Sponge nutritional modes in an Indo-Pacific seagrass ecosystem

By

Ramadian Bachtiar

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This thesis was conducted under the supervision of:

Prof James J. Bell

(Primary supervisor, Victoria University of Wellington)

&

AProf Hawis H. Madduppa

(Co-supervisor, IPB University, Bogor, Indonesia)

Victoria University of Wellington

Wellington, New Zealand

Abstract

Life on earth depends on the constant cycle of nutrients that keep ecosystems in equilibrium and maintain ecosystem functioning, which might be disrupted due to the major and rapid environmental changes we are currently facing. Sponges are a major component of benthic communities in many marine ecosystems worldwide. Most sponges are heterotrophic and gain their nutritional needs from filter feeding dissolved organic carbon and particulate organic carbon from the water column. However, some sponges also form symbioses with photosynthetic symbionts and are autotrophic under some circumstances. These two different nutritional modes may play different roles in the movement of energy flow through benthic systems. Here I studied the sponge assemblages in a seagrass meadow at two spatially separated sites at three tidal zones, located in the Wakatobi National Park, Indonesia, which is representative of shallow-water seagrass ecosystems in the Southeast Asian Indo-Pacific bioregion. My thesis aims to increase our understanding of the importance of these two different sponge nutritional modes in seagrass ecosystems to better understand how environmental change might impact sponge assemblages.

In my first data chapter, I conducted sponge and seagrass surveys to investigate the seagrass and sponge community structure and the potential drivers of the observed distribution patterns. For the identification of the sponges, I combined morphological and molecular techniques, using four DNA markers (18S, 28S, ITS, and CO1-ext). I identified ten sponge species: *Spongia* sp., *Spheciospongia* sp, *Phyllospongia foliascens*, *Haliclona koremella*, *Amphimedon* sp., *Dactylospongia elegans*, *Axinella* sp., *Clathria reinwardti*, *Rhopaloeides* sp., and *Siphonodictyon mucosum*. Sponges were found in all tidal zones of the studied seagrass meadow, including at the high-shore zone that regularly experiences aerial exposure during low tide. I propose that sponge morphological adaptation is important for sponge survival in the different seagrass zones.

My second data chapter determined the dominant nutritional mode of seagrass sponges by measuring *in situ* gross primary production (GPP) to dark respiration (P:R) ratios. I measured the P:R ratios for eight sponges out of the ten found in the studied seagrass meadow. I found that six sponges were autotrophic and net oxygen producers over a full 24 hour period. Based on their high biomass, autotrophic sponges contributed considerably to the total sponge assemblage biomass at all tidal zones and sites. While the high-shore and middle-shore zones

had more similar abundances of heterotrophic and autotrophic sponges, autotrophic sponges dominated the sponge assemblage in the near-reef-flat zone of the seagrass meadow by contributing up to 98% of the total sponge biomass.

In my third data chapter, I measured the sponge-mediated organic carbon flux *in situ* to investigate the influence of sponges on the carbon flow from the water column to benthos. The five most abundant sponge species, comprising two heterotrophic sponges and three autotrophic sponges, representing 75.1–99.8% of the total sponge biomass at my studied seagrass meadow, were investigated. I found that in dark incubations (representing night-time), the heterotrophic and autotrophic sponges did not show significant differences in sponge-mediated carbon flux ($p > 0.05$). However, in light incubations (representing daytime), autotrophic sponges released organic carbon to the water column, while heterotrophic sponges removed organic carbon from the water column. Overall, at the seagrass meadow level, the sponge assemblage was still a net remover of organic carbon over a 24-hour cycle at all tidal zones, including where autotrophic sponges contributed 98% of the total sponge biomass (the near-reef-flat zone).

In my fourth data chapter, I measured the sponge-mediated picoplankton flux of the six most abundant sponges (representing 75.1–99.8% of the total sponge biomass) *in situ* to investigate the influence of sponge assemblages on picoplankton abundance. I found diel variability in all picoplankton observed and for the sponge-mediated fluxes. I found that heterotrophic sponges removed significantly more picoplankton-derived organic carbon than the autotrophic sponges, removing 40–60 times more carbon than the autotrophic sponges over 24 hours. This chapter supports the hypothesis that autotrophic sponges are gaining supplementary nutrition from their hosted photosynthetic symbionts, reducing their need to suspension feed for their nutritional needs.

In summary, autotrophic sponges dominated the sponge assemblages in the seagrass ecosystem I studied in the Southeast Asian Indo-Pacific bioregion. This thesis has shown that autotrophic sponges can be net oxygen producers, releasing organic carbon to the water column (during daytime) and thus are not so dependent on picoplankton feeding. Variation in bentho-pelagic interactions due to autotrophic sponges compared to heterotrophic sponges will influence the impact of any increase or decline in sponges as a result of changing environmental conditions.

...to Anti

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Chapter contributions

Chapter 2:

Study design: Ramadian Bachtiar, Prof James J. Bell (Victoria University of Wellington, New Zealand), and AProf Hawis H. Madduppa (IPB University, Indonesia).

Data collection: RB

Data analysis: RB

Chapter writing and editing: RB, JJB, and HHM.

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Chapter 3:

Study design: Ramadian Bachtiar, Prof James J. Bell (Victoria University of Wellington, New Zealand), and AProf Hawis H. Madduppa (IPB University, Indonesia).

Data collection: RB

Data analysis: RB

Chapter writing and editing: RB, JJB, and HHM.

Publication: **Bachtiar**, R., H.M. Madduppa, and J.J. Bell. (*In review*). Variation in autotrophic and heterotrophic sponge abundance in a shallow water seagrass system. *Marine Biology*.

Chapter 4:

Study design: Ramadian Bachtiar, Prof James J. Bell (Victoria University of Wellington, New Zealand), and AProf Hawis H. Madduppa (IPB University, Indonesia).

Data collection: RB

Data analysis: RB

Chapter writing and editing: RB, JJB, and HHM.

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Chapter 5:

Study design: Ramadian Bachtiar, Prof James J. Bell (Victoria University of Wellington, New Zealand), and AProf Hawis H. Madduppa (IPB University, Indonesia).

Data collection: RB

Data analysis: RB and Dr Kylie Price (Malaghan Institute of Medical Research, Wellington, New Zealand)

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CHAPTER 1.

General introduction

A changing world

The world faces major and rapid environmental changes as human activities have reached the level that is altering the Earth (Vitousek et al. 1997). Back in 1997, Vitousek et al. reported that human actions had transformed one-third to one-half of Earth's terrestrial surface, where humans fixed more nitrogen than all natural terrestrial sources combined, and had caused a 30% higher carbon dioxide concentration in the atmosphere than at the beginning of the Industrial Revolution. These changes and those since have led many authors to describe this current era as the Anthropocene – the geological epoch of mankind (Crutzen 2002). Any major environmental change will potentially cause biodiversity loss, affecting ecosystem stability and may lead to the loss of ecosystem function and services (Hooper et al. 2005; Worm et al. 2006; Cardinale et al. 2012; Hautier et al. 2015).

Currently, 690 million people (about 10% of the world's population) live in coastal areas that are less than 10 m above sea level, and nearly 2.4 billion people (about 40% of the world's population) live within 100 km of the coast, creating tremendous pressure on the marine environment (IPCC 2019). Urban development, aquaculture, fishing activities, and land conversion to agriculture, including rice farming and timber over-exploitation, are the main pressures on tropical coastal regions. These activities have already resulted in major degradation of the marine environment. For example, global mangrove forests lost 35% of their global cover from 1980 to 2000 (Valiela et al. 2001). The world had already lost 19% of its original coral reef areas by 2008 (Wilkinson 2008), and the rate of loss remains high due to ongoing climate change and other local stressors (Hughes et al. 2017). The median return time between severe bleaching events has declined steadily since 1980 and is now only six years, which is not long enough to full recovery of mature coral assemblages (Hughes et al. 2018). Seagrass beds have also experienced significant declines in recent decades (Duarte et al. 2008), with the world loosing 33 million ha of its seagrass area or about 18% of the documented global seagrass area from 1988 to 2008.

Sponges

Sponges (phylum Porifera) are the oldest extant metazoan group (i.e. animal) and are considered the sister group of all other extant animals (reaffirmed by Pisani et al. 2015). Sponges are found across all marine environments (Figure 1.1) from the tropics (e.g. Bell and Smith 2004) to polar regions (e.g. Starmans et al. 1999) and from shallow water (e.g. Barnes

1999) to the deep oceans (McIntyre et al. 2016). Sponges can be found in rocky or reef environments, to sandy and muddy substrates (e.g. Becking et al. 2013), and some sponges have colonized a wide variety of freshwater habitats (Manconi and Pronzato 2008). Based on the World Porifera Database to date (accessed on 8 April 2022), there are 9,227 marine sponge species that have been validated (de Voogd 2022). With this number of species, sponges are considered one of the most species-rich marine assemblages.

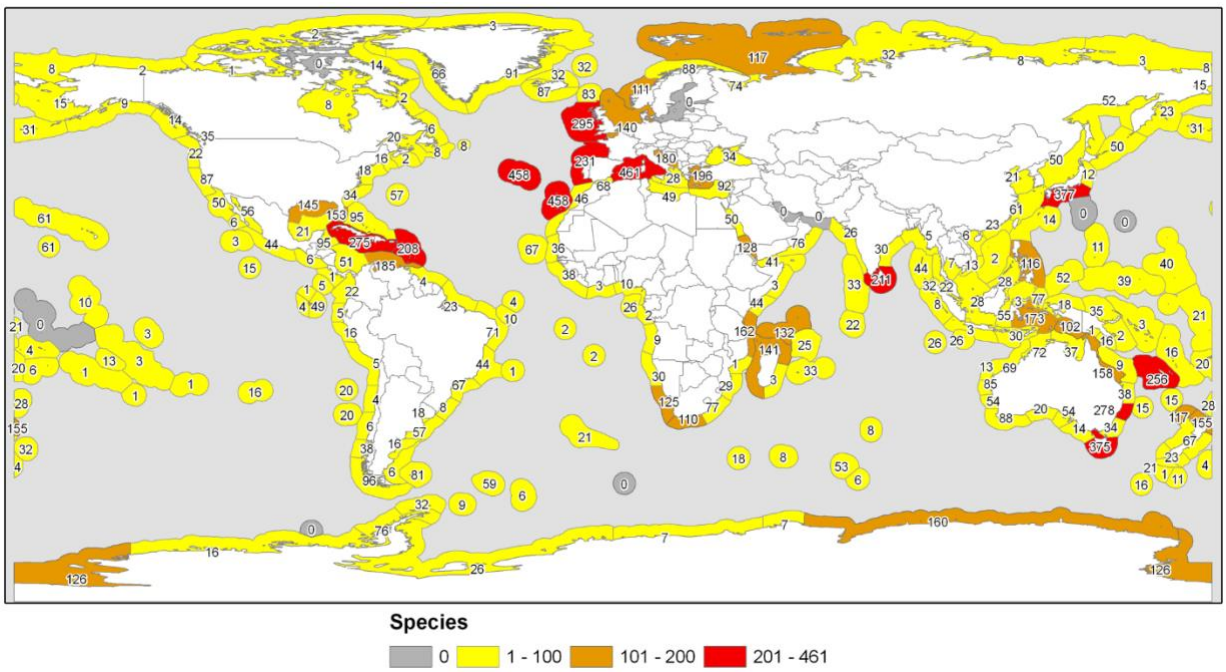

Figure 1.1. The global distribution of sponge richness (Van Soest et al. 2012), noting some area have been much more extensively studied than others.

Sponges are exclusively aquatic animals (Bergquist 1978). They are divided into four classes (based mainly on the composition of their skeletons), which are Demospongiae, Hexactinellida (glass sponges), Calcarea (calcareous sponges), and Homoscleromorpha (de Voogd 2022). Demospongiae is the most diverse class in Porifera; it lives in both marine and freshwater environments, and comprises about 80% of all sponge species, grouped into 14 orders (de Voogd 2022). Hexactinellida, also known as glass sponges, are exclusively marine with more than 600 species (in five orders) and are mostly found in deeper water (200 to >6000 m). However, there are also reports that glass sponges occur in shallow water sub-marine caves in the Mediterranean (Vacelet 1996) and at shallower than 50 m in Fiordland, New Zealand (Battershill and Bergquist 2010). Calcareous sponges are restricted to marine environments, where most of them are found in shallow waters and are characterized by calcium carbonate

spicules. There are about 700 sponge species in the class Calcarea, or 8% of the total sponge species. Homosclemorpha is the most recently recognized class of Porifera and the smallest one, with less than 100 species that are exclusively marine (Gazave et al. 2011). They are the only Porifera class with a true basement membrane and are generally found in shallow water.

The sponge body structure comprises internal water canals and chambers, through which a unidirectional current flows. The water is drawn in through many small inhalant pores (ostia) that bring nutrients and oxygen, and expelled through larger exhalant pores (osculum) that carry out waste and reproductive elements. Most sponges have three body layers. The outer layer, or the "skin" of the sponge, is called the pinacoderm and is formed by pinacocytes cells. The middle layer or mesohyl consists of a collagen matrix and skeleton material responsible for the shape of the sponge. The inner layer or choanoderm is where the feeding cells are located (called choanocytes), which line the canals and chambers and are also responsible for generating a water current. Sponges have a relatively small number of cell types, and each cell type may have several functions and have a high capacity for cell trans-differentiation (Renard et al. 2013). This is an important feature since sponges do not have organs but have specialized cells that perform various functions. The most important cell type is the archaeocyte, which can transform into other cell types when needed and is considered the stem cell of sponges (Muller 2006).

Sponges can reproduce sexually and asexually (Ayling 1980; Maldonado and Riesgo 2009). With respect to sexual reproduction, most sponges are sequential hermaphrodites where they produce sperm and egg cells at different times for cross-fertilization with other sponges of the same species (Goudie et al. 2013). However, sponges can also reproduce asexually through budding or fragmentation (e.g. Wulff 1991; Ereskovsky and Tokina 2007). Some freshwater sponges have gemmules – internal buds resistant to extreme conditions for long periods, and when the environment becomes less hostile, they can develop into new sponges (Simpson and Gilbert 1973).

The functional roles of marine sponges

Sponges are an essential component of many marine benthic communities (Bell et al. 2020). Diaz and Rützler (2001) highlighted three ecological properties of sponges that make sponges critical to ecosystem function. First, sponges are one of the most diverse benthic components and promote diversity through their associated infauna. Second, sponges are one of the most

abundant (in terms of area occupied) organisms. Third, sponge biomass (weight and volume) may exceed that of other reef epibenthos.

Sponges play a wide variety of functional roles. Bell (2008) categorized sponge functional roles into three main categories: their impacts on the substrate, linkages between benthic and pelagic environments, and their association with other organisms. The impacts of sponges on substrate include bioerosion, reef creation, and substrate stabilization, consolidation and regeneration (reviewed by Schönberg et al. 2017). These roles are based on the capability of some sponges to break down solid calcium carbonate into smaller fragments and fine sediments (Rützler 1975), which is expected to accelerate in the future as ocean acidification progresses (Wisshak et al. 2014). Sponges also affect the substrate by 'glueing' together corals rubble, resulting in a more stable substrate necessary for coral recruitment and recovery (Wulff 1984).

Sponges can pump large quantities of water with high filtering efficiency and particle retention (Reiswig 1971a; Reiswig 1971b; Reiswig 1974). This means that sponges can strongly influence the water column and often link pelagic and benthic environments. Sponges link the benthic environment with the pelagic environment through carbon cycling and energy flow, silicon cycling, nitrogen cycling, and localized oxygen depletion in the water column (see review by Maldonado et al. 2012). Sponges are involved in carbon cycling through their feeding activity, with sponges feeding on ultraplankton – planktonic organisms sized less than 10 μm (e.g. Perea-Blázquez et al. 2013), dissolved organic carbon (DOC; Yahel et al. 2003), and also viruses (e.g. Hadas and Marie 2006), and also by providing fixed carbon to higher trophic levels through the 'sponge loop' (de Goeij et al. 2013). Since most sponges build skeletons (spicules) from biogenic silica, sponge populations are one of the major silicon sinks in the marine environment (Maldonado et al. 2005). Sponges also mediate nitrogen cycling by hosting a wide diversity of microbes (e.g. Jiménez and Ribes 2007; Hoffmann et al. 2009). In some circumstances where sponges are dominant (either in coverage area or biomass), sponges might affect the local dissolved oxygen concentration of the water column. Richter et al. (2001) reported that in coral reef framework cavities, when 60% of the cavity-dwellers were sponges, the community respiration rate caused a small but significant reduction of oxygen level compared to water overlying the reef.

Sponge interactions with other organisms also facilitate several functional roles. Sponges contribute to the primary production by having symbiosis with photosynthetic organisms (e.g. Wilkinson 1983). Sponges are also 'home' for many macrofaunae and support biodiversity. For

example, Koukouras et al. (1996) reported that *Agelas oroides* had 135 associated species, *Aplysina aerophoba* had 184 associated species, and *Axinella cannabina* had 84 associated species (Koukouras et al. 1996). Sponges also protect some organisms from predation. For example, the bivalve *Arca noae* lives in association with with the demosponge *Crambe crambe* making use of the sponge's secondary metabolites to deter predators (Marin and López Belluga 2005). Some sponges also as act as secondary settlement substrates for other organisms and may also influence near-boundary and reef level water flow regimes due to their often three-dimensional and erect morphologies (Bell 2008).

Sponge nutritional modes

Heterotrophy in sponges

Filter feeder

Most sponges are filter-feeders. They feed by trapping organic matter filtered from the water. Owing to their specialized aquiferous system, sponges can pump and filter large quantities of water. For example, a study on the tropical sponge *Aplysina lacunosa* reported that a sponge with 500 ml body volume is able to pump 1–6 litres per hour (Gerrodette and Flechsig 1979). Weisz et al. (2008) reported that 1 kg dry weight of *Callyspongia plicifera* can pump 6.48 litres of seawater per second. The water enters the sponge body through ostia distributed over the body surface, flows down the canals and then through choanocyte chambers, and is finally expelled through the osculum. Ostia size is the first filter for the edible particle size of sponges. Large particles (> 50 μm) are filtered by the ostia and taken up by epithelial pinacocytes at the sponge body surface; particles with a size below 50 μm will go through the ostia and are taken up by the pinacocytes lining the canals; and finally, the small size particles (< 5 μm) will reach the choanochambers and are engulfed by the choanocytes (feeding cells). After being captured by pinacocytes and choanocytes, the food particles are then passed to the mesohyl cells by transcytosis. In the mesohyl, food particles are phagocytosed mostly by archaeocytes, resulting in an assimilable compound transported throughout the sponge by archaeocytes.

Sponges ingest plankton or living particulate organic carbon (LPOC), detritus or detrital particulate organic carbon (POC_{det}), and dissolved organic carbon (DOC). For LPOC, sponges prefer nano- and picoplankton, with the highest retention efficiencies found for 0.1–10 μm sized particles, withfiltering efficiency up to 93% (e.g. Pile et al. 1996; Ribes et al. 1999). Hadas et

al. (2009) claimed that detritus is also a significant carbon source since detritus contributes 54% of POC, based on their observation of the nearly-symbiont-free sponge, *Negombata magnifica*. However, sponge consumption of detritus depends on proportion of detritus in the total available food composition (Coma et al. 2001). DOC is another important part of the sponge's diet. de Goeij et al. (2008) found that tropical encrusting sponges take up DOC, which accounted for 90% of their total organic carbon (TOC) removal. Mueller et al. (2014) also reported that the main diet of coral-excavating sponges is DOC, which represented 81–83% of the sponge TOC uptake.

Carnivorous sponges

There are 130 carnivorous sponge species, or less than 2% of the total described sponge species to date (de Voogd 2022). This feeding strategy has long been considered an evolutionary adaptation to the extremely oligotrophic environments of the deep sea, which makes filter-feeding unsuitable (Vacelet and Boury-Esnault 1995). However, Vacelet and Boury-Esnault (1996) also found a carnivorous sponge in shallow water (17–22 m depth) in a Mediterranean cave, occurring between 15–60 m from the cave opening. The aquiferous system of carnivorous sponges is completely absent or is partially reduced and is devoid of choanocytes. Carnivorous sponges use filaments or inflatable spheres with adhesive Velcro-like surfaces to catch their prey. Devoid of a true digestive tract, carnivorous sponges use their entire cells to engulf their prey. The sponge moves its cells towards the prey, concentrates around it, and finally engulfs the prey. This process takes a few hours to finish. After being engulfed, the prey is phagocytosed by archaeocytes and bacteriocytes, which need 8–10 days to complete the entire process.

Autotrophy in sponges

Many marine benthic invertebrates form symbioses with photosynthetic algae and microbes that facilitate photosynthesis, such as the Cnidaria (e.g. Stat et al. 2006; Davy et al. 2012), Platyhelminthes (Serôdio et al. 2010), Mollusca (Trench et al. 1981), Ascidia (Hirose 2015), and also Porifera (sponges). For example, about 85% of all intertidal sponge species examined in Zanzibar (western Indian Ocean) were photosynthetically active (Steindler et al. 2002). Erwin and Thacker (2007) also reported that one third of the sponge species in the Caribbean contain photosynthetic symbionts. Food availability is one factor that led to the development

of sponge relationships with photosynthetic symbionts (Wilkinson 1987a; Wilkinson and Cheshire 1990).

Cyanobacteria are the most common photosymbiont hosted by the sponges (Diaz et al. 2007), but some sponges also form associations with filamentous algae (Carballo and Ávila 2004) and dinoflagellates (Hill et al. 2011). Photosynthetic symbionts can benefit host sponges by providing supplemental nutrition in the form of glycerol and other small organic molecules (Arillo et al. 1993), and by fixing nitrogen (Wilkinson and Fay 1979). Wilkinson (1983) reported that six of the ten most common sponges on the Great Barrier Reef are net primary producers, producing three times more oxygen than they respire, suggesting that phototrophic sponges could provide a significant contribution to reef productivity .

