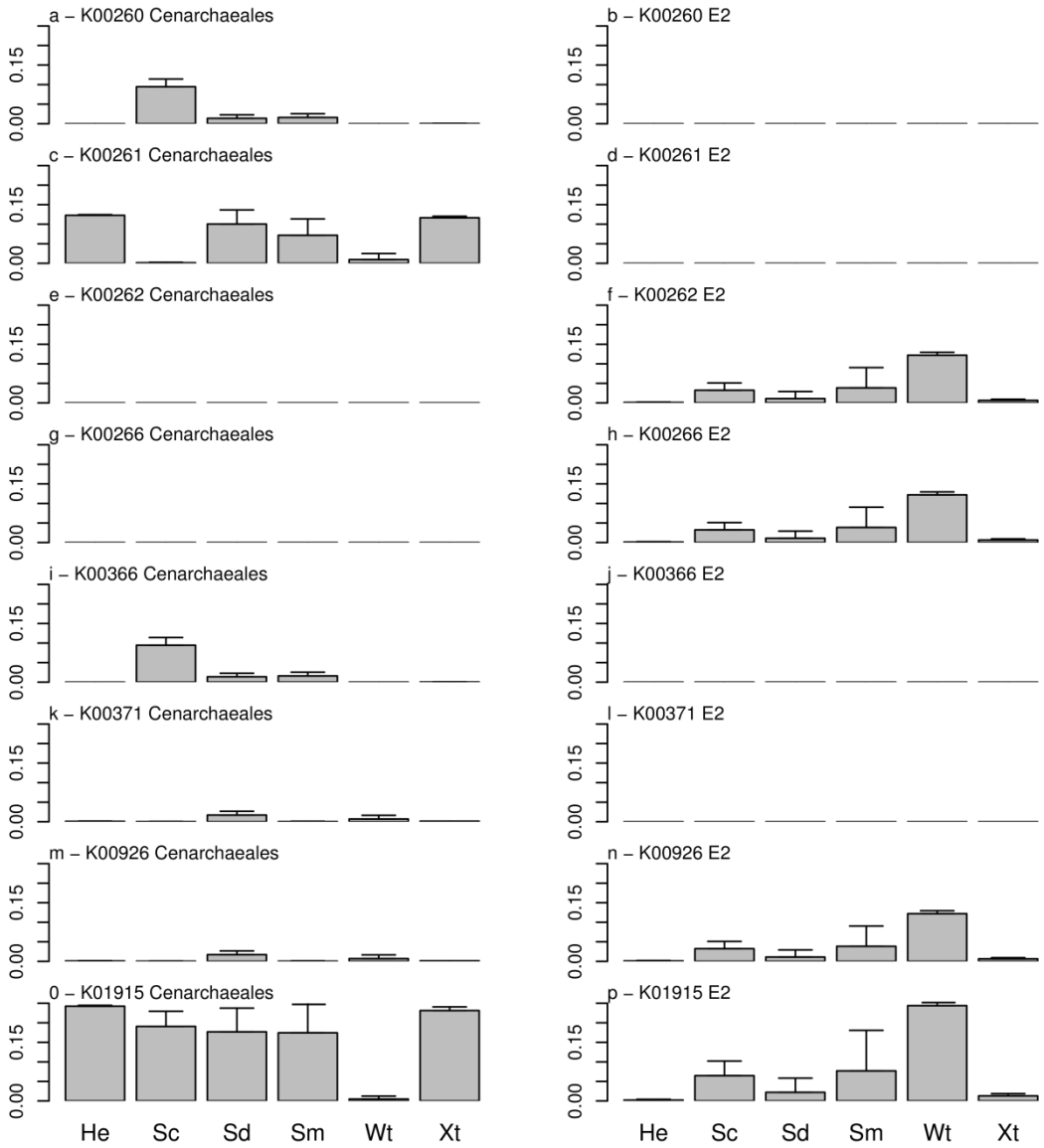

Figure 5.4.5 - The predicted contribution of *Cenarchaeales* and E2 OTUs to the KEGG genes involved in the Nitrogen metabolism from each biotope: seawater (Wt), sediment

(Sd), *S. massa* (Sm), *S. carteri* (Sc), *X. testudinaria* (Xt) and *H. erectus* (He). Error bars represent a single standard deviation. The KEGG genes shown include the following: a) K00260 glutamate dehydrogenase; c) K00261 glutamate dehydrogenase ($NAD(P)^+$); e) K00262 glutamate dehydrogenase ($NADP^+$); g) K00266 glutamate synthase (NADPH/NADH); i) K00366 ferredoxin−nitrite reductase; k) K00371 nitrate reductase beta subunit; m) K00926 carbamate kinase; o) K01915 glutamine synthetase.

5.5. Discussion

With the exception of the seawater community, which was dominated by *Euryarchaeota*, the archaeal community of sediment and sponges was dominated by *Crenarchaeota*. In addition to these two commonly found phyla (Lee et al. 2011; Pires et al. 2012; Polónia et al. 2013) another phylum, *Parvarchaeota*, was detected in this study; however this was only found in sediment. *Parvarchaeota* consists of a newly proposed phylum comprising the genera *Parvarchaeum* and *Micrarchaeum* (Rinke et al. 2013; Nikolaki and Tsiamis 2013). Sediment was the most diverse biotope (3 phyla; 12 classes, 16 orders and 15 families) in the coral reef environment. Moreover, all the unclassified OTUs at the phylum level (36) were found in sediment.

Crenarchaeota have been found to be the dominant phylum in sponge biotopes (Webster et al. 2001; Holmes & Blanch 2007), as was the case in our study. However, the percentage of *Crenarchaeota* communities detected here was lower than in previous studies. Polónia *et al*. (2013) found that 98 and 99.8% of the *S. massa* and *X. testudinaria* archaeal communities respectively in Jakarta (Indonesia) were assigned to the *Crenarchaeota* phylum. In the present study, only 63% of the archaeal community inhabiting *S. massa* and 92% of the archaeal community inhabiting *X. testudinaria* were assigned to *Crenarchaeota.* Polónia et al. (2013) also found that 29% of the seawater community in Jakarta was assigned to *Crenarchaeota.* Here, less than 2% of the seawater community was assigned to this phylum. This would seem to suggest, that in seawater environments dominated by *Euryarchaeota*, the proportion of this phylum in sponge tissues tends to increase; indicating a clear influence of the environment on the sponge microbial communities.

In the tropical surface seawaters of the Georgetown coast (Penang, Malaysia) and Kepulauan Seribu reef system (Java, Indonesia), 65-70% of the archaeal community was

assigned to the *Euryarchaeota* phylum (Chan et al. 2013; Polónia et al. 2013). However, in a South Pacific Gyre, considered one of the cleanest oceanic regions of the world, due to its isolation from sources of pollution, the seawater community was almost entirely composed of *Euryarchaeota* (Yin el al. 2013). These authors suggested that the low ammonium concentration in the seawater of the South Pacific Gyre is the reason for the very low *Crenarchaeota* abundance. *Crenarchaeota* have been shown to be important players in many geochemical cycles. All the cultivated members of the class *Thaumarchaeota* (Mesophilic *Crenarchaeota),* for example, obtain their energy through ammonia oxidation (Offre et al. 2013) and thus play an important role in the nitrogen cycle. A reduced concentration of ammonia may thus limit *Crenarchaeota* abundance. The low abundance of seawater *Crenarchaeota* sequences in the present study is an indication of lower pollution levels when compared, for example, to the Kepulauan Seribu reef system or Georgetown coast. Although rare in the seawater samples, *Crenarchaeota* remained the dominant phylum in all sponge species and sediment. Sponges offer their symbionts a stable and nutrient rich environment, namely a constant supply of ammonia, a metabolic waste product excreted by sponges. This makes sponges suitable habitats for ammonia-oxidizing Archaea (AOA).

Xestospongia testudinaria and *H. erectus* shared a higher number of OTUs with sediment than with seawater. In contrast, *S. carteri* and *S. massa* shared a higher number of OTUs with seawater when compared to sediment. This was reflected both in the phylogenetic tree and in the PCO where samples from both *Stylissa* sponges clustered together with seawater samples. This result may be related to the different morphologies and life strategies of each of the sponge species. The skeleton of the slow growing and long lived *X. testudinaria* consists of a very dense network of silicious spicules (Desqueyroux-Faúndez & Valentine 2002). Sponges belonging to the genus *Stylissa*, in turn, are fast growers and have a loose skeleton of very large spicules (Van Soest et al. 2002), which results in higher amounts of water in their tissues. Indeed, when squeezed, a copious amount of water is expelled from the sponge oscules. *Hyrtios erectus* lives cryptically embedded in the sediment covered in coral sand and the buildup of their skeleton is composed by the incorporation of exogenous materials such as sediment grains. Additionally, sponges belonging to the genus *Xestospongia* have been considered high microbial abundance (HMA) sponges in contrast to sponges belonging to the genus *Stylissa* (Moitinho-Silva et al. 2013). Previous studies have noted that low microbial abundance sponges (LMA) tend to filter much larger volumes of water than HMA sponges, hosting communities with lower specificity and diversity and similar to that of seawater (Weisz et al. 2008; Thacker & Freeman 2012; Moitinho-Silva et al. 2013). This is

consistent with the high amount of water found in the tissues of sponges belonging to the genus *Stylissa* and may account for the greater percentage of shared symbionts in both *Stylissa* species. Our results, however, did not allow us to determine whether the sponge OTUs shared with seawater and sediment belonged to the sponge microbiome (as result of environmental selection) or whether they were merely contaminants.

An ongoing debate in sponge microbial studies is the degree to which sponge microbes are transferred horizontally as opposed to vertically (Hentshel et al. 2002; Sharp et al. 2007; Taylor et al. 2007; Schmitt et al. 2008). In the present study, the most abundant OTU (21591 sequences) was found exclusively in both *Stylissa* species (OTU-1) and represented a phylogenetically distinct lineage. OTU-1, assigned to the genus *Cenarchaeum,* was closely related (with a similarity of 100%) to organisms isolated from *Axinella damicornis* (Margot et al. 2002), *Axinella mexicana* (Preston et al. 1996) and *Phakellia fusca* (Han et al. 2012). Margot et al. (2002) and Holmes and Blanch (2007) previously suggested the existence of a symbiotic association between sponges belonging to the genus *Axinella* and an archaeon closely related to *C. symbiosum*. Here, due to the close taxonomic relationship of these *Axinella* and *Phakellia* sponges with the studied *Stylissa* (all belong to the order *Halichondrida*), we suggest the existence of a possibly order-specific symbiosis between *Halichondrida* and *C. symbiosum*. This also suggests that *C. symbiosum* is transmitted vertically, *i.e*., from parent to offspring. In contrast to our results, Schmitt et al. (2012) showed that the bacterial communities of sponges from the same order were not more similar to one another than the microbial communities of sponges from different orders. However, our results also showed that the majority of abundant OTUs found in both *Stylissa* species were shared with seawater. It is probable that these symbionts are acquired from the surrounding seawater.

The nitrogen metabolism of Archaea assessed in the present study was dominated by KOs related to ammonia.