Sponge-mediated energy pathways

There is a growing discussion about the so-called 'sponge-loop' – the pathway where sponges mediate the transfer of the energy from dissolved organic matter (DOM) in the water column to higher trophic levels on coral reefs through the production of mucus (de Goeij et al. 2013; Rix et al. 2016a; Rix et al. 2016b). de Goeij et al. (2013) hypothesized that sponges might play an important role in maintaining productivity in nutrient-poor environments like coral reefs. There are two pathways proposed for how sponges might fuel higher trophic levels. For cryptic and encrusting sponges, the sponge-loop pathway occurs via sponge detritus production fuelled by DOM and consumed by detritivores (reconfirmed by Rix et al. 2018). However, this pathway is not found in all massive and erect sponges since they do not always produce significant quantities of detritus (McMurray et al. 2018). McMurray et al. (2018) proposed an alternative sponge-loop pathway where sponges might fuel higher trophic levels via the predation on sponges by fish, turtles, and invertebrate spongivores since sponges are likely to retain their assimilated carbon as biomass.

The Indo-Pacific: Centre of the global biodiversity

The Indo-Pacific region has long been recognised as supporting the highest concentration of global marine biodiversity, with a gradient in richness linked to longitudinal and latitudinal distance from its centre (Bellwood and Wainwright 2002; Mora et al. 2003; Carpenter and Springer 2005; Tittensor et al. 2010). This region supports about 3000 species of shallow

marine fish, while the next richest region (the Western Atlantic) having only approximately 1200 species (Helfman et al. 2009). There are 100 genera with 758 species of zooxanthellate corals in this region, compared with 28 genera with 73 species in the Western Atlantic (Veron et al. 2016). Among other marine groups, there are about 1000 species of bivalve mollusc (including all the giant clams, Tridacnidae), twice that of the Western Atlantic; and 49 of the 50 species of sea snakes (Hydrophiidae), compared to only one species in the Eastern Pacific (Helfman et al. 2009).

Many theories have tried to explain this phenomenon. They can be classified into three main hypotheses: (1) centre of origin; (2) area and refuge; and (3) region of overlap (Bellwood and Wainwright 2002; Bowen et al. 2013; Cowman 2014). The centre-of-origin theory assumes that the centre of diversity is also the centre of origin, with each speciation radiating from this centre. The second hypothesis is that the Indo-Pacific's extensive shallow habitats may reduce faunal loss and have acted as a refuge or area of species accumulation during global environmental change (e.g. sea-level changes). Rosen (1988) added that more species may have evolved in the Indo-Pacific due to the region's large size compared with other regions. The third hypothesis is that the Indo-Pacific region is the area where the faunal distribution of several biogeographic realms overlaps. Obviously, one theory alone cannot entirely explain this centre of global biodiversity, but it could be explained by a combination of these hypotheses.

Beside sandy or soft-sediment habitats, coral reefs, seagrass beds, and mangroves are the three main marine ecosystems in the Indo-Pacific (see Figure 1.2–4 for the distribution and species richness). Generally, but not always, these can be found in close proximity and are connected through ecological and biophysical processes (Nagelkerken et al. 2000; Unsworth et al. 2008; Saunders et al. 2014), and provide many environmental services (Field et al. 1998a; Moberg and Folke 1999; Guannel et al. 2016). Early researchers also recognized a triangular region within the Indo-Pacific described as the Coral Triangle region that stretches from the Philippine Archipelago across the Indonesian Wallacea region and West Guinea; this is the area where corals, fish and other organisms have their highest global biodiversity (Allen 2000; Veron et al. 2009). Six hundred and five zooxanthellate coral species, or 76% of the world's total species, have been reported from the Coral Triangle region (CTR; Veron et al. 2009). About 50–60 species out of a total of 73 true mangrove species are also found in the CTR (Duke and Schmitt 2015), and the CTR is considered to be the centre of global seagrass biodiversity, with 12 seagrass species that occur in this region (Spalding et al. 2003).

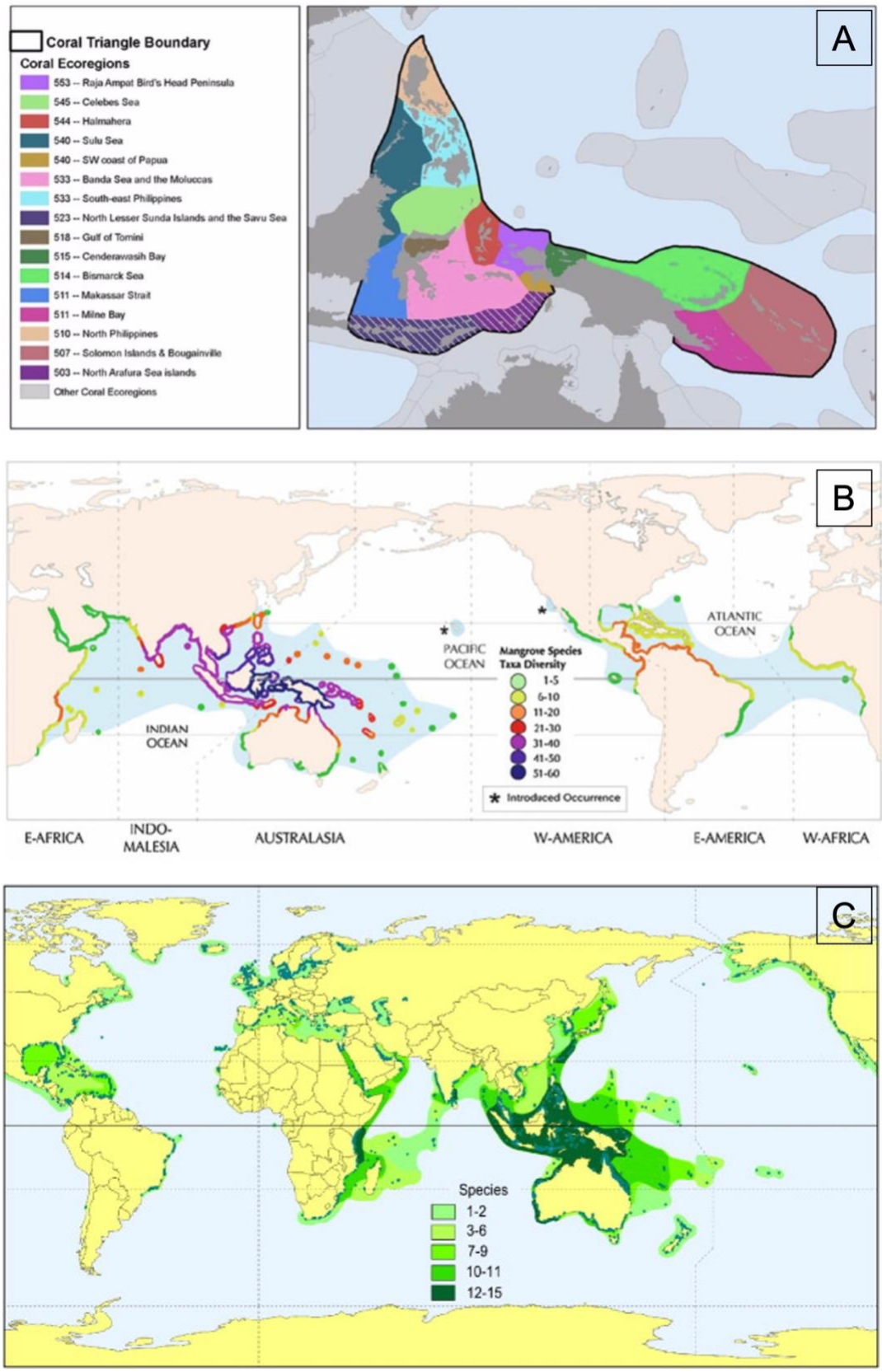

Figure 1.2. (A) Coral Triangle boundaries with coral ecoregions and the zooxanthellate coral species richness. Area north of Australia. Source: Veron et al. (2009); (B) Global distribution and species richness of mangroves. Source: Duke and Schmitt (2015); (C) Global distribution and species richness of seagrass. Source: Short et al. (2007).

Wakatobi National Park

Within the Coral Triangle region, off the south coast of Southeast Sulawesi province of Indonesia, lays the Wakatobi National Park (WNP; 05^{o} 29.6S, 123^{o} 45.26E). It is also a UNESCO World Biosphere Reserve. With about 1.4 million ha and 100,000 inhabitants, the WNP is Indonesia's third-largest and most populated marine national park (BPS-Statistics 2019; Wakatobi 2019).

Three hundred and ninety-six hermatypic scleractinian coral species and 590 reef-fish species have been recorded in the WNP (Pet-Soede and Erdmann 2004). However, the WNP has experienced dramatic declines in coral reef and the mangroves habitat cover from 2002 to 2016. Using Landsat satellite images, Firmansyah et al. (2016) showed that WNP's coral reef habitat area has dropped from 27,968 ha in 2002 to 12,458 ha in 2016 (about 55% drop), while the mangrove area has also dropped from 2,499 ha in 2002 to 1,052 ha in 2016 (about 58% drop). The WNP mean hard coral cover in 2016 was approximately 25.11%, compared to 46.7% in 2002 (McMellor and Smith 2010; Firmansyah et al. 2016). Human-induced activities such as sand and coral mining, along with mangrove logging and destructive fishing practicing (e.g. blast and cyanide fishing) have been identified as the major factors causing this environmental degradation in the WNP (Firmansyah et al. 2016).

Sponges in Wakatobi

One of the earliest oceanographic expeditions in Indonesia, the Snellius II expedition (1984–1985), collected 355 distinct sponge species and estimated that a total of 830 sponge species existed in the eastern part Indonesia including Wakatobi (Van Soest 1989). However, only 100 sponge species across Indonesia have currently been described and validated (de Voogd 2022). In the Wakatobi, Bell and Smith (2004) reported that within two sites with a total observed area of 52.5 m^2, 100 sponge species were found. More recently, 140 sponge species were documented on the fringing reef of Hoga Island, Wakatobi (Rovellini et al. 2019). *Lamellodysidea herbacea* is the most common sponge in Wakatobi reefs: it occurred in 42% of the total observed sponge patches (Powell et al. 2014). Powell et al. (2014) also found that sedimentation rate was the key driver of sponge abundance patterns in the WNP. In WNP, *L. herbacea* harbours photosymbionts that have the ability to photo-acclimate to light availability, which could explain their survival in turbid environments (Biggerstaff et al. 2015). Biggerstaff et al. (2017) discovered that *L. herbacea* could survive high sedimentation by reducing its

respiration rate and removing settled sediment by producing mucus. Mucus production as a sediment clearance mechanism was also observed in the giant barrel sponge (*Xestospongia testudinaria*) in WNP (McGrath et al. 2017). These findings support the hypothesis of Bell et al. (2013) that there is the possibility for a regime shift from coral- to sponge-dominated reefs as sponges and corals have different responses to environmental change.

Goals and aims

Like all tropical regions, the Indo-Pacific bioregion has experienced considerable environmental change, and one of the most understudied ecosystems are the seagrass meadows. In order to understand the ecosystem dynamics and predict the impact of environmental change, we need to understand the role of each organism in the flow of matter and energy. Sponge assemblages are essential benthic organisms in tropical marine ecosystems. While most sponges are heterotrophic, some are autotrophic. The sponges with different nutritional modes might play different roles in the energy flow through marine systems. The primary goal of my thesis is to increase our understanding of the importance of these two different sponge nutritional modes in seagrass ecosystems in order to better understand how environmental change might impact on sponge assemblages.

The specific aims of this thesis are to:

1. Determine the structure of seagrass sponge assemblages and explore their potential drivers of abundance and diversity patterns by conducting a sponge and seagrass survey on the west side of Hoga Island, Wakatobi. Sponges will be identified by utilizing both a morphological and molecular approaches. Sponge biomass (abundance) in the seagrass bed will be quantified and assessment made of the seagrass and sponge assemblage structure and spatial distribution.
2. Determine the sponge nutritional mode: autotrophic or heterotrophic, and their variation across the seagrass bed. The net primary productions and dark respiration of the sponges will be measured, and use the P:R ratios as a proxy for their nutritional mode along with the photosynthetic pigment concentration. I will use the information from the sponge survey to assess the variation in sponge nutritional modes across the studied seagrass bed.
3. Assess how sponges with different nutritional modes may impact on water column carbon dynamics. I will measure the sponge-mediated organic carbon flux in dark and light

incubations (representing night and daytime) and how sponges might influence the water-column at the assemblage level in the studied seagrass bed will be assessed.

4. Assess how sponges with different nutritional modes may influence the picoplankton abundance in the water column. I will measure the sponge-mediated flux for the observable picoplankton groups and their ambient abundance in the water column in dark and light incubations. Then, the sponge assemblage influence on the picoplankton at the habitat level will be assessed by utilising the sponge survey information.

CHAPTER 2.

Contrasting drivers of sponge and seagrass assemblage composition in an Indo-Pacific seagrass meadow

Abstract

Sponges are major components of benthic marine ecosystems across the world. Although seagrasses are one of the key ecosystems in tropical environments, their associated sponge assemblages have been poorly studied. In this study, I investigated seagrass and sponge assemblage composition in an Indo-Pacific seagrass meadow, located at Hoga Island in the Wakatobi National Park, Southeast Sulawesi, Indonesia. I examined a continuous seagrass meadow, spatially separated into two sites, categorised into three tidal zones: high-shore, middle shore, and near-reef-flat zones. I investigated the species richness, composition, and abundance of both seagrass and sponge species across zones and sites. For sponge identification, I combined morphology and molecular technique where four DNA markers (18S, 28S, ITS, and COX-1) were used. Ten sponge species found in the seagrass meadow were identified and I found that the sponge assemblage composition was significantly different among sites and zones, while the seagrass assemblage was not. This suggests that the seagrass and sponge assemblages have different ecological drivers. Based on my observations, I propose that sponge adaptation to the prevailing environmental conditions in different seagrass zones may be aided by sponge morphology. Furthermore, seagrass ecosystem management strategies should consider the different drivers of seagrass and sponge distribution patterns; they might be differentially affected by specific anthropogenic stressors.

Keywords: Porifera, Wakatobi, ecological drivers, morphology

Introduction

Global marine biodiversity is declining at an alarming rate as a result of many human impacts, which has resulted in a reduction in the provision of ecosystem services (Sala and Knowlton 2006). As biodiversity supports the stability of ecological functions and services (Cardinale et al. 2012; Lefcheck et al. 2015), appropriate environmental management and conservation efforts are urgently needed. However, before such measures can be implemented, we need to understand the drivers of organism abundance and distribution patterns, since these patterns strongly influence ecosystem dynamics (Balmford and Gaston 1999).

The Indo-Pacific biogeographic region is an important global biodiversity hotspot, particularly the Indonesia–Philippine archipelago (Mora et al. 2003; Tittensor et al. 2010). Algae, molluscs, fishes, arthropods, and corals all have their maximum diversity in this region (Hoeksema 2007). However, despite considerable research efforts in this area, not all ecosystems have been equally studied. Seagrasses in particular have been the least studied (Waycott et al. 2009; Ooi et al. 2011), despite the Indo-Pacific region having the highest seagrass diversity in the world (Short et al. 2007) and being hypothesised as the centre-of-origin for tropical seagrasses (Short et al. 2001).

Seagrass meadows have a wide range of functional roles, including supporting extensive primary and secondary production (Duarte and Cebrián 1996), habitat provision (Heck et al. 2003), facilitating sediment accumulation and stabilisation (Orth et al. 2006), and nutrient cycling (Romero et al. 2006; McGlathery et al. 2007). As a result, human food security is very dependent on seagrass health, particularly for industrial- and small-scale fisheries production (Unsworth et al. 2019). For example, in the Indo-Pacific, more than 50% of the fish landed by small-scale fishers in the Coral Triangle region are seagrass-associated (Unsworth et al. 2014). Seagrass meadows are also very important for carbon sequestration (Duarte et al. 2005). In the Indonesia Archipelago, seagrass meadows have an average carbon sequestration capacity of about 5.62–8.40 ton C ha^{-1} y^{-1} (Wahyudi et al. 2020), more than double the global average (2.78 ton C ha-1 y-1; Duarte et al. 2013). Therefore, it is critical to maintain the ecological function of seagrass meadows, particularly in the Indo-Pacific. However, despite the importance of these seagrass meadows, they are under widespread threat because of continued population growth and coastal development (Unsworth et al. 2018).

The resilience of seagrass ecosystems is influenced by the interaction of three main factors: the biological features of the seagrass, the supporting ecosystem, and the biophysical environment (Unsworth et al. 2015). Unsworth et al. (2015) identified seagrass species diversity, seagrass cover, macroalgal cover, epiphyte cover, and water transparency as the five most-used resilience parameters that have been incorporated into seagrass monitoring guidelines (e.g. McKenzie et al. 2001; Short et al. 2015), since this data are easy and cheap to collect. Seagrass species diversity is essential to seagrass resilience, with Kilminster et al. (2015) suggesting that each seagrass species has specific disturbance responses. Therefore, higher seagrass diversity likely provides functional redundancy and a better chance of recovery following a disturbance. Seagrass is also more resistant to disturbances when more abundant, making seagrass cover an important resilience assessment parameter (Fonseca and Bell 1998; Duarte et al. 2006). Macroalgal cover and epiphyte presence provide an assessment of the susceptibility of seagrass to being outcompeted and have seagrass blade photosynthetic activity disrupted, respectively (Unsworth et al. 2015). Lastly, water transparency is indicative of the amount of photosynthetically available light reaching the seagrass, thereby affecting photosynthesis and influencing overall seagrass productivity (O'Brien et al. 2018).

Seagrass species life-history traits have also been used to classify seagrass resilience modes (e.g. Carruthers et al. 2002; Carruthers et al. 2007; Waycott et al. 2011). Based on seagrass growth forms and reproductive strategies that may contribute to resilience, seagrass species have been categorised into colonising, opportunistic and persistent species (Kilminster et al. 2015). Colonising species have a short turnover time (< months) of ramets, quickly reach (< 1 month) sexual maturity and produce dormant seeds. In contrast, persistent species have a long turnover time (months–years) of ramets, a very long time to reach sexual maturity (years), and do not typically form seed banks. Opportunistic seagrass species combine the core features of the two groups to colonise quickly, produce seeds or seedlings and maintain persistent biomass, and rapidly recover from seeds if needed. These sets of traits lead to different responses of seagrass species to disturbance. Colonising seagrasses feature low physiological resistance but have a rapid ability to recover after a disturbance. Persistent seagrasses have high physiological resistance but a slow recovery ability, while opportunistic seagrass species are intermediate between the other two groups.

Despite the identification of resilience features of seagrass species, little is known about the role of seagrass-associated benthic communities (Unsworth et al. 2015). Sponges are major

components of benthic communities across the globe, including seagrass meadows (Van Soest et al. 2012; Bell et al. 2020). For example, Barnes (1999) reported that sponges were the dominant sessile form in the seagrass meadows of the Quirimba Archipelago in East Africa. Sponges were also reported as one of the dominant benthic organisms in a *Posidonia australis* seagrass meadow in southeastern Australia (Demers et al. 2015). However, the ecology of seagrass sponges has generally been poorly studied compared to coral reef sponges, and particularly whether the drivers of sponge and seagrass assemblage composition are the same. It is important to determine the drivers of seagrass and sponge distribution patterns in developing appropriate and scale-dependent management strategies.

Sponges are sessile suspension feeders that have many functional roles (Bell 2008), including impacts on the substrate (see review by Schönberg et al. 2017), carbon and nitrogen cycling (e.g. Jiménez and Ribes 2007; Hadas et al. 2009; Hoffmann et al. 2009; Hoer et al. 2018), and facilitating primary production (e.g. Wilkinson 1983; Erwin and Thacker 2008). A number of biotic and abiotic factors have been identified as controlling spatial and temporal variability in sponge assemblages and species, although these drivers are often species-specific (e.g. Ramsby et al. 2017; Beepat et al. 2020) due to the adaptive plasticity of sponges to environmental and physical factors (Carballo et al. 1994). For example, type and availability of substrate have been reported to influence sponge assemblages in shallow-water ecosystems (Hunting et al. 2013; Marlow et al. 2018), along with competition with other taxa, such as macroalgae (e.g. Ávila et al. 2015; Ramsby et al. 2017) and corals (e.g. Kelmo and Attrill 2013; Glynn and Manzello 2015). Morphological variation is also an important adaptation mechanism shown by sponges to environmental conditions, which can determine their survival success in a particular habitat (Palumbi 1984). For example, Bell and Barnes (2000) reported that massive and encrusting morphologies dominated high-energy sites at Lough Hyne in Ireland, while pedunculate, papillate, and arborescent morphologies dominated low current sites. In another example, elephant-ear and vase-like morphologies were reported to strongly correlate with local water movement and sedimentation rate (Pronzato et al. 1998).

Few studies have described spatial variation in sponge abundance and diversity in the Indo-Pacific region, and most of the available studies have been on coral reefs sponges and have highlighted the importance of environmental conditions as the major drivers (e.g. de Voogd et al. 2006; Cleary and de Voogd 2007; de Voogd et al. 2009; Madduppa et al. 2015; Utami et al. 2018). For example, Bell and Smith (2004) reported that depth, surface angle and sedimentation

rates were important drivers for sponge assemblages at two reefs in Wakatobi National Park, Indonesia. Meanwhile, from the western part of the Indonesia Archipelago, de Voogd and Cleary (2008) reported 118 sponge species inhabited the reefs across Jakarta Bay to the north part of Seribu Islands (60 km away from the mainland Jakarta). They observed increased sponge diversity further offshore from the Jakarta mainland, where the environmental conditions and water quality improved. Furthermore, a longitudinal pattern was described in a study from the south coast of Java Island, where sponge species and morphological diversity gradually increased from the west to the east of the island (Hadi et al. 2018). We have less information about the drivers of temporal variation in Indo-Pacific sponge assemblages. Over a 13 year study on a reef in Wakatobi National Park, sponge assemblages had dramatic temporal changes driven by species-specific population variability, although the drivers could not be identified (Rovellini et al. 2019).