Ammonium and ammonia incorporation can occur via two distinct pathways: glutamine synthetase/glutamate synthase and glutamate dehydrogenase (Harper et al. 2008). The Glutamate dehydrogenase pathway is responsible for the catalysis of the glutamate catabolism; *i.e*., for the breakdown of glutamate into ammonium and α-ketoglutarate and thus is also responsible for feeding the tricarboxylic acid pathway (TCA) (Peterson and Smith 1999). The glutamate metabolism is, in this way, an important link between the carbon and nitrogen metabolisms (Belitsky and Sonenshein 1998).

All the biotopes had similar relative abundances of glutamine synthetase (K01915). Due to high ammonium affinity, the glutamate synthase pathway is used under restricted nitrogen availability while the glutamate dehydrogenase pathway, due to its low ammonium affinity, requires higher nitrogen availability (Harper et al. 2008). Here, despite presenting a high relative abundance of $NADP^+$, seawater was the most enriched biotope for glutamate synthase (NADPH/NADH; K00266). Sponges and sediment, in contrast, were enriched for glutamate dehydrogenase (NAD^+, $NAD(P)^+$) in comparison to seawater. These results indicate that seawater is a relatively nitrogen poor environment when compared to sponges and sediment (Hentschel et al. 2012).

The reversible oxidative deamination of glutamate to α -ketoglutarate and ammonia is catalysed by glutamate dehydrogenases. These enzymes can act with only one coenzyme (NAD^+ or $NADP^+$) or with two coenzymes ($NAD(P)^+$). The first case occurs normally in lower eukaryotes or in prokaryotes while the second case occurs mainly in higher eukaryotes (Miñambres et al. 2000).

Here, *S. carteri* was enriched for glutamate dehydrogenase, had a relatively low abundance of glutamate dehydrogenase ($NADP^+$) and a very low relative abundance of glutamate dehydrogenase ($NAD(P)^+$). The opposite was the case for *X. testudinaria* and *H. erectus*, which had a high relative abundances of glutamate dehydrogenase ($NAD(P)^+$) and very low relative abundances of glutamate dehydrogenase ($NADP^+$) and glutamate dehydrogenase. *Stylissa massa* had a higher relative abundance of glutamate dehydrogenase ($NAD(P)^+$) than *S. carteri* but lower than *X. testudinaria* and *H. erectus*. *Stylissa massa* also had higher relative abundances of glutamate dehydrogenase ($NADP^+$) and glutamate dehydrogenase than *X. testudinaria* and *H. erectus*. This suggests the existence of different degrees of specialisation in the oxidative deamination of glutamate to α-ketoglutarate and ammonia in different biotopes varying from sponges such as *S. carteri*, which rely mainly on glutamate dehydrogenase to *X. testudinaria* and *H. erectus*, which rely mainly on glutamate dehydrogenase ($NAD(P)^+$).

Seawater had the highest relative abundance of the coenzyme glutamate dehydrogenase ($NADP^+$), which participates in the glutamate anabolism, *i.e*., the transformation of ammonium and α-ketoglutarate into glutamate. This enrichment of a coenzyme promoting ammonium assimilation is consistent with the above suggestion of seawater as a nitrogen poor environment. Seawater also had a high relative abundance of carbamate kinase (K00926), an enzyme that catalyses the transformation of carbamoyl phosphate to carbamate with the concomitant production of ATP. This may be an import source of energy to planktonic *Archaea* (Uriarte et al. 1999).

Sediment, in turn, had the highest relative abundance of nitrate reductase beta subunit (K00371), a possible indication that sediment archaeal communities use nitrate as a nitrogen source or electron acceptor (Tang et al. 2013); suggesting relatively high rates of reduction of nitrate to nitrite (the first step of denitrification) in this biotope (Shapleigh 2006; Liu et al. 2012).

The pathway ferredoxin-nitrite reductase (K00366) was previously identified in *C. symbiosum* (Hallam et al. 2006), an archaeon restricted to both *Stylissa* species. Here, this enzyme was particularly enriched in *S. carteri*, a possible indication that their archaeal communities catalyze the reduction of nitrite, which may have a toxic effect on sponge tissues, to ammonium for further incorporation.

C. symbiosum (OTU-1) comprised more than 62% on average of the *Stylissa* archaeal community, while unclassified members of the *Cenarchaeaceae* family comprised 90.2% (OTU-3 and OTU-77) and 96.0% (OTU-2, OTU-38 and OTU-84) on average of *X. testudinaria* and *H. erectus* archaeal community, respectively. Similarly, these dominant OTUs were responsible for almost all of the sponge KO counts and thus clearly play a dominant role in the *Archaea*-mediated nitrogen metabolism.

5.6. Conclusion

Our study provides novel insights into the function and distribution of Archaea in coral reefs. We observed that a higher proportion of *Euryarchaeota* in seawater appears to influence the proportion of this phylum in sponge hosts. This results in greater compositional similarity between the archaeal communities inhabiting *Stylissa* host species and seawater. This effect is much less pronounced in *X. testudinaria* and *H. erectus*. Based on this and other studies, we also suggest the existence of a possibly order-specific association between *Halichondrida* and *C. symbiosum.* Our results also showed significant differences among biotopes with respect to functional gene content in archaeal assemblages. These differences were accentuated between host and non host biotopes and resulted in clear differences in dominant OTU functions. In sponges belonging to the genus *Stylissa* and seawater, ammonium assimilation is performed preferentially through the expression of NAD^+ or $NADP^+$ specific glutamate dehydrogenase (typical for prokaryotes) and glutamate synthase (NADPH/NADH; K00266) whereas in *X. testudinaria*, *H. erectus* and sediment ammonium assimilation is performed preferentially through the expression of glutamate dehydrogenase with mixed specificity

($NAD(P)^+$). Our results indicate that archaeal communities in host and non-host biotopes have distinct ecophysiological roles and may thus provide complementary nitrogen cycling functions to coral reef ecosystems.