Despite the paucity of information on the ecology of seagrass sponge assemblages in the Indo-Pacific, some studies have revealed what might drive abundance and diversity patterns. For example, Wulff (1995) suggested that top-down processes prevent reef-sponges from occupying the adjacent seagrass meadows in San Blas Islands, Republic of Panama. However, sponges can also form intra-phyletic mutualistic relationships to overcome predators and increase sponge diversity in seagrass meadows (Wulff 2008). Biophysical features may also be important drivers of seagrass abundance and diversity patterns. Attrill et al. (2000) suggested that seagrass structural complexity did not determine the size and composition of the associated macroinvertebrates, including sponges, but the abundance of the seagrass plants did. Furthermore, a study in the Gulf of Mexico suggested that the proportion of coarse particles in the substrate was the best variable to explain sponge distribution in seagrass meadows (Ávila et al. 2015). Finally, ambient nutrient availability has also been reported as the driver for the outcome of the sponge–seagrass interactions in the Bahamas (Archer et al. 2018).

In the Indo-Pacific region, particularly in the Indonesia Archipelago, ecological studies on seagrass sponges are scarce, and are all concentrated on Java Island. Ismet et al. (2017) reported that a total of 18 sponge species inhabited a multispecies seagrass meadow in the Seribu Islands National Park, Jakarta, which had a species-specific level of correlation with each seagrass species' cover in the meadow. Meanwhile, 16 sponge species were recorded in two seagrass meadows in the Gulf of Pacitan, East Java, where the assemblages were dominated by the boring sponge *Spheciospongia inconstans* (Setiawan et al. 2021). There are still major gaps in

our knowledge on the ecology of sponge assemblages in the seagrass ecosystems, especially in this important biogeographic region.

Detailed surveys are the first step in understanding ecological processes and the drivers of abundance and diversity patterns, which is crucial for effective management plans. Based on sites and zones within my studied seagrass meadow, I recorded the seagrass species diversity, seagrass cover, canopy height, macroalgal cover, epiphytes cover, and water transparency. For the sponge assemblage, I recorded the sponge diversity and abundance, and determined the growth form for each sponge species. In this chapter I aim to: (1) determine the ecological status of the seagrass meadow and its resilience capacity by measuring the habitat resilience parameters (utilizing Seagrass Ecological Quality Index developed by Hernawan et al. 2021) and by classifying the seagrass species composition (grouped by their response to disturbances; after Kilminster et al. 2015); (2) investigate spatial distribution patterns of the seagrass and sponge assemblages in the seagrass meadow; (3) consider the importance of sponge morphology in driving distribution and abundance patterns; and (4) explore the potential drivers of sponge seagrass assemblages.

Materials and methods

Study site and design

My study was conducted in a seagrass meadow on the western side of Hoga Island in the Wakatobi National Park, Indonesia, which lies within the Coral Triangle region. The fieldwork was carried out during June – July 2019. The Wakatobi National Park (WNP) experiences semidiurnal tides with a maximum amplitude of 2.3 m, whereby much of the seagrass in the area is exposed to air during a low spring tide (Unsworth et al. 2008; personal observation). The region's seagrass meadows are dominated by *Thalassia hemprichii* and *Enhalus acoroides*, with minor floral coverage (i.e. under 5% coverage) of three other seagrass species – *Cymodocea rotundata*, *Halophila ovalis*, and *Halodule uninervis* (Unsworth et al. 2007). A seagrass survey in 2006 reported that the studied seagrass meadow had an average seagrass cover of 70 ± 3.2 %, and 1.8 ± 0.1 mm sized coral sand substrate (Unsworth et al. 2008). Across the study area, the seagrass and substrate were visible through the water column from the surface, which is likely due to the very shallow water depth (especially at the high-shore zone).

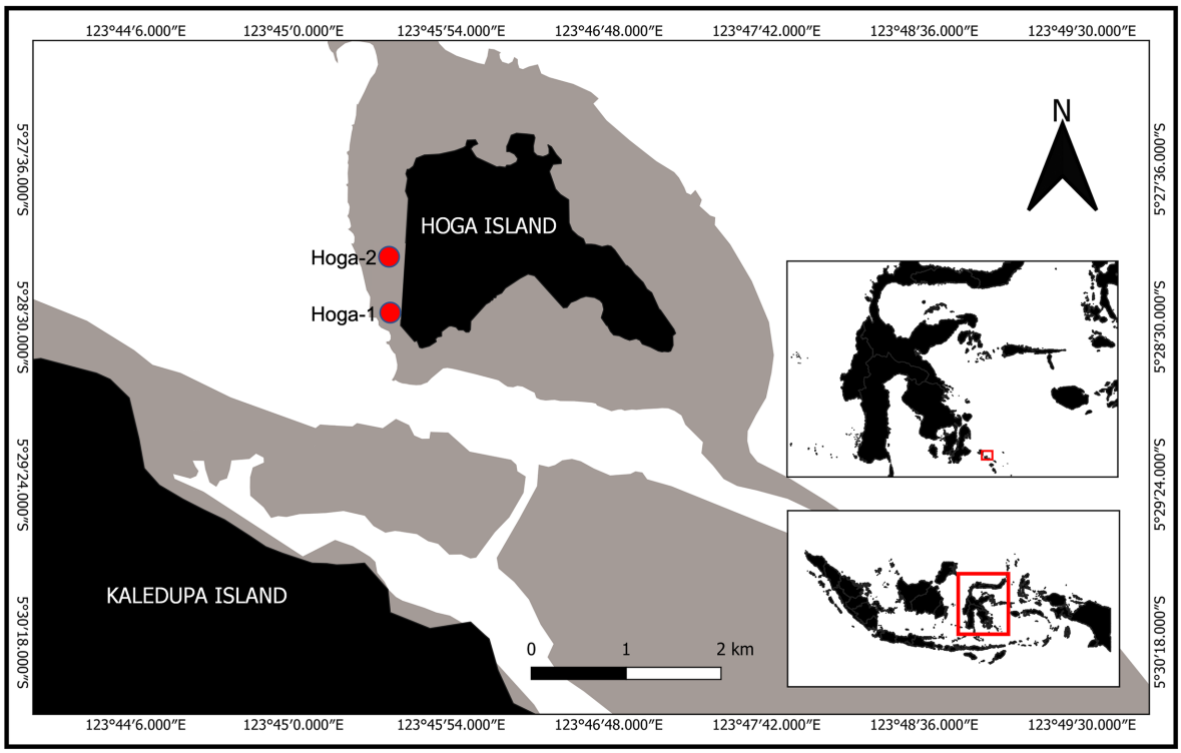

Figure 2.1. Location of the two study sites on the west side of Hoga Island's seagrass meadow, Wakatobi National Park, Southeast Sulawesi – Indonesia.

The seagrass meadow was a continuous meadow, and as a spatially separated replicate, I categorised the study area into two sites and three zones. The sites were separated into Hoga-1 (5° 28' 27" S, 123° 45' 57" E) and Hoga-2 (5° 28' 07" S, 123° 45' 35" E), and the zones within each site were categorised as high shore (HS), middle shore (M), and near-reef-flat (NRF) zones (Figure 2.1). Hoga-1 was at the southern part of the meadow, close to the channel between Kaledupa Island and Hoga Island, while Hoga-2 was further north. The categorisation of the zones was based on the distance to shore, whereby during high tide, the water depth of high-shore, middle and near-reef-flat zone was approximately 0.5, 1.2, and 1.9 m depth, respectively. Three transects were used at each zone at each site, and a tape measure was laid perpendicular to the shoreline, resulting in nine transects (n = 9) at each site (across all zones), and six transects (n = 6) in each zone (across both sites). The transect length was 50 m, and the distance between the parallel transects in the same zone was approximately 150 m and 300 m for Hoga-1 and Hoga-2, respectively. In the high-shore zone, the transect began at 5 m after the seagrass started to grow, perpendicular to the shoreline towards the sea. In the near-reef-flat zone, the transect began at approximately 5–10 m from the reef-flat boundaries towards the island (i.e. shore). In the middle zone, the transect was placed approximately halfway between the two other zones.

Estimation of site area

I used aerial images to calculate the areal coverage of the sites and zones following the methods of Subhan et al. (2018). A drone (DJI Mavic Air 2) shot multiple vertical aerial images from 150 m height in the morning (before 10 am) to avoid glare. I used Pix4Dcapture (PIX4D 2018) to create a route for the drone to take orthophotos for building up an orthomosaic map of the study site. I set the route to take images resulting in an overlap of 80% to merge the pictures afterwards. All the pictures were merged using Agisoft Metashape, and then I calculated the area covered by seagrass from the merged image with QGIS 3.18 (QGIS 2002).

Hoga-1 was a seagrass meadow between two jetties, while the meadow between the north jetty and the national park's field office was considered Hoga-2 (see Figure S.2.1, App. A). The high-shore zone covered the area where the seagrasses started to occur to the point where there was a depth change. The zone was always exposed to air during low tide. The boundary was 70 m from the shoreline, confirmed by the orthophotos, where a change in seagrass density was observed. The near-reef-flat (NRF) zone was the seagrass-dominated area just behind the reef flat. From the orthophoto, I drew the boundaries from the point where seagrasses started to dominate the reef-flat, extrapolated to the point where coral reef was no longer observed. The middle zone was between the high-shore and near-reef-flat zones, where seagrasses entirely dominated the meadow.

Sponge identification

Except for one rarely found sponge species (n = 1 and only a very small sample), five specimens from each of the distinct sponges that inhabited the studied seagrass meadow were collected for identification purposes (n = 5). A small piece of the sponge (approx. 2 cm in length and 2–5 mm in thickness) was cut and placed in 2 ml cryovial tube, preserved in 98% ethanol, and stored in a -20°C fridge. The vials were stored in an insulated thermos when transporting the samples from the study site at WNP to Bogor (Indonesia), and finally to Wellington, New Zealand. *In situ* photographs of the sponges were taken, along with their gross external morphological characteristics. Molecular analyses were used to assist with the identification. I used the morphological characteristics and original descriptions of the likely species, coupled with the genetic data, to confirm final identification. Only three out of five collected specimens (n = 3) were used for both the molecular and morphological identification process.

Molecular identification

Sponge tissue of approximately 1 mm^3 was cut and washed with distilled water, then dried on tissue paper to minimize ethanol content in the sponge as this could potentially interfere with the DNA extraction process. The sponge DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Germany), following the manufacturer's protocols. Four primer sets were used: 18S (18S_2F/18S_5R as in Mortimer et al. (2021)), 28S (C2/D2 primer set designed by Chombard et al. (1998)), ITS (RA2/ITS2.2 primer set as in Adlard and Lester (1995)), and CO1-ext (COX1-R1/COX1-D2 primer set developed by Rot et al. (2006)). See Table 2.1. for the detailed primer information and product sizes.

Table 2.1. Primer information for marker used (18S, 28S, ITS2, and CO1-ext).

Region	Primer		Size (BP)	Reference
	Forward	Reverse		
18S	18S_2F 5'-GGC TCA TTA AAT CAG TTA T-3'	18S_5R 5'-CTT GGC AAA TGC TTT CGC-3'	893	Mortimer et al. (2021)
28S	C2 5′-GAA AAG AAC TTT GRA RAG AGA GT-3′	D2 5′-TCC GTG TTT CAA GAC GGG-3′	450	Chombard et al. (1998)
ITS	RA2 5'-GTC CCT GCC CTT TGT ACA CA-3'	ITS2.2 5'-CCT GGT TAG TTT CTT TTC CTC CGC-3'	680	Adlard and Lester (1995)
CO1-ext	COX1-R1 5′-TGT TGR GGG AAA AAR GTT AAA TT-3′	COX-D2 5′-AAT ACT GCT TTT TTT GAT CCT GCC GG-3′	425	(Rot et al. 2006)

Polymerase chain reaction (PCR) were carried out in a total volume of 25 µL including the following: 8.4 µL of RedMix (Bioline, Taunton, MA, USA) PCR Mastermix, 0.8 µL of each primer (10 mM; forward and reverse), 2 µL of bovine serum albumin (BSA; 100 mg/mL), 3 µL of DNA template, and 10 µL of H_2O. The amplification profiles for 28S and CO1-ext primer sets were as follows: a 3-minute initial denaturification at 95℃; 36 cycles of 95℃ for 30 s, 50℃ for 55 s, 70℃ for 90 s; and a final extension step at 72℃ for 10 min. For the 18S, the amplification profile was as follows: 94℃ for 5 min; then 35 cycles of 94℃ for 30 s, 58℃ for 55 s, 72℃ for 45 s; and a final extension step of 72℃ for 7 min. Meanwhile, the ITS region was amplified as follows: 94℃ for 2 min; 35 cycles of 94℃ for 30 s, 45℃ for 20 s, 65℃ for 60 s; and a final extension step of 72℃ for 10 min. Products were visually checked on a 1.5%

agarose gel stained with RedSafe Nucleic Acid Staining Solution (20,000x) and sequenced using an automated DNA sequencing service at Macrogen Inc (Seoul, South Korea).

Forward and reverse sequences were trimmed, assembled, and edited manually with Geneious Prime version 2022.0.2 software (Kearse et al. 2012). The derived consensus sequences were exported as *fasta* files and checked in the NCBI GenBank Database by running the online Basic Local Alignment Search Tools (BLAST) searches. The sequence with the highest Bit Score, accompanied with the best E-value, query cover (%), and sequence similarity (%) was selected as the identity for every DNA marker, while still considering the top 20 BLAST results to identify any possible errors (following Yang et al. 2017). The top 20 sequences based on their Bit Score were downloaded for constructing phylogenetic trees for each marker, utilizing the Maximum Likelihood and Neighbor Joining methods, to determine the closest sequence match from the phylogenetic analysis.

Morphological analysis

I used the sponge identification guide 'Sponguide' by Hooper (2000) as the basis of my sponge morphological analysis. The sponge characteristics that can be observed *in situ*, such as growth form, point of attachment, live coloration and hue, oscule shape and distribution, texture, surface and the surface sculpturing, was recorded using the description format provided in the 'Sponguide". For spicule preparations, a ~5 mm^3 tissue sample were dissolved in bleach for at least 24 h at room temperature. The remaining spicules were washed and rinsed 3x with distilled water. One millilitre of the spicule solution was mounted on a microscope slide and examined using a compound light microscope (Leica DM 2500 LED) with an on-board camera system (Leica DMC 4500) for photographing and measuring (length or diameter) the spicules. I measured 20 replicates for each spicule type or as many as I could find under the microscope.

Seagrass and sponge assemblage composition and abundance

I surveyed the seagrass meadow following the methods of Seagrass Watch (McKenzie et al. 2001). Using a 0.25 m^2 metal quadrat, I recorded the seagrass species and measured the seagrass cover and canopy height, and macroalgal and epiphyte cover at 5 m intervals along the transect, resulting in 11 observations for each transect. I used the seagrass guidebook produced by the Indonesia National Institute of Sciences (Rahmawati et al. 2014) to identify seagrass species, which was based on Kuo and Hartog (2001). I used the percentage cover standards provided by Seagrass Watch (McKenzie et al. 2001) for seagrass and macroalgal cover. In each quadrat, which was placed haphazardly, I measured the length of five leaf-blades and averaged the measurements for the quadrat's canopy height. The epiphyte cover was estimated as the percentage of covered blades. For the seagrass composition, I assigned each species into one of the three groups based on its life-history traits: colonising, opportunistic, and persistent species (after Kilminster et al. 2015) to understand how the meadow might respond to disturbance.

For the sponge survey, I recorded the sponges that I encountered within 2.5 m on either side of the transect, resulting in total coverage of 250 m^2 per transect. I measured the volume of every sponge that I found in my belt area with the water-displacement method (after Rützler 1978). For sponge volume measurements, I used either a graduated cylinder or measuring cup, depending on the size and shape of the sponge, with a volumetric resolution of 10 ml. I put the sponge in the cylinder or cup and topped it up with seawater to the full volume scale. Then, I took the sponge out and recorded the volume lost as the sponge volume. Sponge volume is the best abundance metric for sponge assemblages with diverse morphologies as it provides a more appropriate measure of the sponge-related ecological process (e.g. feeding rate, respiration rate, and gross productivity) compared to other sponge abundance metrics (Bell et al. 2017).

To better understand sponge adaptations to living in seagrass ecosystems, I qualitatively assessed sponge morphological variation. I determined the morphology of each sponge species based on the classification of Schönberg (2021). Schönberg (2021) divided sponge growth forms into four basic morphologies: crusts, massive, cups, and erect sponges. For finer resolution, these basic morphologies were further divided into: 1) crust – true crusts, endolithic-bioeroding, and creeping sponges; 2) massive – simple massive, globular massive, composite-massive, and fistular sponges; 3) cups – cups, tubes, and barrels; 4) erect – one-dimensionally, two-dimensionally and three-dimensionally erect forms, stalked, and carnivorous sponges. Not all of these morphologies were found in my study system.

Data analysis

Habitat and sponge assemblage structure

I averaged the abundance of each seagrass and sponge species in all areas (i.e. every zone in both sites) and used the mean to calculate the species composition in each area. I also averaged the five habitat resilience parameters: the seagrass species richness, seagrass cover, canopy height, macroalgal cover, and epiphytes cover. I did not average the water transparency since all the areas experienced the same conditions.

The state of seagrass meadow

I used the seagrass Ecological Quality Index (SEQI) to describe the health status of my studied seagrass beds, allowing me to make comparisons with other locations, especially in Indonesia. I calculated the index following the methods described by Hernawan et al. (2021). The index uses five parameters standardised to each maximum possible value; the parameters are the seagrass species richness, seagrass cover, macroalga cover, epiphyte cover and water transparency. The equation to calculate the SEQI was as follows:

$$SEQI = \left(\frac{St}{Sref}\right) \times 0.2 + \left(\frac{Ct}{Cref}\right) \times 0.2 + \left(\frac{Wt}{Wref}\right) \times 0.2 + \left(1 - \left(\frac{Mt}{Mmax}\right)\right) \times 0.2 + \left(1 - \left(\frac{Et}{Emax}\right)\right)$$

where,

- St = seagrass species richness observed
- Sref = max value of seagrass species richness (9)
- Ct = seagrass per cent cover observed
- Cref = max value of seagrass cover (100)
- Wt = water transparency observed
- Wref = max value of water transparency (2)
- Mt = macroalga per cent cover observed
- Mref = max value of macroalga per cent cover (100)
- Et = epiphyte per cent cover observed
- Emax = max value of epiphyte cover (100)

Since I could see the seagrass and the seafloor on all my transects, according to the scoring guide by Hernawan et al. (2021), the water transparency score for all stations was two (Wt = 2;

i.e. the max possible value). Based on SEQI values, seagrass ecological status can be categorised into five levels: bad, poor, moderate, good, and excellent (see Table 2.2. for the SEQI threshold value).

Table 2.2. The seagrass ecological state category based on the value of SEQI (after Hernawan et al. 2021).

SEQI Value	Ecological status
0 – 0.36	Bad
0.37 – 0.52	Poor
0.53 – 0.68	Moderate
0.69 – 0.84	Good
0.85 – 1	Excellent

Statistical analysis

Permutational multivariate analysis of variance (PERMANOVA) was used to determine any differences between zones, sites, and their interactions, for the seagrass and sponge assemblage composition data. The sponge and seagrass abundance data were log-transformed to reduce the effect of rare species. Meanwhile, square-root transformation was applied to the habitat resilience parameter data to reduce the effect of the wide range of values among the parameters. Differences in the assemblage structure were graphically plotted using non-metric multidimensional scaling based on Bray-Curtis similarities. When I found any differences in the multivariate data (i.e. significantly different in PERMANOVA analysis), similarities percentage (SIMPER) analyses were run to identify the species that characterised the differences between zones and sites. All statistical analyses were performed by the software PRIMER v6 (with the PERMANOVA+ add-on; Anderson et al. 2008).

Results

Seagrass sponge identification

Ten morphologically distinct sponges were found in the studied seagrass meadow, coded as Sponges A – J. Good quality PCR products were successfully obtained from all distinct sponge groups with DNA markers 18S, 28S, and ITS. However, the CO1-ext marker only gave clear PCR products for five sponges (Sponges D – H). For Sponge H, I was only able to get clear PCR products for two specimens, out of five collected specimens, probably due to DNA degradation. For Sponge J, I was able to produce a clear PCR product from only one collected specimen. See Table S.2.1 (Appendix A) for the summary of BLAST results and phylogenetic analysis with every DNA marker for each acquired sponge DNA sequences.

Sponge A

Sponge A is an endopsammic sponge whose body is mostly buried in the sand (substrate) of the seagrass bed. Oscules were irregularly dispersed on the apex of the sponge, forming a short fistule that protruded through the substrate. Its colour while alive was dark, black on the surface, and light gray or light brown on the inside. The average size of the sponge was up to 25–30 cm in width. The sponge texture was firm and rubbery.

Spicules were rare (Figure 2.2C). However, three spicule types were observed: oxea (~100 µm in length; Figure 2.2D), tylostyles (~150 µm in length; Figure 2.2E), and sigma (~70 µm in length; Figure 2.2F).

The BLAST results for Sponge A were similar across the three specimens for each marker. The 18S data showed *Petrosaspongia nigra* and *Spongia zimocca* as the sponge species highest matches (Bit Score, % query cover, % sequence similarity, and E-value), while *Hippospongia ammata* and *Spongia zimocca* matched with the 28S marker, again with identical scores, but the Bit Score was not as high as for the 18S data. The ITS data matched with *Hippospongia ammata*. Phylogenetic analysis for this sponge species gave different results for each marker, whereby *Spongia zimocca* and *Hippospongia ammata* were the most frequent sponge IDs that showed the closest proximity with the Sponge A sequences.

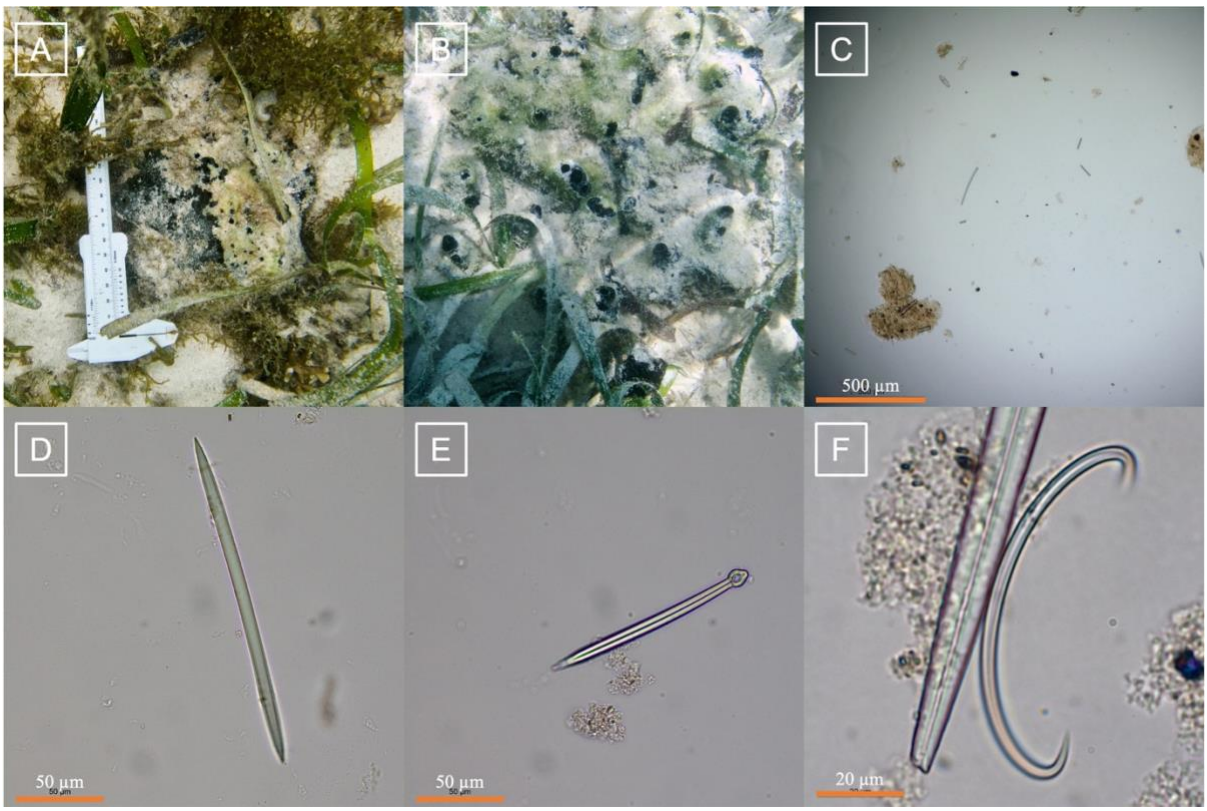

Figure 2.2. Pictures of Sponge A in situ at the studied seagrass bed (A and B) and the observed spicules (C–F). Spicules were photographed in different scale, please look at the scale bar for reference.

The morphological characteristics of Sponge A matched most closely with those of *Spongia zimocca* and *Hippospongia ammata*, but not those of *Petrosaspongia nigra*. Based on the original description by Bergquist (1995), the texture of *Petrosaspongia nigra* is hard and incompressible, and also the oscules are flush with the surface, which differed from the characteristics of Sponge A. Since *Spongia zimocca* had higher scores from the BLAST results than *Hippospongia ammata*, the identity of Sponge A most strongly aligns with *Spongia zimocca*. However, there is no record of either *Spongia zimocca* or *Hippospongia ammata* in the Indonesia archipelago or wider Indo-Pacific bioregion (de Voogd 2022). Due to no record of occurrence for this particular species in my study site's bioregion, I assign *Spongia* sp. as the identity for Sponge A.

Sponge B

Sponge B is a massive endopsammic sponge, where most of the body was buried in the sand (substrate), leaving only its apex on the sand surface (see Figure 2.3A). The average size of an sponge was about 25–30 cm in width, and the largest specimens could reach about 50–70 cm in diameter. The external colour of the living sponge was brownish orange, while the internal colour was beige. Conspicuous and discrete oscules of various sizes were scattered over the exposed side of the sponge(Figure 2.3A). The texture of the sponge was firm and incompressible.

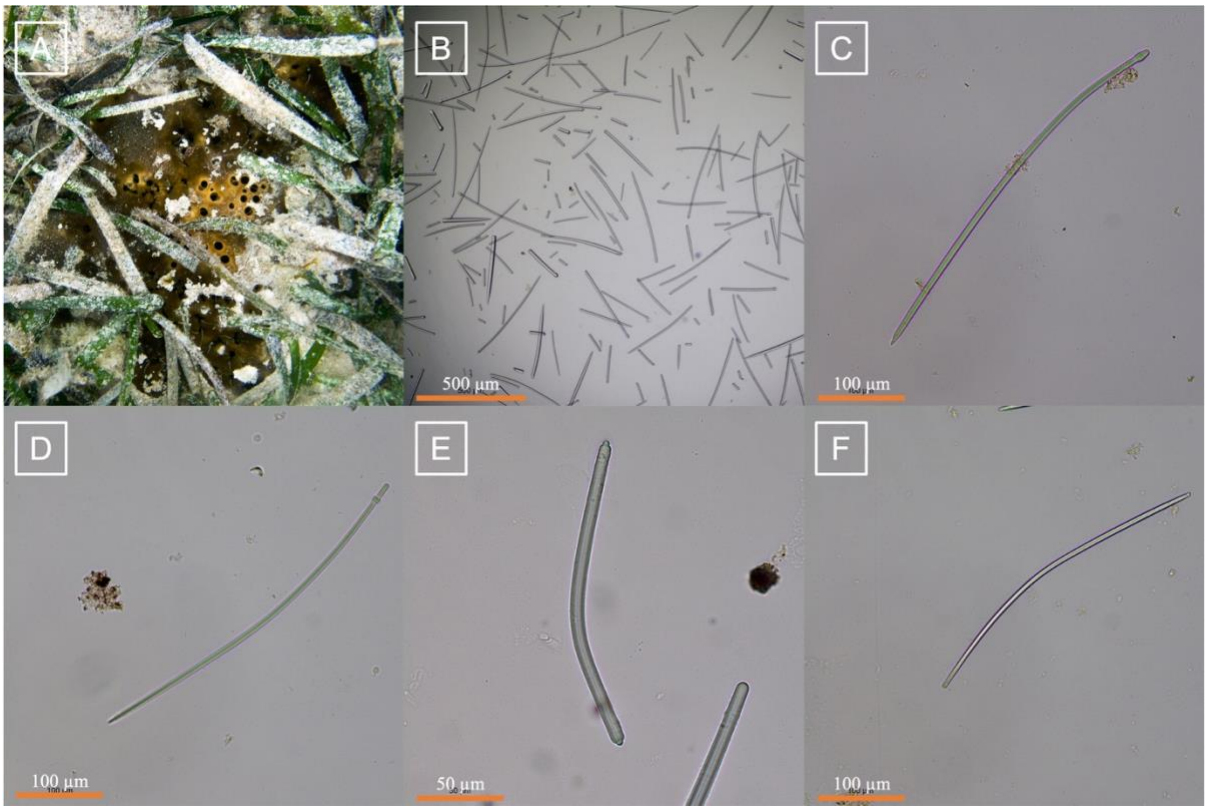

Figure 2.3. *In situ* photograph of Sponge B (A) and the spicules found (B–F). Note: spicules were photographed at different scales.

Sponge B contained many spicules, and tylostyle (Figure 2.3B–C; 200–300 µm long), bended oxea with extended conule-like shape at both ends (Figure 2.3E; length about 150 um), and styles (Figure 2.3F; ~250 µm long), were observed. No microscleres were observed.

The BLAST results of the three DNA markers gave different species as the sponge ID but all were from the same genus *Spheciospongia*: *Spheciospongia vesparium* from 18S; *Spheciospongia inconstan* from 28S; and *Spheciospongia solida* from ITS. The sequence

similarity in 28S and ITS DNA markers was relatively low (< 96%), compared to the results from the 18S marker that gave sequence similarity of 99.9%. However, the morphological characteristics of Sponge B were closer to *Spheciospongia inconstan*, than to *Spheciospongia vesparium*, based on their original descriptions (Dendy 1887; Vicente et al. 1991). Furthermore, there is no record of *Spheciospongia vesparium* occurring in the Indo-Pacific, as the sponge only has been recorded on the eastern coast of the tropical America continent (de Voogd 2022). It is important to note that BLAST results with the 18S DNA marker also showed *Spheciospongia* sp. as the top hit with identical scores for *Spheciospongia vesparium*. Given these conflicting results I have assigned *Spheciospongia* sp. as the sponge identity for Sponge B.

Sponge C

Sponge C is a lamellate sponge, 10–40 cm in diameter or height, with the external colour of living specimens being yellowish green or brown (Figure 2.4A). The outer surface is verrucose, with conspicuous punctate raised oscules. Sponge texture is firm, flexible, but not easily compressible.

Spicules were very rare (Figure 2.4B), and may represent contaminants. However, I observed two groups of oxea (40–50 µm and 80-100 µm long), tylostyles (~300 µm long), euasters (20–30 µm in diameter), and oxyspherasters (40–50 µm in diameter). See Figure 2.4.C-F for the spicule photographs.

The molecular identification with the three DNA markers gave different results at the species level, but they all belong to the same sub-family of Phyllospongiinae (Family Thorectidae). The 18S data consistently showed *Strepsichordaia lendenfeldi* as the top hit based on the Bit Score across the three specimens, but it also showed that *Phyllospongia papyracea* and *Phyllospongia lamellosa* have more than 99% sequence similarity with a 100% query cover, although the Bit Score was slightly lower. The 28S showed *Carteriospongia foliascens* as the sponge identity for the three specimens. *Carteriospongia flabellifera* and *Carteriospongia foliascens* were identified as the sponge ID with the ITS marker. It is important to note that *Phyllospongia lamellosa* and *Carteriospongia foliascens* are actually the same sponge species, and the accepted species name for these two sponges is *Phyllospongia foliascens*. So, *Phyllospongia foliascens* was the sponge that consistently came out as the top hit BLAST searches across the three DNA markers.

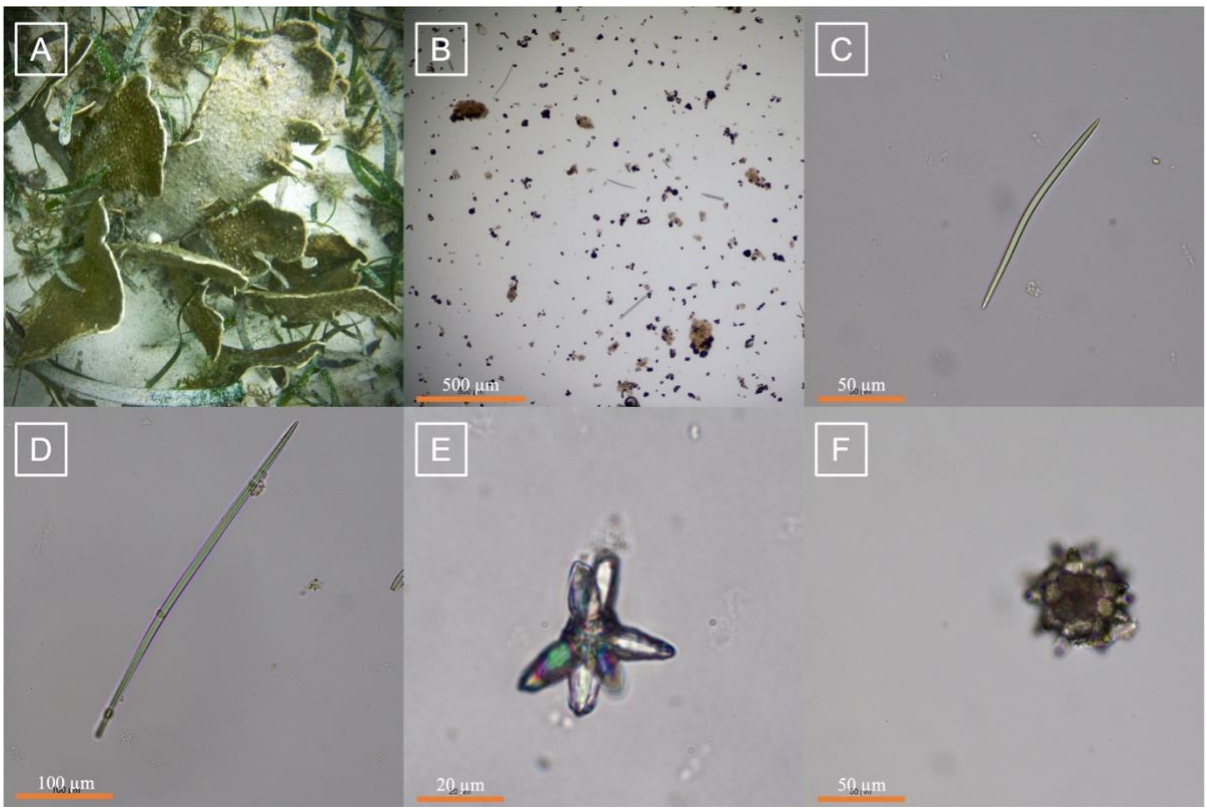

Figure 2.4. *In situ* photograph of Sponge C (A) and the contained spicules (B–F). Spicules were photographed in different scale, please look at the scale bar for reference.

With respect to the morphological characteristics, the oscules on *Strepsichordaia lendenfeldi* and *Carteriospongia flabellifera* (for which the accepted name is *Polyfibrospongia flabellifera*) are flush with the sponge surface (Bowerbank 1877; Bergquist et al. 1988), which does not match with the characteristics of the Sponge C. The sponge *Phyllospongia papyracea* has an extremely thin body – about 1–2 mm thick); this feature also does not match with Sponge C's characteristics. All observed characteristics of Sponge C matched with the morphological description of *Phyllospongia foliascens* by Bergquist et al. (1988). In addition, there are records that *Phyllospongia foliascens* occurs in the Banda Sea (de Voogd 2022), where my study site is located. Considering all of this information, I conclude that the identity of Sponge C is *Phyllospongia foliascens*.

Sponge D

Sponge D is a bushy sponge (i.e. a ramose growth form) that lays on the canopy of the seagrasses (Figure 2.5A). The colour of the living sponge is bright blue-green and appears heavily pigmented. The surface is smooth and not shiny. Oscules are small, dot like structures. The texture is spongy but easily torn. Strongyle spicules were often observed (Figure 2.5B-C), between 70–80 µm in length. Toxa (~40 µm long) were also observed but were rare (Figure 2.5D); possibly a contaminant.

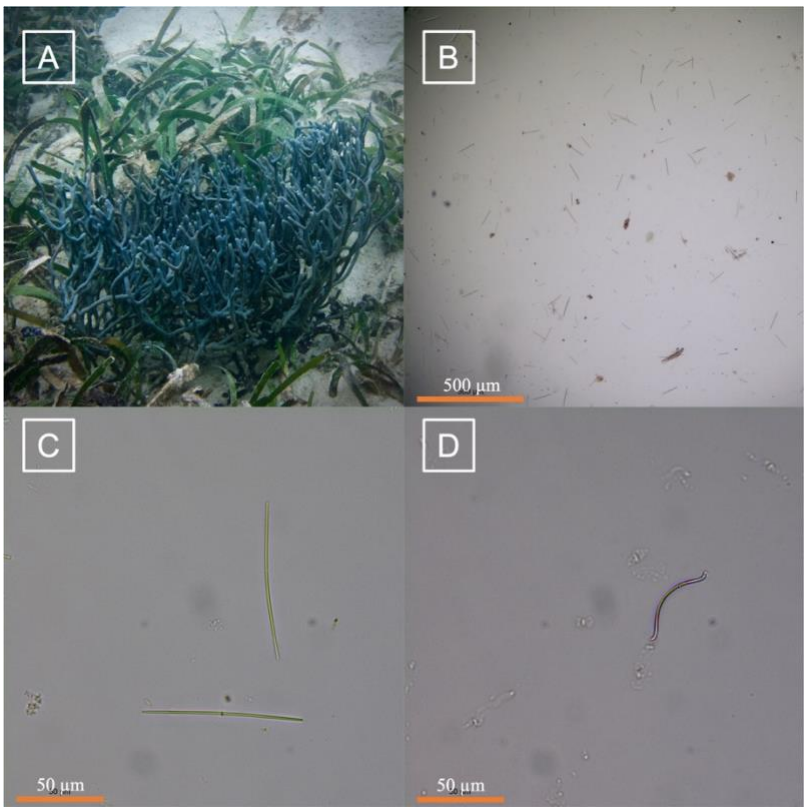

Figure 2.5. *In situ* photograph of Sponge D (A) and its spicules (B–D). Note: spicules were photographed with different scale.

The BLAST results with the 18S DNA marker consistently showed *Callyspongia* sp. as the sponge identification across the three specimens. BLAST search with the 28S DNA marker only gave a result at the Class level, which showed the identity of Sponge D as a member of Demospongiae. The ITS marker showed *Haliclona* sp. as the identity of Sponge D, but the query cover was below 50%. The CO1-ext marker showed that the sequence of Sponge D has a 100% similarity with *Haliclona koremella*. From the phylogenetic analysis, only the 18S marker showed *Callyspongia* sp. as the closest identity. Both ITS and CO1-ext showed the *Haliclona* genus as being the closest match, and in particular *Haliclona koremella,* based on

the CO1-ext marker. The observed morphological characteristics of Sponge D matched with the original description of *Haliclona koremella* (De Laubenfels 1954). There are also several records for its occurrence in the wider Indo-Pacific region (de Voogd 2022). Therefore, I conclude that *Haliclona koremella* is the identity of Sponge D.

Sponge E

Sponge E is a grey bulb-like sponge that was attached a limestone base in the seagrass bed (Figure 2.6A). The height of the bulb is about 5–10 cm. Oscules are large and prominent at the top of the bulb, with a slightly elevated membranous lip surrounding each oscule. Sponge texture is compressible but it returns to its initial shape quickly when compressed (i.e. it is resilient). Spicules are numerous (Figure 2.6B) with straight or slightly bent oxeas, size ~100 µm in length (Figure 2.6C). Euaster size was ~20 µm in diameter, but they were observed in very low numbers (Figure 2.6D).

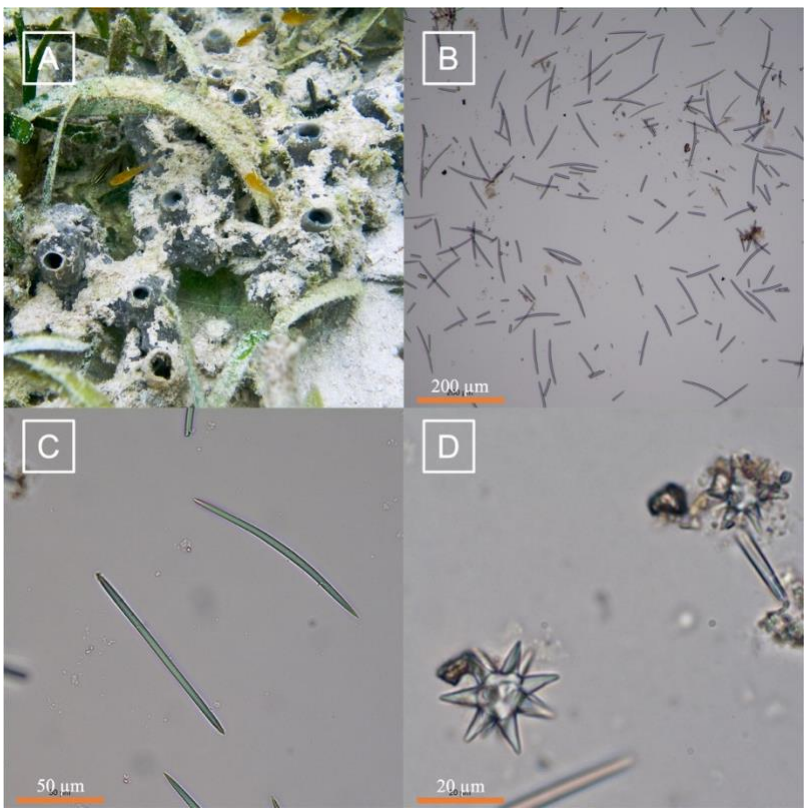

Figure 2.6. *In situ* photograph of Sponge E (A) and its spicules (B–D). Note: spicules were photographed with different scales.

Molecular identification with the four DNA markers consistently showed *Amphimedon queenslandica* across three specimens. Moreover, the observed morphological characteristics of Sponge E matched the description of *Amphimedon queenslandica* by Hooper and Van Soest (2006). However, *Amphimedon queenslandica* has only been recorded from the Great Barrier Reef, Australia, but not anywhere else in the wider Indo-Pacific bioregion (de Voogd 2022). Therefore, I assign *Amphimedon* sp. as the sponge identity for Sponge E.