5.7. Acknowledgments

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5.8. References

Belitsky BR & Sonenshein AL (1998) Role and regulation of *Bacillus subtilis* Glutamate dehydrogenase genes. *Journal of Bacteriology* 180: 6298–6305.

Bowen JL, Morrison HG, Hobbie JE & Sogin ML (2012) Salt marsh sediment diversity: a test of the variability of the rare biosphere among environmental replicates. *ISME Journal* 6: 2014–2023.

Bromke MA (2013) Amino acid biosynthesis pathways in diatoms. *Metabolites* 3: 294–311.

Cabello P, Roldán MD, Moreno-Vivián C (2004) Nitrate reduction and the nitrogen cycle in Archaea. *Microbiology 150*: 3527-3546.

Capone DG, Dunham SE, Horrigan SG & Duguay LE (1992) Microbial nitrogen transformations in unconsolidated coral reef sediments. *Marine Ecology Progress Series* 80: 75–88.

Chan XY, Arumugam R, Choo SW, Yin WF & Chan KG (2013) Metagenomic sequencing of prokaryotic microbiota from tropical surface seawater. *Genome Announcements* 1: e00540–13.

Cleary DFR (2003) An examination of scale of assessment, logging and ENSO-induced fires on butterfly diversity in Borneo. *Oecologia* 135: 313–321.

Cleary DFR, Becking LE, Voogd NJ, Pires ACC, Polónia ARM, Egas, C & Gomes, NCM (2013) Habitat-and host-related variation in sponge bacterial symbiont communities in Indonesian waters. *FEMS Microbiology Ecology* 85: 465-482.

Costa R, Keller-Costa T, Gomes NCM, da Rocha, Ulisses N, van Overbeek L & van Elsas JD (2013) Evidence for Selective Bacterial Community Structuring in the Freshwater Sponge *Ephydatia fluviatilis*. *Microbial Ecology* 65: 232–244.

Dang H, Zhou H, Yang J, Ge H, Jiao N, Luan X, Zhang C & Klotz MG (2013) Thaumarchaeotal Signature Gene Distribution in Sediments of the Northern South China Sea: an Indicator of the Metabolic Intersection of the Marine Carbon, Nitrogen, and Phosphorus Cycles? *Applied and Environmental Microbiology* 79: 2137–2147.

de Voogd N, Cleary DFR, Hoeksema B, Noor A & van Soest R (2006) Sponge beta diversity in the Spermonde Archipelago, SW Sulawesi, Indonesia. *Marine Ecology Progress Series* 309: 131–142.

de Voogd NJ & Cleary DFR (2007) Relating species traits to environmental variables in Indonesian coral reef sponge assemblages. *Marine and Freshwater Research* 58: 240–249.

Demain AL (1998) Induction of microbial secondary metabolism. *International Microbiology* 1: 259–264.

Desqueyroux-Faúndez and Valentine (2002) Family *Petrosiidae*. In: Systema Porifera: A Guide to the Classification of Sponges (eds. Hooper JNA and Van Soest RWM). Kluwer Academic/Plenum Publishers, New York.

Diaz MC & Rutzler K (2001) Sponges: an essential component of Caribbean coral reefs. *Bulletin of Marine Science* 69: 535–546.

Ejim L, Farha MA, Falconer SB, Wildenhain J, Coombes BK, Tyers M, Brown ED & Wright GD (2011) Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. *Nature Chemical Biology* 7: 348–350.

Erwin PM, Olson JB & Thacker RW (2011) Phylogenetic diversity, host-specificity and community profiling of sponge-associated bacteria in the northern Gulf of Mexico. *PloS One* 6: e26806.

Fan L, Reynolds D, Liu M, Stark M, Kjelleberg S, Webster NS & Thomas T (2012) Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. *Proceedings of the National Academy of Sciences of the United States of America* 109: E1878–E1887.

Francis CA, Beman JM, Kuypers MMM (2007) New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *ISME J* 1: 19–27.

Francis CA, Roberts KJ, Beman JM, Santoro AE & Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing Archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America* 102: 14683–14688.

Hallam S, Mincer T, Schleper C, Preston C, Roberts K, Richardson P & DeLong E (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. *PloS One Biology* 4: 520–536.

Hansen AK & Moran NA (2011) Aphid genome expression reveals host symbiont cooperation in the production of amino acids. *Proceedings of the National Academy of Sciences of the United States of America* 108: 2849–2854.

Harper C, Hayward D, Wiid I & van Helden P (2008) Regulation of nitrogen metabolism in Mycobacterium tuberculosis: a comparison with mechanisms in *Corynebacterium glutamicum* and *Streptomyces coelicolor*. *IUBMB life* 60: 643-650.

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J & Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. *Applied and Environmental Microbiology* 68: 4431–4440.

Hentschel U, Piel J, Degnan SM & Taylor MW (2012) Genomic insights into the marine sponge microbiome. *Nature Reviews Microbiology* 10: 641-654.

Holmes B & Blanch H (2007) Genus-specific associations of marine sponge with group I Crenarchaeotes. Marine Biology 150: 759–772.

Hoppert M, Krüger M, Reitner J & Cockell C (2013) Archaea in Past and Present Geobiochemical Processes and Elemental Cycles. *Archaea*: *2013*.

Hou W, Wang S, Dong H, Jiang H, Briggs BR, Peacock JP, et al. (2013). A comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan Province China using 16S rRNA gene pyrosequencing. *PloS one 8*: e53350.

Jackson SA, Flemer B, McCann A, Kennedy J, Morrissey JP, OGara F & Dobson AD (2013) Archaea appear to dominate the microbiome of *Inflatella pellicula* deep sea sponges. *PloS One* 8: e84438.

Jiao N & Zheng Q (2011) The Microbial Carbon Pump: from Genes to Ecosystems. *Applied and Environmental Microbiology* 77: 7439–7444.

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, Knight R, Beiko RG & Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 31: 814+.

Lauro FM, DeMaere MZ, Yau S, Brown MV, Ng C, Wilkins D, et al. (2011) An integrative study of a meromictic lake ecosystem in Antarctica. *The ISME Journal 5:* 879-895.

Lee OO, Wang Y, Yang J, Lafi FF, Al-Suwailem A, Qian PY (2011) Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. *The ISME Journal* 5: 650–664.

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia* 129: 271–280.

Liu M, Fan L, Zhong L, Kjelleberg S, & Thomas T (2012) Metaproteogenomic analysis of a community of sponge symbionts. *The ISME Journal 6:* 1515-1525.

Miñambres B, Olivera ER, Jensen RA, & Luengo JM (2000) A New Class of Glutamate Dehydrogenases (GDH) Biochemical and Genetic Characterization of the First Member, The Amp-Requiring Nad-Specific GDH of *Streptomyces Clavuligerus*. *Journal of Biological Chemistry 275:* 39529-39542.

Moitinho-Silva L, Bayer K, Cannistraci CV, Giles EC, Ryu T, Seridi L, et al. (2013). Specificity and transcriptional activity of microbiota associated with low and high microbial abundance sponges from the Red Sea. *Molecular Ecology* 23: 1348–1363.

Nikolaki, S., & Tsiamis, G. (2013). Microbial Diversity in the Era of Omic Technologies. *BioMed Research International* 2013: ID 958719.

Offre P, Spang A & Schleper C (2013) Archaea in Biogeochemical Cycles. *Annual Review of Microbiology* 67: 437-457.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson G, Solymos P, Stevens M, Wagner H (2009) vegan: Community ecology package. R package version: 1:15-2.

Peterson PE & Smith TJ (1999) The structure of bovine glutamate dehydrogenase provides insights into the mechanism of allostery. *Structure* 7: 769-782.

Pires ACC, Cleary DFR, Almeida A, Cunha Â, Dealtry S, Mendonça-Hagler LCS, Smalla K & Gomes NCM (2012) Denaturing Gradient Gel Electrophoresis and Barcoded Pyrosequencing Reveal Unprecedented Archaeal Diversity in Mangrove Sediment and Rhizosphere Samples. *Applied and Environmental Microbiology* 78: 5520–5528.

Polónia ARM, Cleary DRF, Duarte LN, de Voogd NJ, Gomes NCM (2013) Composition of Archaea in seawater, sediment and sponges in the Kepulauan Seribu reef system, Indonesia. *Microbial Ecology* 67: 553–567.

Preston CM, Wu KY, Molinski TF & DeLong EF (1996) A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. *Proceedings of the National Academy of Sciences* 93: 6241–6246.

Previsic A, Walton C, Kucinic M, Mitrikeski PT & Kerovec M (2009) Pleistocene divergence of Dinaric Drusus endemics (*Trichoptera, Limnephilidae*) in multiple microrefugia within the Balkan Peninsula. *Molecular Ecology* 18: 634–647.

R Core Team (2013) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available from http://www.R-project.org/.

Radax R, Rattei T, Lanzen A, Bayer C, Rapp HT, Urich T & Schleper C (2012) Metatranscriptomics of the marine sponge *Geodia barretti*: tackling phylogeny and function of its microbial community. *Environmental Microbiology* 14: 1308–1324.

Reigstad LJ, Richter A, Daims H, Urich T, Schwark L & Schleper C (2008) Nitrification in terrestrial hot springs of Iceland and Kamchatka. *FEMS Microbiology Ecology* 64: 167–174.

Renema W, (2010) Is increased calcarinid (foraminifera) abundance indicating a larger role for macro-algae in Indonesian Plio-Pleistocene coral reefs? *Coral Reefs* 29: 165-173.

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, et al. (2013) Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 499: 431 - 437.

Schmitt S, Angermeier H, Schiller R, Lindquist N & Hentschel U (2008) Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. *Applied and Environmental Microbiology* 74: 7694–7708.

Schmitt S, Tsai P, Bell J, Fromont J, Ilan M, Lindquist N, Perez T, Rodrigo A, Schupp PJ, Vacelet J, Webster N, Hentschel U & Taylor MW (2012) Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *The ISME Journal* 6: 564–576.

Shapleigh, JP (2006). The denitrifying prokaryotes. In:The prokaryotes (eds. Dworkin M, Falkow S, Rosenberg E, Schleifer KH,Stackebrandt E), pp. 769–792.Springer, New York.

Shapleigh JP (2013) Denitrifying Prokaryotes. In:*The Prokaryotes: Prokaryotic Physiology and Biochemistry* (eds. Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F), pp. 405-425. Springer, Berlin Heidelberg.

Sharp KH, Eam B, Faulkner DJ & Haygood MG (2007) Vertical transmission of diverse microbes in the tropical sponge *Corticium sp*. *Applied and Environmental Microbiology* 73: 622–629.

Shi Y, Tyson G W, Eppley JM, DeLong EF (2011) Integrated metatranscriptomic and metagenomic analyses of stratified microbial assemblages in the open ocean. *The ISME Journal 5*: 999-1013.