Sponge F

Sponge F is an arborescent cylindrical digitate branching sponge (Figure 2.7A). The external colour of a living specimen is bright yellowish green, and the texture is firm and barely compressible. The sponge surface is conulose, with scattered oscules across the surface. A few spicules (Figure 2.7B) were observed in only one of the specimens, along with oxyspherasters, size ~40 um in diameter were observed (Figure 2.7C–D). The other two specimens did not have any spicules.

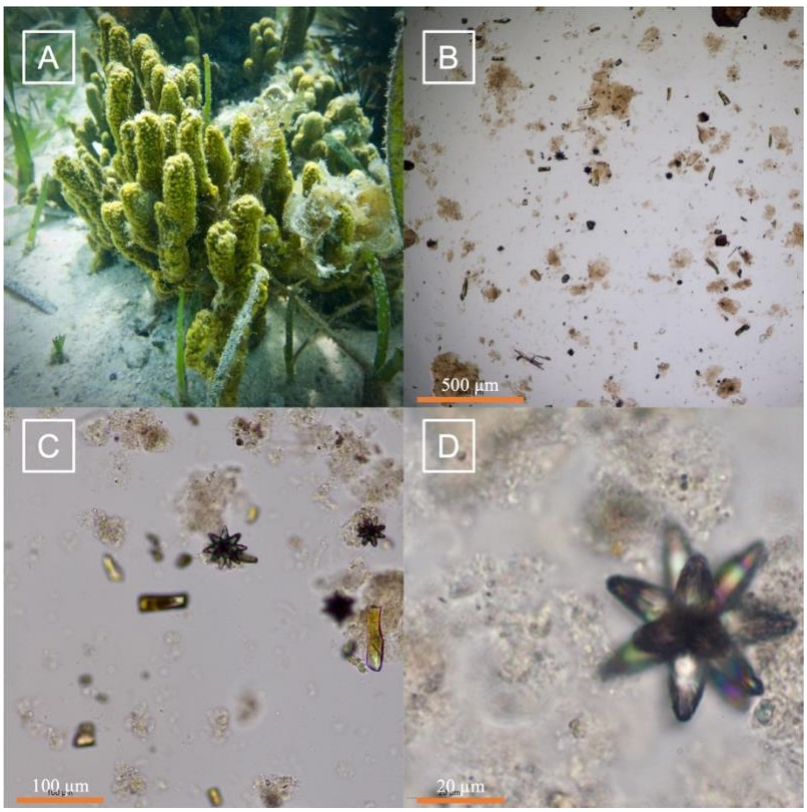

Figure 2.7. *In situ* photograph of Sponge F (A) and its spicules (B–D). Note spicules were photographed with different scales.

Dactylospongia elegans showed the closest match from the BLAST searches with the 18S, 28S, and ITS DNA markers, consistently across all three specimens. The sequences of Sponge F had 100% query cover and 99.9 and 100% similarity with *Dactylospongia elegans* at the 18S region. However, the CO1-ext DNA marker gave each specimen a different possible sponge identity. All observed morphological characteristics of Sponge F matched with the description of *Dactylospongia elegans* in Hooper et al. (2004). Furthermore, *Dactylospongia elegans* is common in the my study area (de Voogd 2022). Therefore, I conclude that Sponge F is *Dactylospongia elegans*.

Sponge G

Sponge G is an erect massive composite sponge that attaches itself to a limestone base in the seagrass bed. The external living colour is bright orange, with an irregular conulose surface. Oscules are conspicuous, with slightly membranous lips that are scattered all over the surface. The texture is firm but compressible. Straight and bent spicules (200–300 µm in length) as well as oxea (~100 µm long; see Figure 2.8B-D) were observed.

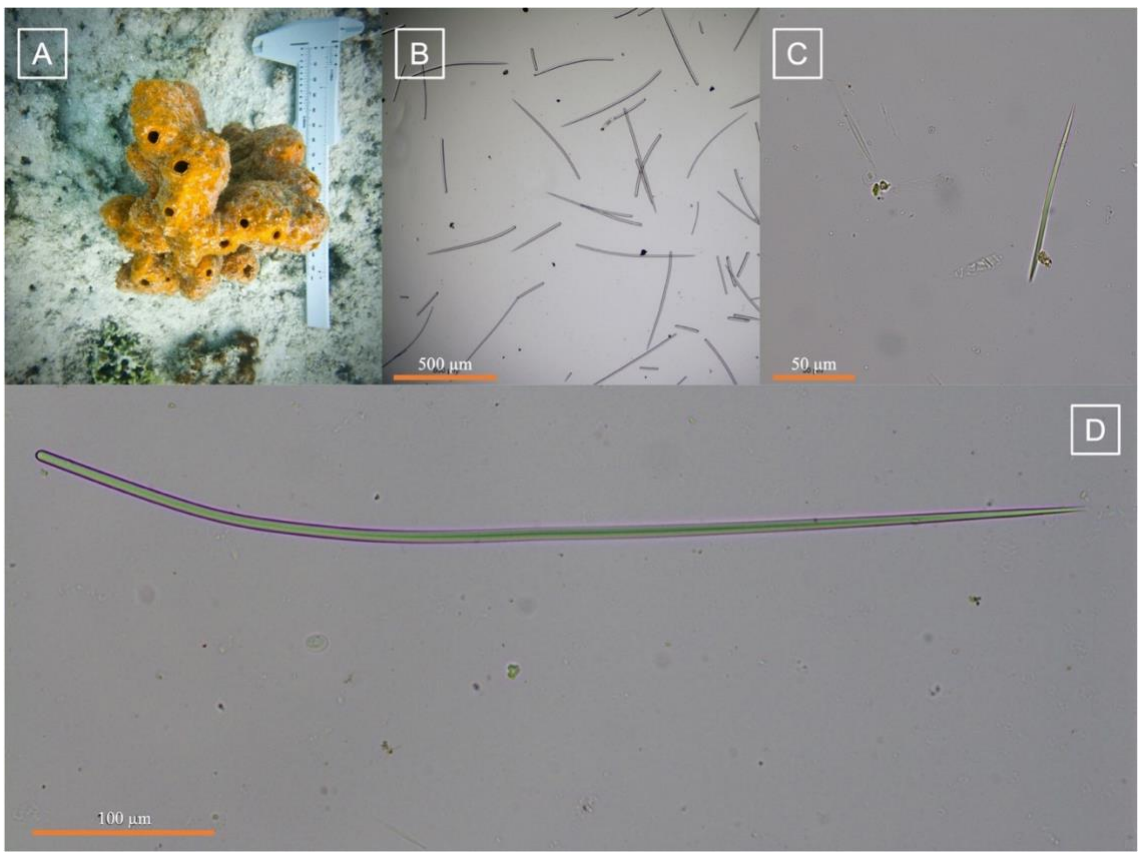

Figure 2.8. *In situ* photograph of Sponge G (A) and its spicules (B–D). Spicules were photographed with different scales.

Axinella verrucosa and *Axinella corrugata* had the closest matches from the BLAST search with the 18S marker; both species showed a sequence similarity of 99.9% from 100% query cover. BLAST results from the ITS and CO1-ext marker gave *Axinella corrugata* as the sponge identity across the three specimens, with sequence similarity of 97.5–99.1 % from ~100% query cover. Meanwhile, BLAST results with 28S gene gave three different hits with identical scores: *Stylissa carteri*, *Axinella* sp., and *Timea lowchoyi*. My morphological observations of Sponge G cannot differentiate the BLAST result top hits. However,*Axinella* appeared to be the common genus that matched across all three DNA markers. However, based on the World Porifera Database (de Voogd 2022), *Axinella verrucosa* and *Axinella corrugata* do not occur in the Indo-Pacific bioregion. Therefore, I assign *Axinella* sp. as the identity of Sponge G.

Sponge H

Sponge H is a cylindrical finger-like sponge that was usually found creeping on limestone boulders in the seagrass bed (Figure 2.9A). The external colour of living specimens is reddish brown. Oscules are discrete, with a slightly raised membranous lip, and are located mainly at the tips of the digits. Texture is firm and barely compressible, and the surface is smooth and even.

Subtylostyles in several size groups (~100, 200, and 400 µm in length) were observed, both in a straight and slightly bent form. Acanthostyle spicules in the size of about 40 µm in length were also observed(Figure 2.9B-E).

Clathria reinwardti was the strongest match for the two observed specimens based on the four DNA markers. Sponge G had 96.9% sequence similarity with *Clathria reinwardti* with 100% query cover for the 18S marker. The other markers either gave a query cover below 50% (ITS), or a sequence similarity below 96% (28S and CO1-ext). Based on the morphological characteristics, the description of Genus *Clathria* in Hooper et al. (2004) and the species *C. reinwardti* (Vosmaer 1880) matched Sponge G's characteristics. Regarding the sponge distribution, *Clathria reinwardti* is known to occur in my study site (de Voogd 2022). Therefore, I conclude that *Clathria reinwardti* is the identity of Sponge G.

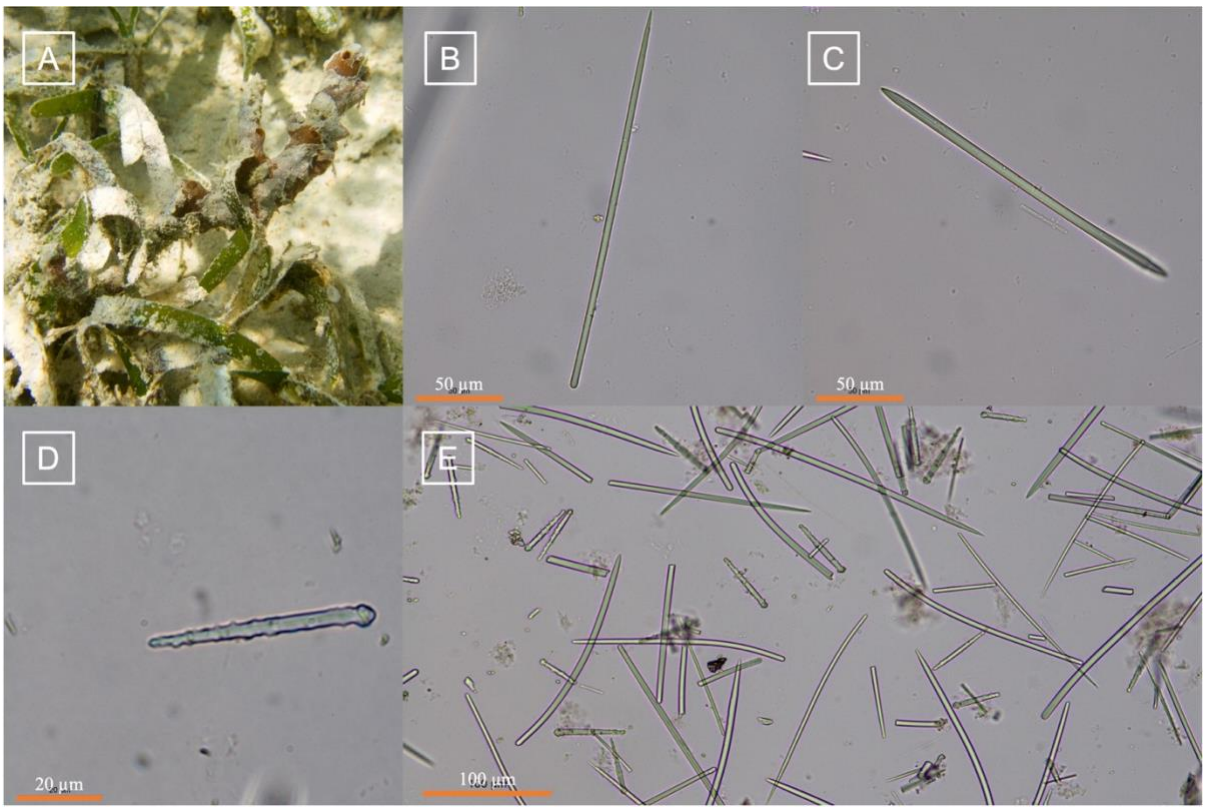

Figure 2.9. *In situ* photograph of Sponge H (A) and its spicules (B–E). Spicules were photographed at different scales.

Sponge I

Sponge I is a massive sponge that can be found living on the bottom of the seagrass bed, not attached to the substrate. The sponge can be easily picked up, as if it is a "free living" sponge (see Figure 2.10A). The size of the sponge is about 20 cm in length. The external colour of the living upward surface is black, in various colour hues. The bottom part (i.e. the side that is facing/touching the substrate) is yellowish-green. The sponge surface is opaque and even, and oscules are not visible. Sponge texture is firm and rubbery. Spicules are very rare. Two specimens showed only a few spicules (Figure 2.10B); the other specimen had more spicules but still low numbers (Figure 2.10C). I found thin bent oxea (~400 µm long) and tylostyle (~300 µm long). See Figure 2.10D-F for the photographs of spicules.

The BLAST results of the three specimens with three DNA markers were different for each specimen. One specimen (specimen #1) showed similar BLAST results with Sponge A across three DNA markers (18S, 28S, and ITS), while *Petrosaspongia nigra* and *Spongia zimocca.* came out as the sponge's identity, based on the 18S marker; *Hippospongia ammata* and *Spongia zimocca* based on the 28S marker; and *Hippospongia ammata* based on the ITS marker. The

sponge identity for this specimen appears to be *Spongia zimocca*, having a 99.9% sequence similarity out of 100% query cover.

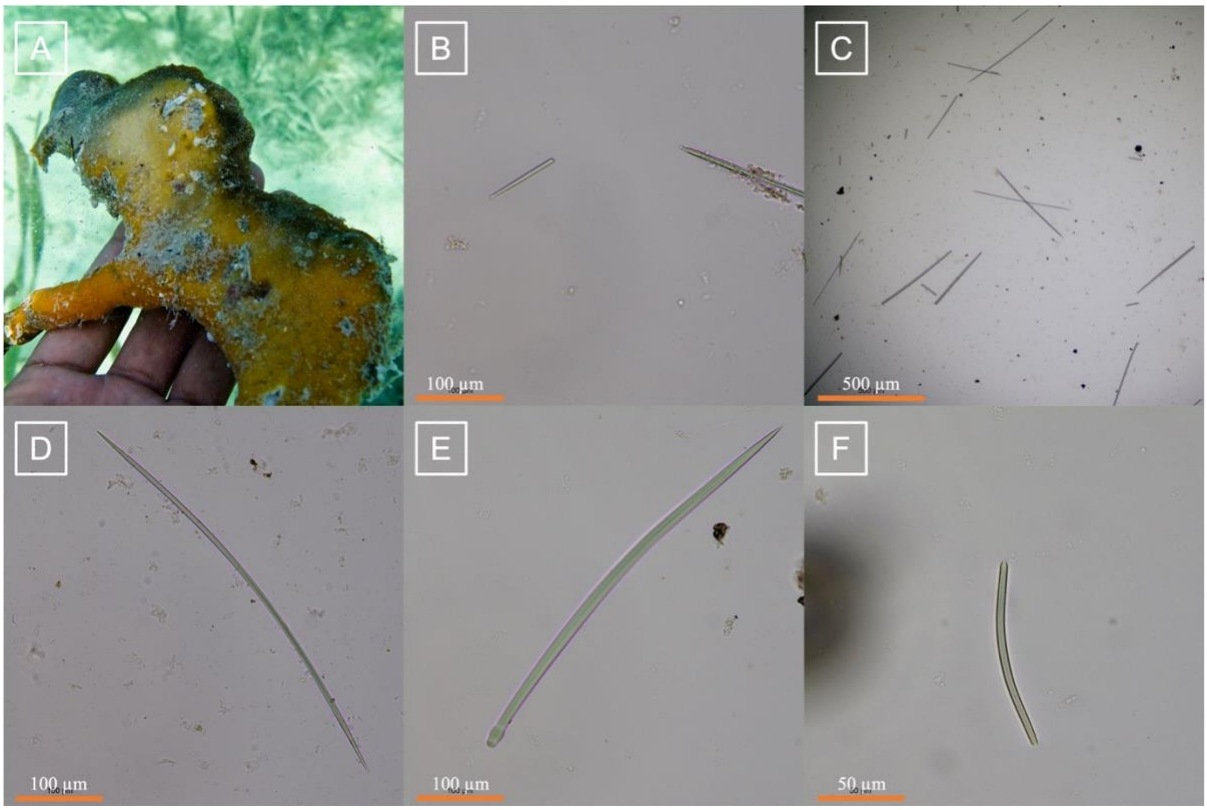

Figure 2.10. *In situ* photograph of Sponge I (A) and its spicules (B–F). Spicules were photographed at different scales.

The other specimen (specimen #2) only had a good quality 18S sequence, which yielded *Axinyssa topsenti* as the sponge's identity with a 99.7% sequence similarity out of 100% query cover. But the third specimen (specimen #3) gave *Rhopaloeides* sp. as the sponge's identity with a 100% sequence similarity out of 100% query cover in the 18S marker.

Based on the observed morphological characteristics, Sponge I is most likely a keratose sponge as it does not contain spicules (or only in very low numbers). Therefore, *Axinyssa topsenti* could not be the sponge's identity as it is not a member of the keratose sponge group. So that leaves *Spongia zimocca.* and *Rhopaloeides* sp. as the possible identity for Sponge I. However, since my morphological observations cannot differentiate those two sponges (*Spongia zimocca* and *Rhopaloeides* sp.), I have relied on the BLAST results. I conclude that *Rhopaloeides* sp. is the most likely identity of Sponge I.

Sponge J

Sponge J is an endopsammic sponge and only the protruding fistules are visible in the seagrass bed. The fistules have a chimney-like shape, and the colour of living specimens is dark black. Oscule shape is simple and located at the top of the chimney-like fistules. The sponge excreted a lot of mucus, even after being preserved in the ethanol. The fistules contain a lot of spicules, where the straight and bent oxeas (~150 um in length) were observed (Figure 2.11B-D).

Figure 2.11. *In situ* photograph of Sponge J (A) and its spicules (B–D). Spicules were photographed at different scales.

BLAST searches with the 18S sequence did not yield the specimen's identity as a member of Porifera. Meanwhile, the 28S and ITS markers gave the same BLAST results for the sponge's identity, namely *Siphonodictyon mucosum*. In addition, *Siphonodictyon mucosum* has been reported in the wider area of Indo-Pacific bioregion (de Voogd 2022). Since I only collected

and observed the fistules of Sponge I, I do not have any knowledge of the morphological characteristics of its main body. For this reason, I base my sponge identification decision on the molecular technique, which gave *Siphonodictyon mucosum* as the possible identity of Sponge J.

Sponge assemblage structure

I recorded ten sponge species across the studied seagrass meadow: *Spongia* sp., *Spheciospongia* sp., *Phyllospongia foliascens*, *Haliclona koremella*, *Amphimedon* sp., *Dactylospongia elegans*, *Axinella* sp., *Clathria reinwardti*, *Rhopaloeides* sp., and *Siphonodictyon mucosum*, which had a species-specific abundance at each site and zone (Figure 2.12a-b). I found all ten sponges at both sites. However, while I found all ten sponges in the middle and near-reef-flat zones, only five sponge species inhabited the high-shore zone: *Spongia* sp., *Spheciospongia* sp., *Amphimedon* sp., *Siphonodictyon mucosum*, and the *Rhopaloeides* sp. species.

The most sponge-abundant (sponge volume per m^2) zone in Hoga-1 and -2 were the high shore and middle zone, respectively (Figure 2.12c-d). As the zone covered the largest area of the meadow, the middle shore zone was estimated to have the highest total sponge biomass in Hoga-1 and -2, where sponge biomass was estimated at 1458 and 6181 litres, respectively (see Table S.2.3, App. A).

I found all four basic growth forms in the sponge assemblage, whereby six sponges had a massive growth form, two sponges had an erect growth form, one had a sponge cup-like, and there was one sponge crust-like growth form (Table S.2.4, App. A). In the more refined classification, I found seven morphologies out of 14 growth forms. There were three sponges with fistular–endopsammic morphology, two sponges with massive composite morphologies, and the other five morphologies were only represented by one sponge species.