Simister RL, Deines P, Botté ES, Webster NS, Taylor MW (2012) Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. *Environmental Microbiology* 14: 517-524.

Sipkema D, Franssen M, Osinga R, Tramper J & Wijffels R (2005) Marine sponges as pharmacy. *Marine Biotechnology* 7: 142–162.

Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM & Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored “rare biosphere”. *Proceedings of the National Academy of Sciences of the United States of America* 103: 12115–12120.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.

Tang K, Liu K, Jiao N, Zhang Y, & Chen CTA (2013) Functional Metagenomic Investigations of Microbial Communities in a Shallow-Sea Hydrothermal System. *PloS One*, 8: e72958.

Taylor MW, Radax R, Steger D & Wagner M (2007) Sponge associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews* 71: 295–347.

Thacker RW & Freeman CJ (2012) Sponge-microbe symbioses: recent advances and new directions. *Advances in Marine Biology* 62: 57–111.

Tourna M, Stieglmeier M, Spang A, Koenneke M, Schintlmeister A, Urich T, Engel M, Schloter M,Wagner M, Richter A & Schleper C (2011) *Nitrososphaera viennensis*, an ammonia oxidizing archaeon from soil. *Proceedings of the National Academy of Sciences of the United States of America* 108: 8420–8425.

Urakawa H, Martens-Habbena W & Stahl DA (2010) High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method. *Applied and Environmental Microbiology* 76: 2129–2135.

Uriarte M, Marina A, Ramón-Maiques S, Fita I, & Rubio V (1999) The carbamoyl-phosphate synthetase of Pyrococcus furiosus is enzymologically and structurally a carbamate kinase. *Journal of Biological Chemistry* 274: 16295-16303.

Van Soest, Rob WM, Dirk Erpenbeck, and Belinda Alvarez (2002). "Family Dictyonellidae Van Soest, Diaz & Pomponi, 1990." In: Systema Porifera (eds Hooper JNA; Van Soest RWM & Willenz P), pp. 773-786. Springer, US.

Wang S, Xiao X, Jiang L, Peng X, Zhou H, Meng J & Wang F (2009) Diversity and Abundance of Ammonia-Oxidizing Archaea in Hydrothermal Vent Chimneys of the Juan de Fuca Ridge. *Applied and Environmental Microbiology* 75: 4216–4220.

Webster NS, Watts JEM & Hill RT (2001) Detection and phylogenetic analysis of novel *Crenarchaeote* and *Euryarchaeote* 16S ribosomal RNA gene sequences from a Great Barrier Reef sponge. *Marine Biotechnology* 3: 600–608.

Weisz JB, Lindquist N & Martens, CS (2008) Do associated microbial abundances impact marine demosponge pumping rates and tissue densities?. *Oecologia* 155: 367-376.

Wemheuer B, Wemheuer F & Daniel R (2012) RNA Based Assessment of Diversity and Composition of Active Archaeal Communities in the German Bight. *Archaea-an International Microbiological Journal* 2012: 695826.

Wilson, M. S., Siering, P. L., White, C. L., Hauser, M. E., & Bartles, A. N. (2008). Novel archaea and bacteria dominate stable microbial communities in North America's largest hot spring. *Microbial Ecology* 56: 292-305.

Yan B, Hong K, Yu ZN, et al. (2006) Archaeal communities in mangrove soil characterized by 16S rRNA gene clones. *Journal of Microbiology-Seoul* 44: 566.

Yin Q, Fu B, Li B, Shi X, Inagaki F & Zhang XH (2013) Spatial Variations in Microbial Community Composition in Surface Seawater from the Ultra- Oligotrophic Center to Rim of the South Pacific Gyre. *PloS One* 8: e55148.

Yung PY, Burke C, Lewis M, Kjelleberg S & Thomas T (2011) Novel Antibacterial Proteins from the Microbial Communities Associated with the Sponge *Cymbastela concentrica* and the Green Alga *Ulva australis*. *Applied and Environmental Microbiology* 77: 1512–1515.

Zhang LM, Offre PR, He JZ, Verhamme DT, Nicol GW & Prosser JI (2010) Autotrophic ammonia oxidation by soil *Thaumarchaea*. *Proceedings of the National Academy of Sciences of the United States of America* 107: 17240–17245.

Zhang Z, Schwartz S, Wagner L & Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7: 203–214.

Chapter 6: Conclusions and Future research directions

The percentage of the world's reefs reported to be threatened has increased dramatically in recent decades. The increase of urban settlements in coastal zones has been affecting these environments through various forms of disturbance namely river runoff, land based pollution, untreated sewage, deforestation, sedimentation, tourism and overfishing. In order to design effective conservation strategies, a deep understanding of how the different coral reef taxa deal with these disturbances is required. This thesis has contributed to increase the understanding of the coral reef responses to human-imposed stresses.

The analysis of the response of the different studied taxa to environmental and spacial variables demonstrated that environmental variables are the most important in explaining variation in composition in the Spemonde, but that distinct taxa react differently to the same environmental variable and this response varies strongly with depth. Of the studied environmental variables CDOM was the most important explanatory variable and thus should be taken in consideration in any future conservation management programs. A study assessing the temporal and spatial variation of Chlro_a and SST in our two focal study regions over the last 17 years is already underway, and a study assessing coral cover variation in Spermonde Archipelago over the last 18 years is also projected.

Despite the growing number of studies accessing archaeal communities, few have accessed coral reef environments and most of them have focused on a single taxon. This thesis reported, for the first time, archaeal composition and function in different sponge hosts, seawater and sediment in coral reef environments. The results of the two different studies on archaeal communities showed *Crenarchaeota* as the dominant phylum in sponge species and sediment and *Euryarchaeota* as the dominant phylum in seawater samples. The proportion of these two phyla in the surrounding seawater can influence the sponge archaeal community in such a way that phylogenetic similarities between the archaeal community of sponges belonging to the genus *Stylissa* and seawater were observed. Indications of vertical transmission in sponges belonging to the genus *Stylissa* were also shown, with the suggestion of an order-specific association between *Halichondrida* and *Cenarchaeum symbiosum*. To further investigate the role of *Archaea* in coral reefs additional studies on the functional patterns observed could be undertaken by analyzing in detail the functional genes responsible for the top level functional categories. Furthermore, cross-shelf studies should allow us to understand how ecological gradients and anthropogenic disturbances affect archaeal composition, diversity and function in the different reef environments and biotopes.

Chapter 7: Appendix

7.1. Supplementary Materials and Methods

7.1.1. 16S rRNA gene barcoded-pyrosequencing

Using DNA as template, the V3V4 region was amplified, using barcoded fusion primers (524F-10- ext (5'– TGYCAGCCGCCGCGGTAA -3') and Arch958R-mod (5'-CCGGCGTTGAVTCCAATT -3') (Pires et al. 2012) with the Roche-454 A and B Titanium sequencing adapters, an eight-base barcode sequence in adaptor B and specific sequences for the ribosomal region. These regions were amplified using two consecutive amplification reactions. Two replicate PCR reactions were performed using 0.2 mM of each archaeal specific primers, 1x Advantage 2 Polymerase Mix (Clontech, Mountain View, CA, USA), 1x Advantage 2 PCR Buffer, 0.2 mM dNTPs (Bioron, Ludwigshafen am Rhein, Germany), 5% (vol/vol) dimethyl sulfoxide (DMSO) (Roche Diagnostics GmbH, Mannheim, Germany) and genomic DNA template. After a denaturation step at 94°C during 4 min, 30 thermal cycles of 30sec at 94°C, 45sec at 50°C and 1 min at 68°C were carried out followed by an extension step at 68°C for 10 min. The PCR products were quantified fluorimetrically with PicoGreen (Invitrogen, CA, USA), pooled at equimolar concentrations and sequenced in the A direction with GS 454 FLX Titanium chemistry, according to manufacturer's instructions (Roche, 454 Life Sciences, Brandford, CT, USA).

7.1.2. Sequence analyses of 16S rRNA gene fragments

The barcoded pyrosequencing libraries were analysed using the QIIME (Quantitative Insights Into Microbial Ecology; (Caporaso et al. 2010) software package (http://www.qiime.org/; last checked 2014-01-20) on a computer running the BioLinux 7 operating system (http://nebc.nerc.ac.uk/; checked 2014-01-20). In QIIME, fasta and qual files were used as input for the split_libraries.py script. Default arguments were used except for the minimum sequence length, which was set at 218 bps after removal of forward primers and barcodes; backward primers were removed using the 'truncate only' argument and a sliding window test of quality scores was enabled with a value of 50 as suggested in the QIIME description for the script. In addition to user-defined cut-offs, the split_libraries.py script performs several quality filtering steps (http://qiime.org/scripts/split_libraries.html). OTUs were selected using UPARSE with usearch7 (Edgar 2013). The UPARSE sequence analysis tool (Edgar 2013) provides clustering, chimera checking and quality filtering on de-multiplexed sequences. Chimera checking was performed using the UCHIME algorithm, which is the fastest and most sensitive chimera checking algorithm currently available (Edgar et al. 2011). The quality

filtering as implemented in usearch7 filters noisy reads and preliminary results suggest it gives results comparable to other denoisers such as AmpliconNoise, but is much less computationally expensive (http://drive5.com/usearch/features.html; last checked 2014-01-20). First reads were filtered with the -fastq_filter command and the following arguments -fastq_trunclen 250 -fastq_maxee 0.5 -fastq_truncqual 15. Sequences were then dereplicated and sorted using the -derep_fulllength and -sortbysize commands. OTU clustering was performed using the -cluster_otus command. An additional chimera check was subsequently applied using the -uchime_ref command with the gold.fa database (http://drive5.com/uchime/gold.fa). AWK scripts were then used to convert the otus to QIIME format. In QIIME, representative sequences were selected using the pick_rep_set.py script in QIIME using the 'most_abundant' method. Reference sequences of OTUs were assigned taxonomies using default arguments in the assign_taxonomy.py script in QIIME with the rdp method (Wang et al. 2007). In the assign_taxonomy.py function, we used a fasta file containing reference sequences from the Greengenes 13_5 release for and the rdp classifier method. We used a modified version of the taxonomy file supplied with the Greengenes 13_5 release to map sequences to the assigned taxonomy. Finally, we used the make_otu_table.py script in QIIME to generate a square matrix of OTUs x samples. This was subsequently used as input for further analyses using the R package (R Core Team 2013).

7.1.3. Blast analysis

Sequence Identifiers of closely related taxa of numerically dominant OTUs (≥ 100 sequences) were downloaded using the NCBI Basic Local Alignment Search Tool (BLAST) command line 'blastn' tool with the -db argument set to nt (Zhang et al. 2000). BLAST identifies locally similar regions between sequences, compares sequences to extant databases and assesses the significance of matches; functional and evolutionary relationships can subsequently be inferred. Each run produces a list of hits based on significant similarity between pairs of sequences, *i.e.,* the target sequence and taxa present in the database (or no hits if no significantly similar sequences are found). A discussion of how significance is determined can be found at http://www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html. We used the blastn command line tool in a Linux environment to query representative sequences of selected taxa including all the most abundant OTUs (≥ 100 sequences) against the online NCBI nucleotide database. We then generated a vector containing sequence identifiers (GI's) of the ten top hits of all representative sequences and used the Entrez.efetch function in

BioPython with the rettype argument set to 'gb' to download genbank information of aforementioned top hits including the isolation source of the organism and the host.