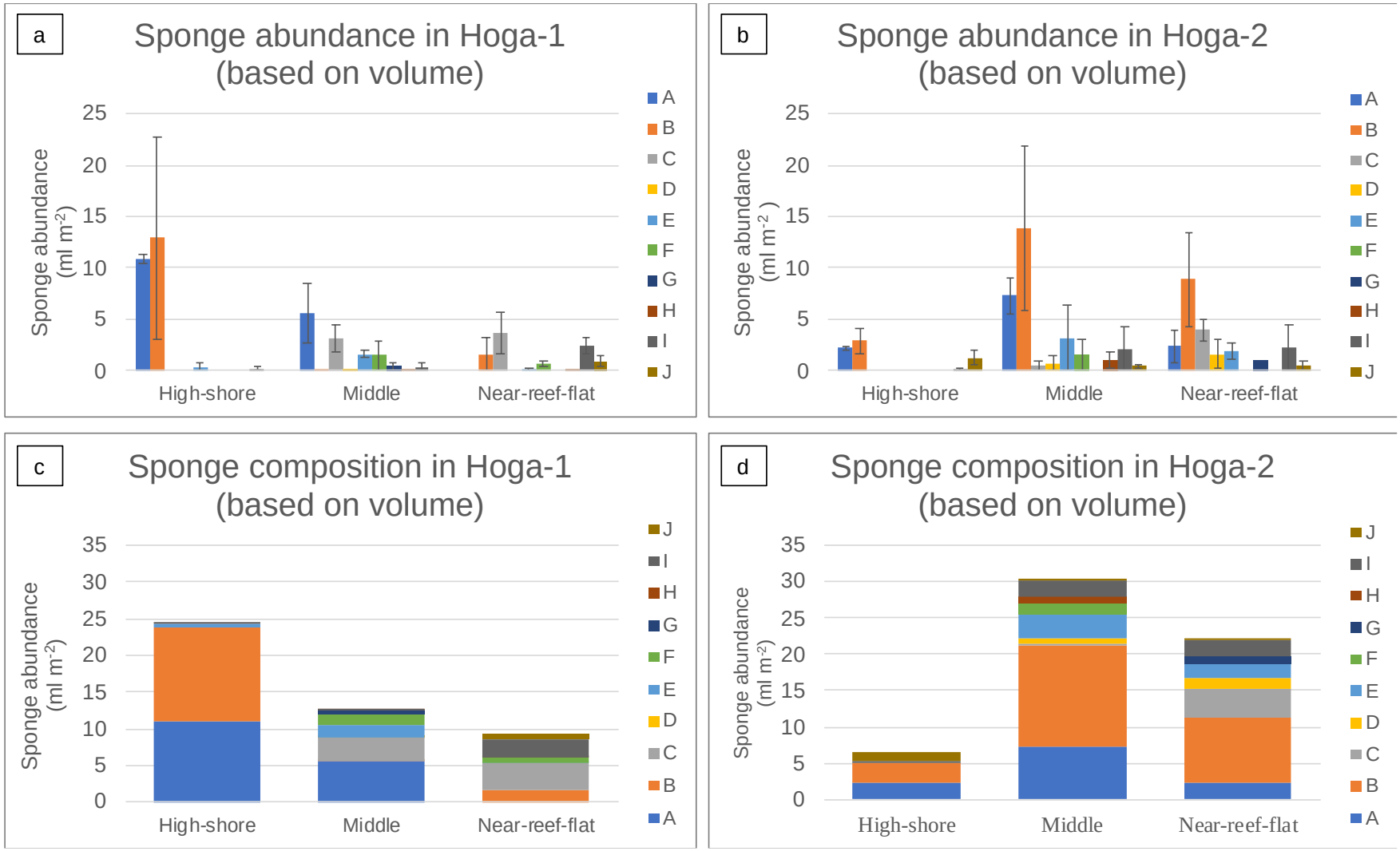

Figure 2.12. Sponge abundance (top) and species composition (down) at each zone at Hoga-1 and Hoga-2. Sponge species key: A= *Spongia* sp.; B= *Speciospongia* sp.; C= *Phyllospongia foliascens*; D= *Haliclona koremella*; E= *Amphimedon* sp.; F= *Dactylospongia elegans*; G= *Axinella* sp.; H= *Clathria reinwardti*; I= *Rhopaloeides* sp.; J= *Siphonodictyon mucosum*.

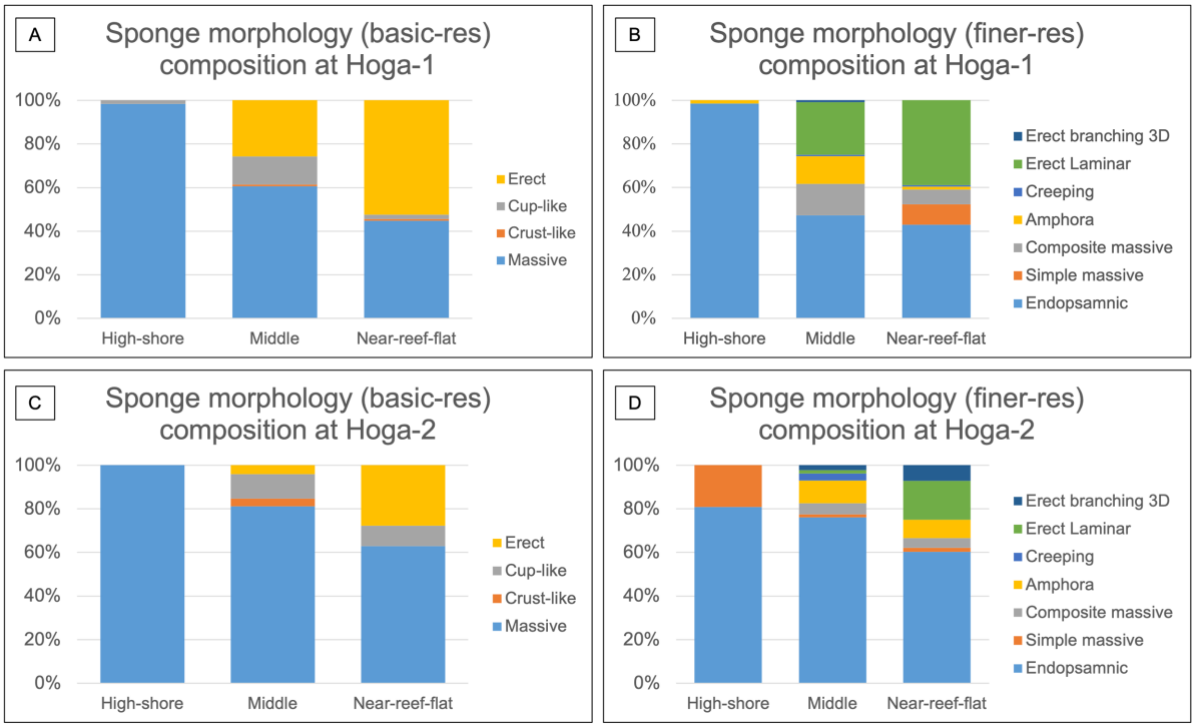

Figure 2.13. Sponge morphology composition at Hoga-1 (A and B) and Hoga-2 (C and D) in basic (A and C) and more refined (B and D) resolution based on the classification by Schönberg (2021).

Based on the basic growth form classification, I found that massive morphologies dominated the seagrass meadow at all sites and zones (Figure 2.13A–B). However, its contribution decreased further away from the shore. At the high-shore zones (both sites), almost 100% of the sponges were massive morphologies, declining to 60% and 80% at Hoga-1 and -2, respectively, in the middle zone. The abundance of this morphology further decreased to 44 and 63% in the near-reef-flat zone at Hoga-1 and -2, respectively. The massive morphologies were dominated by endopsamnic sponges at both sites and all zones (Figure 2.13C–D). I also observed an increase in erect growth forms, dominated by erect-laminar morphology, towards the near-reef flat zone shore at both sites. Meanwhile, cup-like growth forms, represented only by the amphora morphology, were most common in the middle zone at both sites.

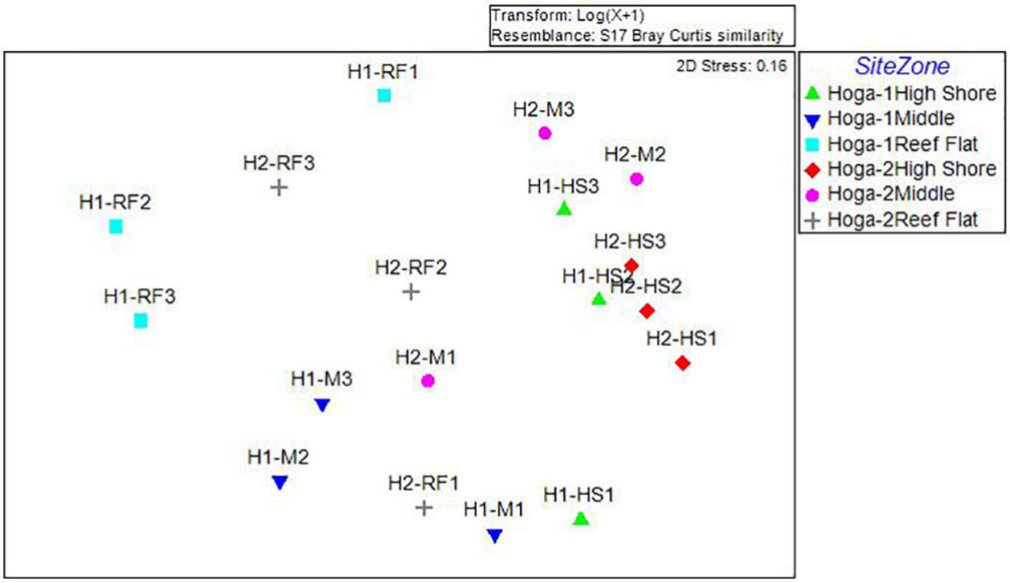

Figure 2.14. Ordination of sponge assemblage structure for each site and zone based on non-metric multidimensional scaling (n-MDS).

The sponge assemblage in my studied seagrass meadow varied with each combination of site and zone (Figure 2.14). The two-way PERMANOVA test showed that the sponge composition was significantly different between sites (Pseudo-F = 2.7285, $p < 0.05$), zones (Pseudo-F = 5.2376, p = 0.001), and there was an interaction between sites and zones (Pseudo-F = 3.4014, p = 0.003). The significant zone–site interaction for the sponge composition means that the way the factor zone influenced the sponge assemblages was different between sites. Therefore, sponge assemblages at Hoga-1 and -2 had different zonation patterns. The n-MDS plot (Figure 2.14) showed that while the sponge assemblage at every zone in Hoga-1 was different, the sponge assemblage in the high-shore and middle zone of Hoga-2 were much more similar to each other.

The similarities percentage (SIMPER) analysis showed that *Spongia* sp. characterized Hoga-1 similarities (44 %), while at Hoga-2, both *Spongia* sp. and *Spheciospongia* sp. were similarly representative of the site (35% and 34%, respectively). Based on zones (across sites), *Spongia* sp. contributed 62% to the high-shore zone's similarities (across sites). *Spongia* sp. contributed 40% in the middle zone, followed by *Spheciospongia* sp. by 19% to the zone's similarities. In the near-reef-flat zone, *Phyllospongia foliascens* and *Rhopaloeides* sp. were the most contributing sponges to the zone's similarities by 36% and 22%, respectively (see Table S.2.5, App. A for full results of SIMPER).

Habitat and Seagrass assemblage structure

The total area of the two studied seagrass meadows were 0.151 km^2 and 0.213 km^2 for Hoga-1 and Hoga-2, respectively. Meanwhile, the high-shore, middle and near-reef-flat zone coverage across both sites was 0.064, 0.213 and 0.087 km^2, respectively.

Table 2.3. Seagrass species richness, cover, canopy height, macroalgal and epiphyte cover, and seagrass ecological quality index (SEQI) of Hoga Island's seagrass meadow, Wakatobi National Park, Indonesia. Means are expressed ± SE.

Site/Zone	Species Richness	Seagrass cover (%)	Canopy height (cm)	Macroalgal cover (%)	Epiphyte cover (%)	SEQI
Site (all zones)						
Hoga-1	8	57.01 ± 4.90	13.5 ± 2.3	13.9 ± 3.6	53.8 ± 3.7	0.76 ± 0.04
Hoga-2	7	49.69 ± 2.83	10.5 ± 2.1	14.4 ± 3.5	45.8 ± 5.7	0.74 ± 0.04
Zone (all sites)						
High Shore	7	58.11 ± 4.21	10.0 ± 1.1	10.4 ± 3.1	38.5 ± 6.2	0.77 ± 0.03
Middle	8	47.80 ± 5.75	11.2 ± 3.9	15.9 ± 4.3	50.4 ± 5.2	0.74 ± 0.05
Near Reef Flat	8	54.14 ± 4.83	14.8 ± 2.3	16.4 ± 5.3	60.5 ± 2.1	0.73 ± 0.05
Total area (all transects)	8	53.35 ± 2.89	12.0 ± 1.5	14.2 ± 2.4	49.8 ± 3.4	0.76 ± 0.03

The seagrass cover of the studied meadow ranged between 48–58 %, with an overall average of 53 ± 3 % (see Table 2.3). I recorded eight seagrass species at my study sites, which included two persistent species (*Enhalus acoroides* (Linnaeus f.) Royle and *Thalassia hemprichii* (Ehrenberg) Ascherson in Petermann); four opportunistic species (*Cymodocea rotundata* Ehrenberg et Hemprich ex Ascherson, *Cymodocea serrulata* (R.Brown) Ascherson et Magnus, *Halodule uninervis* (Forsskål) Ascherson, and *Syringodium isoetifolium* (Ascherson) Dandy); and two colonising species (*Halophila minor* (Zollinger) den Hartog, *Halophila ovalis* (R.Brown) J. D. Hooker). I found all the seagrass species at all sites and all zones, except for *Syringodium isoetifolium*, which was only found in the middle and near-reef-flat zones of Hoga-1. Opportunistic species dominated the seagrass assemblage, contributing 77–84 % of the total seagrass cover (Figure 2.15). Both sites were dominated by *Cymodocea rotundata*, which contributed 48 ± 8 and 65 ± 9 % of the seagrass cover at Hoga-1 and Hoga-2, respectively.

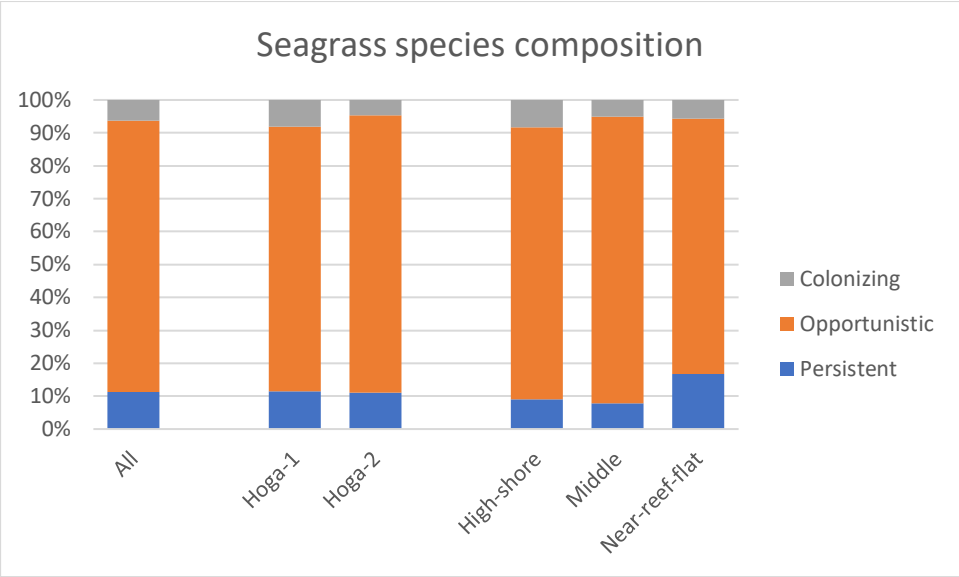

Figure 2.15. The seagrass species composition of the studied seagrass meadow, grouped into three categories based on its life-history traits: colonising, opportunistic, and persistent species (after Kilminster et al. 2015).

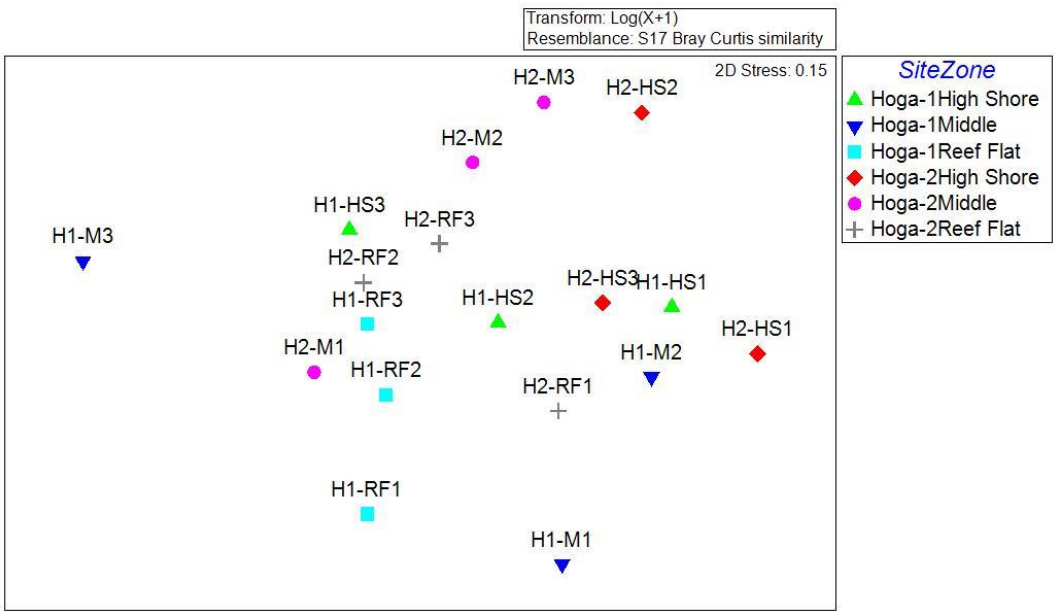

Figure 2.16. Non-metric Multidimensional Scaling (n-MDS) plot of seagrass assemblage structure for both sites and all three zones using.

The macroalgal cover in the seagrass sites was relatively low, ranging between 10–16 % with an average of 14.2 ± 2.4 for the entire meadow. The epiphyte cover in the seagrass meadow ranged between 39–61 % with an average of 49.8 ± 3.4 %. Based on the five measured parameters, the SEQI of the meadow ranged between 0.73–0.77, with an average of 0.76 ± 0.03

for the entire meadow. Based on SEQI, the studied seagrass meadow was categorised as being in good condition.

Seagrass assemblages were highly variable among sites and zones (Figure 2.16). However, the two-way PERMANOVA test did not show significant differences in seagrass assemblage composition between sites, zones, and their interactions (see Table S.2.2, App. A). Of the seagrass habitat's resilience parameters (i.e. seagrass cover, seagrass diversity, canopy height, macroalgal cover, and epiphytes cover), I did not observe any significant difference between sites and zones, or for any interactions (see Table S.2.2, App. A), suggesting a homogenous seagrass meadow.

Discussion

Very little is known about the ecology of sponge assemblages in Indo-Pacific seagrass ecosystems, including the drivers of abundance and distribution patterns, their interactions with seagrass species, and whether the drivers of seagrass and sponges are the same and co-dependent. This information is important for developing effective conservation and management plans. My study is the first to show how sponge assemblages change across seagrass zones from the shore to the reef. I found that even though seagrass beds were generally homogenous between sites and zones, the sponge assemblage compositions were significantly different between the zones, suggesting they have different drivers. I propose that these patterns are at least partly driven by morphological adaptation to the local environmental conditions. My study also revealed that the sponge assemblages varied significantly between two sites in a continuous seagrass meadow with little spatial separation (i.e. based on sites across all zones), suggesting that water depth or level of aerial exposure area not the only drivers of sponge assemblage patterns.

Sponge identification

Sponges are notoriously difficult to identify based on their morphology since the taxa have relatively few morphological traits and yet have been able to develop so many distinct sponge species (Hooper et al. 2004). I used a combination of molecular techniques and morphological approaches to identify ten sponges found in my studied seagrass meadow. However, some care needs to be taken in the use of molecular tools for sponge identification.

First, primer selection is important since no single pair of DNA markers worked across all specimens (see Table 2.1, App. A). The same issue was also reported by Yang et al. (2017), who examined the use of multiple DNA markers to identify sponges and reported that different markers had different amplification success rates. From my results, the COI-ext DNA marker had the worst PCR success rate of all the other markers that I used. The COI-ext marker only produced good PCR products for five sponge species out of ten (i.e. 50% success rate). However, the 18S, 28S, and ITS-2 markers produced clear PCR results for all ten sponge species, although some did not yield the target organism (i.e. did not identify the sample as a sponge) on the BLAST searches. The low success rate for the amplification of COI-ext from my specimens is consistent with previous studies. For example, Vargas et al. (2012) reported an average of 27 ± 17 % (ranging from 0–55 %) success rate in amplification of the COI region out of 3,360 specimens. Vargas et al. (2012) suggested that the age of the samples and the existence of thiocyanate salts (indicated by a low 260/230 ratio) were the reasons behind the amplification failure. This might also explain my results, since my samples were analysed almost 1.5 years after being collected in the field, so they might have degraded to some extent.

Second, the results of the BLAST searches for the sequences are only as good as the database being used. Currently, there are 326,932 records of nucleotide sequences, resulting in 5347 sponge species identifications in the NCBI database (NCBI 2022). The number of sponge species recorded in the NCBI database is only slightly above 50% of the total valid sponge species (9490 valid species of marine and non-marine sponges; de Voogd 2022). Therefore, there is still a high probability that the BLAST searches did not give the true match to my sponge sequences. The different records for each of the different DNA markers might also strongly affect the BLAST search results. In the NCBI database, the number of sponge species recorded via DNA markers 28S, COX-1, 18S, and ITS-2 is 2159, 1641, 1311, and 417 species respectively (NCBI 2022). As expected, the ITS-2 DNA marker had the worst sequence similarity percentage among all DNA markers. Ten BLAST searches out of 22 sequences (i.e. almost half) amplified with the ITS-2 marker came out with a similarity percentage below the threshold used (96%). Surprisingly, it was the 18S marker that consistently had the highest bit score and sequence similarity across all specimens, not the 28S marker that has the highest number of database records.

It is important to acknowledge that there have not been many sponge sequence submissions from my study area – Indonesia, which covers a vast area of the Southeast Asian Indo-Pacific

bioregion. Currently, only 488 nucleotide records from Indonesia covering 84 sponge species are found in the NCBI database (NCBI 2022). The number of records is small, considering the vast area of Indonesia's archipelago and when compared to the global records (326,932 nucleotide sequences). This means the number of sponge sequence records from Indonesia is only about 0.15% of the global records of nucleotide sequences. The deficiency of database records potentially limits the identification resolution only to the genus level, not down to the species level.

My results highlighted the importance and usefulness of combining morphological and molecular approaches for sponge identification , although different markers have their strength and limitations (Duran et al. 2004; Wörheide 2006; Redmond and McCormack 2009; Voigt et al. 2012). More importantly, there is an urgent need to do taxonomic work on sponges in Indonesia.

The state of the seagrass meadow

My survey found that the meadow had 53.35 ± 2.89 % seagrass cover, and I recorded eight seagrass species. This is considerably different from the results from previous studies on the same seagrass meadow approximately 13–14 years earlier, where the meadow had been reported to have approximately 70% seagrass cover and was occupied by only five seagrass species (Unsworth et al. 2007; Unsworth et al. 2008). The sampling effort is likely the reason for the higher seagrass diversity in my study since my sampling effort was about ten times the sampling effort of the previous study. My study showed a decline of almost 20% seagrass cover from these previous studies across 2005 and 2006 (i.e. Unsworth et al. 2007; Unsworth et al. 2008). Even though my study is only a snapshot of the seagrass meadow condition, it still suggests a large decline in seagrass cover over the last 13–14 years.

My study also showed that the meadow had experienced a shift in assemblage composition. The seagrass bed was dominated by *Thalassia hemprichii* and *Enhalus acoroides* in 2005-2006 (Unsworth et al. 2007; Unsworth et al. 2008), but is now dominated by *Cymodocea rotundata* (the present study). Based on seagrass life-history traits, *Thalassia hemprichii* and *Enhalus acoroides* are persistent seagrass species, while *Cymodocea rotundata* is an opportunistic seagrass (Kilminster et al. 2015). Coupled with *Cymodocea serrulata*, *Halodule uninervis*, and *Syringodium isoetifolium*, opportunistic seagrasses dominated the meadow consistently across sites and zones accounting for over 80% of the cover. The decline in seagrass cover and the

shift in assemblage composition may have resulted from environmental pressures on the seagrass meadow. With respect to local disturbances, there has been no intensive local coastal development that might have directly affect the seagrass meadow in my studied area. The clear water transparency that I observed during my survey reflected this condition. However, I did observe considerable invertebrate gleaning activities in my studied seagrass meadow. Invertebrate gleaning is a small-scale fishery that is mostly unreported and unregulated globally, including Indonesia (Unsworth et al. 2019). Therefore, the impacts of this fishing method are mostly unknown despite its direct effect on the seagrass habitat (Nordlund et al. 2011). Nordlund et al. (2011) reported that the people who have been doing this fishing method for 5–10 years observed a 30% decline in harvest and approximately 60% decline after three decades. This indicates how severe and chronic invertebrate gleaning impacts could be to the seagrass and its inhabitant macrofauna.

The seagrass cover of my studied meadow fell in the range of the Wakatobi National Park's average seagrass cover. The periodic monitoring programme by the Indonesian Institute of Sciences reported an average of 55.28% and 53.28% seagrass cover in 2015 and 2018, respectively, across the Wakatobi National Park (Sjafrie et al. 2018; Hernawan et al. 2021). My studied meadow's seagrass ecological quality index (SEQI) score ranged between 0.73–0.77, similar to the national park's SEQI score (SEQI = 0.75; Hernawan et al. 2021), and was therefore categorised as a 'good' seagrass meadow. Indonesia's national average SEQI is currently 0.68 ± 0.02 with 39 ± 4 % seagrass cover, which puts my seagrass meadow's condition as one of the less impacted seagrass meadows. However, the considerable decline in seagrass cover (approximately 20%) and the shift in seagrass composition in about a decade experienced in my studied meadow, suggests that the SEQI score does not necessarily reflect a high resilience capacity that might be expected from a 'good' seagrass meadow. In the future, I suggest applying different weights to each parameter to give a better indication of the seagrass condition, such as those used the calculation of coral reef and mangrove health indices (Kaufman et al. 2011; Faridah-Hanum et al. 2019). I also argue that the seagrass composition based on life-history traits categories (i.e. persistent, opportunistic, and colonising seagrass) may better describe seagrass meadow condition, rather than the seagrass diversity (i.e. the number of seagrass species) alone. In addition, water transparency did not differentiate my seagrass sites and may not be that useful as an assessment parameter for intertidal seagrass meadows with relatively very shallow water depth.

Seagrass assemblage patterns

My studied meadow was a 1.1 km long continuous seagrass meadow that covered approximately 0.364 km^2 area. The two-way PERMANOVA test did not show any significant differences in the abundance or assemblage composition between zones or sites (Table S.2.2, App. A), suggesting that the meadow was a homogenous seagrass meadow. The spatial scale of seascape studies is critical to understanding the ongoing physical and biological processes that drive the organism distributions (Boström et al. 2011). In my case, the scale of spatial separation between sites and zones was not large enough to capture any major drivers of biological patterns for the seagrass species.

Sponge species richness and abundance

I found ten sponge species in my studied seagrass meadow, similar to other studies. For example, 9 to 11 sponge species were reported inhabiting seagrass meadows in East Java – Indonesia (Setiawan et al. 2021). One to ten sponge species were also found in sub-temperate seagrass meadows on the southeastern Australia coast (Demers et al. 2015). However, compared with the adjacent coral reefs in the Wakatobi, the sponge species richness in my studied seagrass meadow was much lower. There are over 140 sponge species reported on the coral reefs adjacent to my studied seagrass meadow (Rovellini et al. 2019). A similar ratio was observed in Seribu Islands National Park – Jakarta, where 118 sponge species were recorded in the coral reefs (de Voogd and Cleary 2008), while the seagrass meadows were inhabited by only 13 to 15 sponge species (Ismet et al. 2017). The extreme condition of salinity, temperature, sedimentation, and air exposure that seagrass meadows experience likely explain the much lower species richness of sponges in seagrass beds compared to coral reefs (Pawlik et al. 2007).

Despite much lower sponge species richness than on the local coral reefs, my studied seagrass meadow had considerable sponge biomass. I estimated that the total sponge biomass across the entire meadow (in volume) was 2938 and 7681 litres at Hoga-1 (0.151 km^2) and Hoga-2 (0.213 km^2), respectively (Table S.2.5, App. A; see Figure 2.12 for the average sponge biomass per m^2). This sponge biomass would be expected to have a substantial impact on the seagrass meadow environment. Sponges can efficiently remove particulate (POM) and dissolved organic matter (DOM) from the water column (e.g. de Goeij et al. 2008; Hadas et al. 2009) and potentially affect the picoplankton abundance (e.g. Pile et al. 1996; Perea-Blazquez et al. 2012). Some sponges also facilitate primary production by hosting phototrophic symbionts that can

support their nutritional needs (Wilkinson 1983; Erwin and Thacker 2008). These interactions have the potential to affect the flow of energy in seagrass beds. Sponges could also potentially contribute to nitrogen cycling, since the sponges may harbour a high abundance of microbes (Southwell et al. 2008; Ribes et al. 2012).

Unfortunately, only a few studies have reported sponge biomass (Bell et al. 2020), making it difficult to compare my results. The one study that did record sponge volume in seagrass meadows was Demers et al. (2015) in Jervis Bay, southeastern Australia. These authors reported that the total sponge volume at their most abundant site was 4.4 ± 0.8 ml m^{-2}. Meanwhile, in the middle zone of Hoga-2, the sponge abundance (expressed in volume) was about 30 ml m^{-2} (Figure 2.12), about six times higher. Further research will be required to understanding these biogeographical patterns.

Sponge assemblage patterns and morphological variation

Sponges had conservable biomass at both sites and all zones of my studied seagrass (Figure 2.12). I found that sites, zones, and their interactions affected sponge assemblage distribution (see full PERMANOVA in Table S.2.4, App. A). Five sponge species inhabited the high-shore zone, while the other two zones had ten sponge species. The high-shore zone is likely only habitable for sponges that can survive extended periods of aerial exposure. The zonation down the shore is likely based on the water depth during low tide, resulting in a different period of air exposure.

Morphological adaptation plays an important role in the survival of sponges to different environmental conditions (e.g. Palumbi 1984; Bell and Barnes 2000; Rützler et al. 2007). The high-shore zone was characterised by *Spongia* sp. and *Speciospongia* sp. (see full SIMPER in Table S.2.4, App. A), with their biomass contributing more than 90% of the total sponge biomass (Figure 2.12). These two species were endopsamnic sponges and are mostly buried in the sediment, which is thought to be an adaptation to aerial exposure (e.g. Schönberg 2000; Vinod et al. 2009). By having most of their tissue buried in the sediment, the sponges can reduce water loss due to aerial exposure and prevent overheating. The convex or erect parts of the sponge (i.e. fistules) also avoid sediment build-up on their surfaces and prevent blocking of the sponge's aquiferous system. The middle zone was characterised by *Spongia* sp., *Speciospongia* sp., and *Amphimedon* sp. (Table S.2.5, App. A). While *Spongia* sp. and *Speciospongia* sp. are endopsamnic sponges, *Amphimedon* sp. occurs in an amphora or sack-like morphology, hollow

inside, with a narrowed apical osculum that increases the oscular size and prevents sediment settlement (Krautter 1998; Bell 2004). Schönberg (2021) suggested that amphora or sack-like morphology is suited to moderate sedimentation levels. Meanwhile, the near-reef-flat zone was characterised by erect-laminar sponge *Phyllospongia foliascens*, which dominated the sponge biomass (Figure 2.12). This sponge has a flexible and vertically flattened morphology with high spongin content that can bend when it experiences strong water movement. These features are well suited to areas that experience considerable water movement, which is often experienced in this zone as waves break over the reef crest (Schönberg and Fromont 2011).

Sponge assemblages were also significantly different between sites. However, no single sponge species contributed substantially to this difference (i.e. more than 30% of contribution) (see Table S.2.5, App. A for SIMPER results). I also found a significant difference in the interaction between site and zone, meaning the way that zones influence the sponge assemblage varied between the sites. The sponge biomass in each zone at each site showed different patterns. At Hoga-1, the high-shore had the highest sponge biomass, followed by the middle and near-reef-flat zones. In contrast, the high-shore zone at Hoga-2 had the lowest sponge biomass among the three zones. The pattern at Hoga-2 was similar to what was reported from a seagrass meadow in the Gulf of Mexico, where sponge abundance and biomass was relatively higher at the most distant sites from shore (> 150 m; Ávila et al. 2015). However, this was not the case at Hoga-1. This shows that the zonation based on shore height is not the only driver of the sponge assemblages in the studied seagrass meadow. Other factors that might explain these patterns include differences in shore slope, seafloor sediment composition, and nutrient supply (Bell and Smith 2004; Hunting et al. 2013; Archer et al. 2018). These should be a focus of future studies.

Conclusion

My studied seagrass meadow was in good condition based on the SEQI despite having undergone a major loss of seagrass cover over the last 15 years. Sponges appeared to have considerable biomass across the seagrass meadow and are an important component of the benthic community. Importantly, while I found no difference in seagrass assemblages between sites or zones, sponge assemblage compositions were significantly different, suggesting these assemblages have different environmental or biological drivers. I propose that the degree of aerial exposure is an important driver of the differences between zones to which sponges show

some degree of morphological adaptation. The differences between sites are harder to explain and will require a further detailed examination of the differences in the physical conditions at the two sites.

CHAPTER 3.

Variation in autotrophic and heterotrophic sponge abundance in a shallow water seagrass system

Abstract

Sponges are well known to feed heterotrophically through suspension feeding, but their relationships with photosynthetic symbionts means they also have the potential to utilise or release photosynthetically-derived carbon. Here I determined the nutritional mode of abundant seagrass sponge species at two sites in the Wakatobi National Park, Indonesia from the near-reef flat (d = 1.9 m), middle-shore (d = 1.2 m) and the high shore (d = 0.5 m) to assess the role they might play in nutrient fluxes. I measured *in situ* net primary production (NPP) to dark respiration (P:R) ratios and photosynthetic pigment concentrations of eight sponge species representing 75–100% of the total sponge assemblage biomass in the meadow, and estimated the sponge-mediated primary production across the seagrass bed.. I found that six out of eight sponge species were autotrophic, based on their instantaneous P:R ratios and daily carbon budget. The proportion of autotrophic sponge biomass in the sponge assemblage in the high- and middle-shore of the seagrass meadow ranged between 40–70%, and accounted for 98% and 81% of the biomass in the near-flat-zone of Hoga-1 and -2, respectively. At the assemblage level, sponges were net oxygen consumers in the high-shore and middle-shore zones, but net oxygen producers in near-reef-flat zone of the seagrass meadow over a 24 h cycle. My findings challenge the current view that sponges are generally consumers of carbon from the water column, as autotrophic sponges may be releasing more photosynthetically-derived carbon to the environment than they consume.

Keywords Porifera, P:R ratio, photosynthesis, symbiont, Wakatobi, gross primary production

Introduction

Determining trophic relationships are important for understanding ecosystem dynamics and this requires an understanding of the nutritional mode of community members (Krebs 2009). It is important to determine which species are autotrophic – organisms that are able to meet their nutritional needs through photosynthesis or chemosynthesis (Allan 1995), and which ones are heterotrophic – organisms that obtain their nutrition from the external acquisition of organic carbon (Lazcano 2016). These distinctions can be more complicated for animals that form symbioses with photosynthetic organisms, and thereby interact with the environment as one entity (Bosch and McFall-Ngai 2011; McFall-Ngai et al. 2013). A single entity that is comprised of a host organism with associated microorganisms has been termed a 'holobiont' (Knowlton and Rohwer 2003; Bordenstein and Theis 2015), which interacts with the environment as a single organism (Webster and Taylor 2012; Webster and Thomas 2016). In such cases, one way to determine the overall nutritional mode of the holobiont is to measure the gross primary productivity to respiration (P:R) ratio. Odum (1956) first introduced the P:R ratio as a simple index to determine the relative dominance of autotrophic or heterotrophic metabolism in ecosystems. When gross primary production exceeds the respiration rate (P_G:R > 1; i.e. P_N > 0), an ecosystem is considered to dominated by autotrophic processes, and where gross primary production is lower than the respiration rate (P_G:R < 1; i.e. P_N < 0) an ecosystem is considered to be dominated by heterotrophic processes.

At the individual level, the utilization of the P:R ratio to determine whether a holobiont is autotrophic or heterotrophic requires some caution as natural light intensity changes through the day, and there is a limit to how much light a photosynthetic organism can utilise. Primary production as a result of photosynthesis can occur at low light intensities and generally increases as light intensity increases, until it reaches a plateau at some higher light intensity (Blackman 1905; Blackman and Matthaei 1905). When light intensity increases further, the light intensity may trigger damage to the photosystem, resulting in decreased photosynthesis (i.e. photoinhibition; Kok 1956).

In the interpretation of P:R ratios as an indicator of the holobiont's nutritional mode, there are several caveats that need to considered (see Muscatine et al. 1981). Importantly, the ratio itself is an indirect measurement of carbon use as it is derived from oxygen measurements. In addition, the ratios do not provide any information on amount of fixed carbon that is translocated to the host animal, which is needed to fully evaluate and confirm how much of

carbon requirement of the holobiont is actually being fulfilled by primary production (e.g. Burgsdorf et al. 2021). However, it is likely that any fixed carbon not able to be used by the host or symbiont would be released to the environment altering local carbon fluxes.

Sponges are generally considered to be heterotrophs, feeding on particulate (e.g. Hadas et al. 2009; Perea-Blazquez et al. 2012) and dissolved (e.g. de Goeij et al. 2008; Hoer et al. 2018) organic carbon. However, sponges (Porifera) also form extensive symbioses with a range of organisms, including those capable of photosynthesis (Taylor et al. 2007). The most common photosynthetic symbionts of sponges are cyanobacteria (Rützler 1985; Usher 2008), but sponges also form relationships with zooxanthellae, chlorophytes, rhodophytes, and diatoms (Rützler 1985). Together, the sponge-host and the associated symbionts interact with the environment as one entity termed the sponge 'holobiont' (Webster and Taylor 2012; Webster and Thomas 2016). Although poorly studied compared to coral-dinoflagellate symbioses, sponges are thought to gain supplemental nutrition from their photosynthetic symbionts (Wilkinson 1980; Weisz et al. 2010). Achlatis et al. (2018) demonstrated that photosynthetic symbionts obtain some of the inorganic resources from the host sponge and, in return, translocate some organic nutrients to the sponge host. These authors also found that this translocated photosynthate stimulates the ability of this bioeroding sponges to erode calcium carbonate in a tropical coral reef habitat (Achlatis et al. 2019; Achlatis et al. 2021). Photosymbiont-containing sponges have been found in tropical (eg. Wilkinson 1987b; Steindler et al. 2002) and temperate waters (Roberts et al. 1999; Lemloh et al. 2009), although the true extent of these relationships is poorly known (Usher 2008). In addition, there are only a few studies that have measured gross primary production to respiration (P:R) ratios to estimate the nutritional mode of sponge holobionts (see review by Bell et al. 2020). Most previous studies of sponge–photosymbiont interactions have been focused on the existence of photosynthetic symbionts in sponges by measuring pigment concentrations – particularly chlorophyll-*a* (e.g. Biggerstaff et al. 2017), and photosymbiont fluorometric responses (e.g. Beer and Ilan 1998). However, sponges have complex interactions with their hosted microbes (Freeman and Thacker 2011; Freeman et al. 2021). Besides the quantity of the photosynthetic symbionts that live within sponges, the symbionts' photosynthetic productivity also plays an important role in the nutrient transfer to the sponge host (Freeman et al. 2013).

Early work on sponges has led to a broad assumption that a higher degree of autotrophy characterizes sponges in the shallow tropical waters of the Indo-Pacific region, while

heterotrophs dominate in Atlantic-Caribbean coral reefs. Wilkinson (1983) found that six out of the ten most common sponges on the Great Barrier Reef of Australia (GBR) were net oxygen producers, where their gross primary production exceeded that required for respiration. Subsequent research on the GBR led to the hypothesis that higher light intensity in the Indo-Pacific has led to the evolution of a higher proportion of autotrophic sponges (Wilkinson and Trott 1985) as establishing symbioses with photosymbionts enabled survival in more oligotrophic environments (Wilkinson 1987a; Wilkinson and Cheshire 1990). However, more recent studies have not found any significant differences in particulate food, light intensity, and dissolved organic matter and inorganic nutrients between the two geographical areas considered by (Wilkinson 1987a) suggesting other factors might drive the geographical variation in the prevalence of different sponge nutritional modes (see review by de Goeij et al. 2017). Furthermore, a more recent study from Panamanian coral reefs (in the Caribbean) reported that 20 out of 60 sponge species measured contained high chlorophyll-*a* concentrations (> 125 μg g^{-1} sponge), and another two sponges had intermediate concentrations (between 50 – 125 μg g^{-1} sponge), and were therefore all considered to be photosynthetically active sponges (Erwin and Thacker 2007). Erwin and Thacker (2008) further reported that two photosymbiont-containing sponges in the Caribbean were heterotrophic under low irradiance but autotrophic under high irradiance, based on their GPP to respiration ratios. These more recent studies cast doubt on the general view that heterotrophic sponges dominate on Caribbean reefs.

Understanding the nutritional mode of abundant organisms is critical for understanding how they interact with the wider ecosystem and therefore how any changes in their abundance might impact overall ecosystem functioning. In this study I conducted *in situ* incubations of sponges inhabiting a shallow (< 2 m) seagrass meadow in the Wakatobi National Park, Southeast Sulawesi, Indonesia from the upper to lower areas of a seagrass bed. The aims of this chapter were to: (1) determine the nutritional mode of the sponges based on P:R ratios and how this varies spatially across the seagrass bed; (2) determine the photosynthetic pigment concentrations in the sponge species; (3) estimate the contribution of the entire sponge assemblage to the seagrass meadow oxygen flux.

Methods

Study area and sponge collection

This study was conducted in a seagrass meadow at the western side of Hoga Island, Wakatobi National Park (WNP), Southeast Sulawesi, Indonesia (5° 28' S, 123° 4" E). The WNP is a chain of islands located off Sulawesi's southeast peninsula and lies in the western part of the Banda Sea. The Banda Sea is known to have a high upwelling intensity that leads to high productivity (Gieskes et al. 1990; Moore et al. 2003). With respect to sponges, over 140 sponge species have also been recorded in the fringing coral reefs of Hoga Island, adjacent to the studied seagrass meadow (Rovellini et al. 2019).

The seagrass meadow of Hoga Island is a multispecies seagrass bed that occupies the lower intertidal and shallow subtidal zone with a maximum depth of approximately 1.9 m. Earlier surveys (Chapter 2) showed that the meadow contained eight seagrass species, which were *Enhalus acoroides* (Linnaeus f.) (Royle, 1839), *Thalassia hemprichii* (Ehrenberg) (Ascherson, 1871), *Cymodocea rotundata* (Asch. & Schweinf.), *Cymodocea serrulata* (R.Brown) (Ascherson & Magnus, 1870), *Halodule uninervis* (Forsskål) Ascherson, and *Syringodium isoetifolium* (Ascherson) Dandy, *Halophila minor* (Zollinger) (Hartog, 1957), and *Halophila ovalis* (R.Brown) (Hooker f., 1858). The meadow was dominated by *Cymodocea rotundata*, which contributed 48–65% to the total seagrass cover, while the overall average seagrass cover in the meadow was 53 ± 3%. The substrate of the meadow was 1.8 ± 0.1 mm sized fine coral sand (Unsworth et al. 2008).

From earlier sponge surveys (Chapter 2), I found ten sponge species that inhabited the seagrass meadow: *Spongia* sp. (Linnaeus, 1759), *Spheciospongia* sp., *Phyllospongia foliascens* (Pallas, 1766), *Haliclona koremella* (de Laubenfels, 1954), *Amphimedon* sp. (Duchassaing & Michelotti, 1864), *Dactylospongia elegans* (Thiele, 1899), *Axinella* sp. (Schmidt, 1862), *Clathria (Thalysias) reinwardti* (Vosmaer, 1880), *Rhopaloeides* sp., and *Siphonodictyon mucosum* (Bergquist, 1965). This earlier survey divided the studied seagrass meadow into three tidal zones based on the distance to shore, where the maximum depth during high tide at the high-shore, middle, and near-reef-flat zones was 0.5, 1.2, and 1.9, respectively. During low tide, the high-shore zone was completely exposed to air, while the middle and near-reef-flat zones were still covered by water but with different water depths. There was considerable sponge biomass in all tidal zones at the study site (see Table 3.1). This biomass information (sponge

abundance) was used to estimate the sponge-mediated oxygen flux at the assemblage level in our studied seagrass meadow.