7.1.4. Phylogenetic analysis

The phylogenetic tree was built using the Mega5 program (http://www.megasoftware.net/; last checked 2012/11/20; Tamura et al. 2011) with the Nearest-Neighbor-Interchange and Generalised Time-Reversible model (Tavaré 1986) with Gamma distributed and invariant sites. In the results, we present a bootstrap consensus tree based on 100 replicates (Felsenstein 1985). Branches reproduced in less than 50% of the bootstrap replicates are collapsed. The bootstrap value is shown next to each branch when this exceeds 50%. This value represents the percentage of replicate trees in which the associated taxa clustered together.

7.1.5. Metagenome analysis

PICRUSt is a bioinformatics tool that uses marker genes, in this case 16S rRNA, to predict metagenome gene functional content. These predictions are precalculated for genes in databases including KEGG (Kyoto Encyclopedia of Genes and Genomes) and COG (Clusters of Orthologous Groups of proteins). In the present study we used the KEGG database. In PICRUSt we used the pick_closed_reference_otus.py script to produce a table of OTUs and the normalize_by_copy_number.py script to normalise this table by marker gene copy number. The normalised data was used as input for the predict_metagenomes.py script, which produces a table of metagenome functional predictions for a given OTU table. Output of the *predict_metagenomes.py* script consists of a table of gene (or functional) counts assigned to KEGG orthologs (KOs). KOs are sets of orthologous (high sequence similarity and consistent phylogenetic position; Smit and Mushegian 2000) biosynthesis genes that have been shown to catalyze the same reaction within the same pathway and are thus functionally correlated (Aoki-Kinoshita and Kanehisa 2009). These ortholog groups are graphically represented as nodes in KEGG individual pathways (Kanehisa and Goto 2000). Finally, we used the metagenome_contributions.py script to partition the metagenome functional contributions according to function, OTU, and sample. Given data on the taxonomic assignment of OTUs, this enabled us to assess the metagenome functional contributions of taxa at varying degrees of taxonomic resolution (e.g., at phylum, class or order level).

7.1.6. KEGG pathway database

The KEGG pathway database (Ogata et al. 1999) is a collection of functional pathways complemented with a series of ortholog group tables. These functional pathways are graphical representations of molecular interactions and relations of gene products (proteins, enzymes) responsible for various cellular functions (Kanehisa et al. 2004).The KEGG pathway functional hierarchy consists of 7 top level categories. These include 1. Metabolism; 2. Genetic Information Processing; 3. Environmental Information Processing; 4. Cellular Processes; 5. Organismal Systems; 6. Human Diseases and 7. Drug Development. These in turn consist of from 3 to 12 subcategories; each of the subcategories in turn contains a number of individual pathways. For example, the Metabolism category consists of 12 subcategories including carbohydrate metabolism, lipid metabolism and energy metabolism. The energy metabolism subcategory in turn consists of 8 individual pathways. These are 1. Oxidative phosphorylation; 2. Photosynthesis; 3. Photosynthesis - antenna proteins; 4. Carbon fixation in photosynthetic organisms; 5. Carbon fixation pathways in prokaryotes; 6. Methane metabolism; 7. Nitrogen metabolism and 8. Sulfur metabolism. The individual pathways in turn consist of KEGG Orthologs (KOs) (Kanehisa *et al*. 2004). Note that because of functional overlap, some orthologs can be represented in multiple pathways. Since KOs can belong to several pathways, we used the categorize_by_function.py script in PICRUSt to collapse the PICRUSt predictions at the level of the individual pathways.

7.2. References

Aoki-Kinoshita KF, Kanehisa M (2009) Using KEGG in the Transition from Genomics to Chemical Genomics. In: Bioinformatics for Systems Biology (eds Krawetz S), pp. 437-452. Humana Press.

Caporaso, JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Tumbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME Allows Analysis of High-Throughp Community Sequencing Data. *Nature Methods* 7: 335–336.

Edgar RC (2013) UPARSE: Highly Accurate OTU Sequences from Microbial Amplicon Reads. *Nature Methods* 10: 996–998.

Edgar RC, Haas B, Clemente J, Quince C, Knight R (2011) UCHIME Improves Sensitivity and Speed of Chimera Detection. *Bioinformatics* 27: 2194–2200.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 783–791.

Kanehisa M, Goto S (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research* 28: 27–30.

Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M (2004) The KEGG Resource for Deciphering the Genome. *Nucleic Acids Research* 32: D277–D280.

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, Knight R, Beiko RG, Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 31: 814+.

Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M (1999) KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research* 27: 29–34.

Pires A, Cleary D, Almeida A, Cunha Â, Dealtry S, Mendonça-Hagler L, Smalla K, Gomes N (2012) Denaturing Gradient Gel Electrophoresis and Barcoded Pyrosequencing Reveal Unprecedented Archaeal Diversity in Mangrove Sediment and Rhizosphere Samples. *Applied and Environmental Microbiology* 78: 5520–5528.

R Core Team (2013) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available from http://www.R-project.org/.

Smit A, Mushegian A (2000) Biosynthesis of Isoprenoids via Mevalonate in Archaea: The Lost Pathway. *Genome Research* 10: 1468–1484.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) Mega5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. *Lect. Math. Life Sci* 17: 57–86.

Wang Q, Garrity G, Tiedje J, Cole J (2007) Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences Into the New Bacterial Taxonomy. *Applied and Environmental Microbiology* 73: 5261–5267.

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7: 203–214.