Table 3.1. Sponge biomass estimation (litres) in the seagrass meadow and the average size of the measured sponges (expressed in mean ± SE) for P:R ratio measurements. The estimates of sponge biomass are from Chapter 2.

Sponge	Average volume of the sponge (ml)	Hoga-1 (L)			Hoga-2 (L)		
		High-shore	Middle	Near-reef-flat	High-shore	Middle	Near-reef-flat
Spongia sp.	480 ± 154	267 - 291	207 - 649	0	78 - 91	759 - 1229	28 - 150
Spheciospongia sp.	540 ± 209	79 - 584	0 - 8	1 - 158	62 - 159	796 - 2976	162 - 510
Phyllospongia foliascens	78 ± 7	0	136 - 334	77 - 282	0	0 - 127	109 - 191
Haliclona koremella	134 ± 9	0	0 - 16	0	0	0 - 186	9 - 112
Amphimedon sp.	202 ± 73	0 - 19	93 - 152	3 - 10	0	0 - 874	45 - 99
Dactylospongia elegans	208 ± 63	0	0 - 221	15 - 47	0	0 - 415	0
Axinella sp.	190 ± 64	0	0 - 61	0	0	0	9 - 64
Clathria reinwardti	100 ± 25	0	0 - 12	0 - 5	0	38 - 235	0

I collected seven to ten specimens of each sponge species in a non-destructive way to maintain the holobiont integrity (i.e. by not cutting them). For endopsammic sponges (sponges that buried most of their body in the substrate; *Spongia* sp., *Spheciospongia* sp., and *Siphonodictyon mucosum*), the sponges were removed by digging the surrounding sandy substrate and removing the entire sponge holobiont from the substrate. For sponges that attached themselves to a hard substrate (*Phyllospongia foliascens*, *Amphimedon* sp., *Dactylospongia elegans*, and *Axinella* sp.), the sponges were chiselled from the limestone ensuring the sponge holobiont was kept intact and the tissue not damaged. Meanwhile, I was able to remove *Clathria reinwardti* and *Haliclona koremella* from their substrate gently by hand. These sponges were collected from the tidal zone where they were most abundance. *Spongia* sp. and *Siphonodictyon mucosum* were collected from the high-shore zone, *Phyllospongia foliascens* and *Haliclona koremella* from the near-reef-flat zone, and the rest of the sponge species were from collected the middle-shore zone of the seagrass meadow. The sponges were then moved to our working station located in

the middle-shore zone of the meadow, about 180 m away from shore, keeping the sponges under water at all times. Sponges were acclimated for four-weeks at our working station, before we finally chose five specimens of each species (n = 5) that showed no sign of necrosis for our experiments. With the exception of *Spongia* sp. and *Spheciospongia* sp. that were able to maintain their natural upward orientation without any assistance, all sponges were tied to an artificial substrate – a rounded concrete block (see Figure S.3.1, App. B), to maintain their natural orientation. The sponges and substrates were regularly checked and cleaned from epibionts by gently rubbing them. Unfortunately, I was unable to keep *Rhopaloeides* sp. and *Siphonodictyon mucosum* alive during the acclimation period, so I was only able to work with eight sponge species.

The average size of each sponge species varied due to their different morphologies (see Table 3.1). I measured the sponge volume with the water displacement method (after Rützler 1978) at the end of the experiment. Each sponge specimen was placed into a graduated cylinder or measuring cup (depending on the shape and size of the sponge), topped up with seawater until the maximum volume scale, and the sponge removed and the volume loss recorded as the sponge volume. I then measured sponge wet weight and dry weight at our field laboratory. The dry weight was measured by oven drying the sponges at 60°C and recording the weight every 30 minutes until it did not show any further weight reduction. The final weight was considered to be the sponge dry weight. I measured the ash-free dry-weight of each sponge sample at IPB University in Bogor, Indonesia, using a muffle furnace at 550°C for 4.5 hours. The weight loss during the process was considered to the ash-free dry-weight. See Table 3.2 for details of sponge growth form and average sizes of the measured sponges in multiple metrics (volume, wet weight, dry weight and ash-free dry-weight).

Study design

I measured the net primary productivity (P_N) and dark respiration (R_D) of the sponges *in situ* using incubation chambers at our working station. Six incubation chambers were used for each series of measurements, where five chambers were used for sponges and another served as a control (no sponge, only water). The light incubations to measure the net primary productivity rate were conducted at midday (between 11 AM – 2 PM), using transparent incubation chambers. For dark respiration measurements, the incubations were performed in morning

(started at 7 AM) using black incubation chambers. Each sponge species (n = 5) was measured in three batches over three days to capture any between day variability.

Table 3.2 Growth form and average size of the measured sponges in multiple metrics: volume (mL), wet weight (g), dry weight (g), and ash-free dry-weight (g). Values are expressed in mean ± SE, n = 5. The asterisk denotes the autotrophic sponges, based on their P:R ratios.

Sponge	Growth form	Average size of the measured sponges			
		Volume (mL)	Wet weight (g)	Dry weight (g)	Ash-free dry-weight (g)
Spongia sp.	Massive - endopsammic	480 ± 154	95 ± 29	79.9 ± 28.3	11.67 ± 4.27
Spheciospongia sp.*	Massive – endopsammic	540 ± 209	201 ± 95	132.2 ± 20.5	25.18 ± 11.02
*Phyllospongia foliascens**	Erect - lamellate	78 ± 7	68 ± 8	13.0 ± 1.1	8.46 ± 0.78
*Haliclona koremella**	Erect - ramose (bushy)	134 ± 9	105 ± 6	8.0 ± 0.6	5.30 ± 0.60
Amphimedon sp.	Bulb-like	202 ± 73	74 ± 12	20.0 ± 6.5	13.13 ± 4.28
*Dactylospongia elegans**	Arborescent cylindrical digitate branching	208 ± 63	87 ± 22	23.4 ± 9.6	16.56 ± 6.77
Axinella sp.*	Erect massive composite	190 ± 64	61 ± 11	17.6 ± 4.6	12.61 ± 3.93
*Clathria reinwardti**	Cylindrical finger-like - creeping	100 ± 25	55 ± 16	7.7 ± 1.9	5.10 ± 1.37

Light intensity and temperature measurement

I installed a Hobo pendant logger in each incubation bag, at the bottom of the seagrass bed (n = 1; d = 1.2 m), and above the water surface (n = 1) to record the light intensity and temperature during the incubations. I set the loggers to read light intensity and temperature every minute,

and the readings from the entire incubation duration was averaged to determine the incubation light intensity and temperature.

The light intensity reading from the Hobo pendant loggers were converted into photosynthetically active radiation (PAR) following the protocol described by Long et al. (2012), where the paired readings from Hobo loggers and a PAR light meter were used to establish a fitting function. I used a modified laboratory quantum scalar irradiance meter from Biospherical Instruments (QSPL-2200) as the PAR light meter (μmol photons m^{-2} s^{-1}), recording side-by-side with the Hobo pendant loggers (lux) at the bottom of the seagrass bed. The generated fitting function (see Figure S.3.2, App. B) was used to convert the light intensity recorded by Hobo pendant loggers into PAR (μmol photons m^{-2} s^{-1}).

To determine how the light intensity changed through time in the seagrass meadow, I compiled the records from the Hobo logger deployed on the bottom of the seagrass meadow, from 7 AM to 5 PM, over 12 days. The light intensity value for each hour was obtained by averaging the readings for every minute within the hour. Then, I averaged the light intensity of each hour from the 12 days and plotted a polynomial trendline to identify any patterns. See Figure S.3.3 (Appendix B) for the daily cycle in light intensity at our working station (d = 1.2 m).

Incubation bags and incubation duration

I used modified incubation bags (after Hansen et al. 2000) for our incubation experiment, which were built from an airtight plastic container and plastic sheets. The lid of the container acted as a base for the incubation, fixed onto a flat platform, which was pegged to the seafloor of the seagrass bed to prevent movement of the measured sponges. The main part of the plastic container acted as the frame for the incubation bag, where its bottom was removed to give room for the incubation bag. The plastic sheet was mounted to the container by tapping it around the container wite tape. The tape was also used to seal the incubation bag edges. The plastic sheet was somewhat flexible, which allowed wave action derived interstitial water flow to pass through to the incubation bag (Malan and McLachlan 1991), providing water circulation in the incubation bag. A three-way valve was installed on the plastic sheet to withdraw the water samples and sealed with a silicon tube. Water samples were withdrawn with a syringe from the valve. See Figures S.3.1A, B, and C (Appendix B) for the incubation bags set-up, three-way valve installation, and water sample withdrawing process, respectively.

Transparent plastic sheets were used for the light incubation bags, and black plastic sheets for the dark incubation bags. I only used one layer of plastic sheet for the incubation bags. From the light and temperature recordings across all the incubations, I found no significant difference in light intensity ($t(20) = 0.2$, $p = .4$) and temperature ($t(16) = 0.1$, $p = .46$) between inside and outside the light incubation bags. Meanwhile, the dark incubation bags were able to block the light entirely, resulting in total darkness inside the bags (I = 0 μmol photons m^{-2} s^{-1}). With respect to temperature, there was no significant difference between inside and outside the dark incubation bags ($t(16) = 0.7$, $p = .24$). See Figure S.3.4 (Appendix B) for the average light intensity (PAR) and temperature at the water surface and at the bottom of seagrass meadow (d = 1.2 m), and inside the incubation bags (light and dark) during the incubations (light and dark).

To determine the suitable size of incubation chambers and the incubation duration, I conducted a preliminary experiment with three sponge species: *Spongia* sp., *Spheciospongia* sp., and *Phyllospongia foliascens* (n = 5). I measured the dissolved oxygen (DO) concentration every 18 minutes under dark incubation to determine the respiration rate (Figure S.3.5, App. B). I aimed for an experimental duration where the oxygen depletion was less than 25% of the normal level, but no longer than two hours as suggested by Muscatine (1980) to avoid animal stress. I found a combination of ~10 L incubation bags and 72 minutes of dark incubations gave 10–15% oxygen depletion across the species, which was the set-up used to run the subsequent incubations (see Figure S.3.5, App. B).

P:R ratio determination

The net primary productivity (P_N) represented the net oxygen produced during the light period (i.e. daytime) when photosynthesis and respiration co-occur. Meanwhile, dark respiration (R_D) represented the oxygen respired (i.e. consumed) during the dark period (i.e. night-time) when photosynthesis does not occur. Gross primary production (P_G) was calculated by adding net primary productivity to the dark respiration rate ($P_N = P_G - R_D$). The oxygen fluxes were then converted to carbon fluxes using a photosynthetic and respiration quotient of 1.0 (after Wilkinson 1983). I acknowledge that this approach may overestimate the gross primary productivity (P_G) as I did not measure directly the daytime respiration rate. Instead, I used dark respiration to calculate P_G, which is presumably lower than light respiration.

The incubations were run as follows: one sponge was placed inside each chamber and left for 10 minutes to acclimate after the chambers were sealed. After the acclimation period, I took a

60 ml water sample from the chamber through the three-way valve with a syringe and transported it to the surface. I measured the dissolved oxygen (DO) concentration using a luminescent dissolved oxygen probe HQd LDO101 (resolution of 0.01 mg/L) within 1 minute (or less) of the sample being collected. After 72 minutes, I took another water sample using the same procedure. The difference between these two-point measurements (i.e. oxygen flux in incubation chambers containing the sponges were corrected with the flux recorded in the control chamber. I then normalized the net primary productivity rate (from light incubations) and dark respiration rate (from dark incubations) by the ash-free-dry-weight (AFDW) of each sponge and for a one-hour period.

The net primary productivity rate (P_N) to dark respiration rate (R_D), termed with P:R ratio, was used to determine if each sponge species was autotrophic or heterotrophic. A positive ratio (P:R > 0) means that the sponge holobiont produces more oxygen than it requires for respiration and is considered a net primary producer (i.e. autotrophic). A negative ratio (P:R < O) means the sponge holobiont oxygen production is less than it respires and is considered to be a net primary consumer (i.e. heterotrophic). Wilkinson (1987a) added another criterion to characterize autotrophic sponges, where an autotrophic sponge is the one that derives at least 50% of its energetic requirement from the photosynthetic symbionts using oxygen production and respiration as a proxy for carbon consumed. The daily flux was calculated by summing up the daytime flux (P_N x 12 h) with the night-time flux (R_D x 12 h), which was used to confirm whether the sponge was autotrophic or heterotrophic over a full day. I acknowledge that I may overestimate the daily fluxes since we did not measure P_N at all light intensities, which is changing through time. Instead, I used P_N that was measured at midday only, for the extrapolation. The potential contribution of photosynthesis to sponge daily oxygen consumption was estimated by extrapolating P_G by 12 h and R_D by 24 h.

Since the incubations were conducted on different days means that each batch of incubations were potentially performed under different light intensities and temperatures. To test the effect of light intensity and temperature on differences in net primary productivity rate (P_N), I ran a two-factor permutational analysis of variance (PERMANOVA). Based on the daily cycle of light intensity at our working station (see Figure S.3.3, App. B), light intensity was categorized into three levels: low (0 – 500 μmol photons m^{-2} s^{-1}), moderate (500 – 1100 μmol photons m^{-2} s^{-1}), and high (> 1100 μmol photons m^{-2} s^{-1}) for this analysis. However, none of the light incubations were performed below 500 μmol photons m^{-2} s^{-1} light intensity. In regard to

temperature, based on the temperature range during the light incubations (see Table S.3.1, App. B), I categorized it into four levels: low (27°C), moderate (28°C), high (29°C) and very-high (30°C) for the analysis.

In the dark incubations, the light intensity inside the incubation chambers was always zero μmol photons m^{-2} s^{-1} (i.e. total darkness). Therefore, only temperature varied between the batches of incubations. I ran a one-factor PERMANOVA to test the effect of temperature on differences in dark respiration of the sponges. Based on the range of temperatures during dark incubations, temperatures were categorized into three levels: low (25°C), moderate (26°C), and high (27°C). PERMANOVA was performed by the software PRIMER v6 with PERMANOVA+ add-on (Anderson et al. 2008)

The light intensity and temperature for the different incubation batches were determined by averaging the readings of all six loggers that were installed in the incubation bags (n = 6). The information on the light intensity and temperature in the incubations for each sponge species is provided in Table S.3.1 (Appendix B).

After I had determined the P:R ratio for each sponge species, I ran a one-way ANOVA to test if there were any statistically significant differences between the sponge P:R ratios. To determine which sponges differed from other sponges, I did the Tukey post-hoc test. ANOVA and the post-hoc test were performed by the software IBM SPSS v28.

Photosynthetic pigment concentration

At the end of incubation measurements, two small pieces of sponge tissue (approximately 1 x 0.2 x 0.2 cm) from the part of sponges that was exposed to sunlight were collected for each sponge specimen. I took sponge tissue from the upper part of the endopsammic sponges (*Spongia* sp. and *Spheciospongia* sp.), the part that emerged through the substrate's surface and was exposed to sunlight. The sponge tissue was cut with a scalpel, cleaned with clean seawater and placed in 1.5 ml cryo-vials, kept in a light-blocking container, and frozen at 20°C for storage and transportation to the laboratory for pigment analysis. The pigment concentrations were determined following Pineda et al. (2016), where I cut and weighed the sponge sample (I aimed for approximately 10–20 mg), placed it in a 1.5 ml cryo-vial, and let it thaw. I added 1 ml of 95% ethanol to the vials, wrapped them with foil to prevent chlorophyll degradation, and held

them for 6 hours at -20°C for the chlorophyll extraction. Subsequently, the samples were shaken by a Tissuelyser shaker (Tissuelyser LT, Qiagen Inc, CA, USA) for 5 minutes and centrifuged at 10,000xg for 5 minutes. Then, I transferred 700 µl of the supernatant to a new vial and centrifuged it again at 10,000xg for another 5 minutes. Finally, I transferred 300 µl of extract and the 95% ethanol blanks to a 96-well microplate for the light absorbance reading by a spectrophotometer.

Absorbance at 470, 632, 649, 665, 696, and 750 nm was read on a PerkinElmer EnSpire 2300 multimode plate reader (PerkinElmer, Inc. Waltham, MA, USA). I used the blank-corrected readings minus the absorbance at a wavelength of 750 nm (E_x) to calculate chlorophyll-a, chlorophyll-b and total carotenoid concentrations using the following equations (Lichtenthaler 1987; Ritchie 2008):

Chl a ($\mu g\ ml^{-1}$) = $[(-0.9394 \times E_{632}) + (-4.2774 \times E_{649}) + (13.3914 \times E_{665})]/1.1021$

Chl b ($\mu g\ ml^{-1}$) = $[(-4.0937 \times E_{632}) + (25.6865 \times E_{649}) + (-7.3430 \times E_{665})]/1.1021$

Total carotenoids ($\mu g\ ml^{-1}$) = $[((1000 \times E_{470})/1.1021) - (2.13 \times \text{Chl-a}) - (97.64 \times \text{Chl-b})]/209$

The factor 1.1021 is a path length correction that was calculated according to the formula of Warren (2008), using 300 µl of sponge extract at 632 nm wavelength. Pigment concentrations were normalized to wet weight of the sample (as measured in the beginning of the process) using the equation:

[pigment concentration ($\mu g\ ml^{-1}$) x extraction volume (ml)]/wet weight (g)]

Abundance and composition of autotrophic and heterotrophic sponges in the seagrass meadow

After I determined whether each sponge species was autotrophic or heterotrophic, I calculated the contribution of autotrophic and heterotrophic sponges to the seagrass sponge assemblage biomass in each tidal zone at both sites (sponge abundances are summarised in Table 3.1). I summed the biomass for the autotrophic and heterotrophic sponges separately (based on P:R ratios) and calculated their relative contribution (percentage) to the total sponge biomass. Permutational multivariate analysis of variance (PERMANOVA) was performed to determine

any differences in the composition of autotrophic and heterotrophic sponges. between tidal zones, sites, and their interactions.

Assessments of sponge-mediated primary production in the seagrass meadow

I calculated the sponge-mediated primary production in the seagrass meadow by multiplying each sponge's daily flux with its abundance in each tidal zone at each site (summarized in Table 3.1). I then summed the oxygen flux by all sponges in each area to estimate the sponge assemblage oxygen balance in the seagrass meadow.

Results

Net primary productivity and dark respiration

Six out of the eight sponge species showed a positive net primary productivity rate (P_N), but at species-specific rates (see Table 3). *Haliclona koremella* had the highest P_N with 0.3039 ± 0.0273 mol g_{AFDW}^{-1} h^{-1}, followed by *Phyllospongia foliascens* with 0.0518 ± 0.0122 mol g_{AFDW}^{-1} h^{-1}. *Spheciospongia* sp., *Dactylospongia elegans*, and *Clathria reinwardti* had similar P_N, which was about 0.03 mol g_{AFDW}^{-1} h^{-1}. *Axinella* sp. had the lowest P_N among the sponge species with a positive P_N, and a rate of 0.0134 ± 0.0036 mol g_{AFDW}^{-1} h^{-1}. Meanwhile, *Spongia* sp. And *Amphimedon* sp. were the two sponges with a negative P_N with a rate of -0.0056 ± 0.0018 and -0.0275 ± 0.0102 mol g_{AFDW}^{-1} h^{-1}, respectively. Light intensity, temperature and their interactions between during light incubations did not have a significant effect on the net primary productivity rate measurement in any of the light incubations (all P(perm) > .05; see Table S.3.3-A (Appendix B) for full results of PERMANOVA).

For dark respiration, *Haliclona koremella* had the highest respiration rate among the nine seagrass sponges consuming -0.0890 ± 0.0180 mol g_{AFDW}^{-1} h^{-1}, followed by *Spongia* sp. and *Amphimedon* sp. with a rate of -0.0538 ± 0.0348 and -0.0373 ± 0.0082 mol g_{AFDW}^{-1} h^{-1}, respectively. Four sponges (*Spheciospongia* sp., *Phyllospongia foliascens*, *Dactylospongia elegans*, and *Clathria reinwardti*) showed similar respiration rates, where the mean values ranged between -0.015–0.03 mol g_{AFDW}^{-1} h^{-1}. *Axinella* sp. had the lowest respiration rate among the eight seagrass sponges, consuming -0.0066 ± 0.0018 mol g_{AFDW}^{-1} h^{-1}. From the PERMANOVA, the difference in temperature between dark incubation batches did not significantly affect the measurement of the dark respiration rates (P(perm) > .05; see Table

S.3.3-B, App. B). See Table S.3.2 (Appendix B) for the respiration rate in other sponge biomass metrics (volume, wet weight, and dry weight). For P_N, P_G, and R_D derived for other sponge biomass metrics (volume, wet weight, and dry weight), see Table S.3.2 (Appendix B).

Table 3.3. Net primary productivity rate (P_N), gross primary productivity rate (P_G), dark respiration rate (R_{dark}), daily flux, and the contribution of photosynthesis to daily respiration requirements of the seagrass sponges in the Wakatobi National Park, Indonesia. The unit for P_N, P_G, and R_D is mol g_{AFDW}^{-1} h^{-1}; the daily flux is in mol g_{AFDW}^{-1}; and contribution of photosynthesis to daily respiration requirements is in percentage (%). The values are expressed in means ± SE, n = 5.

Sponge	P_N	P_G	R_D	Daily flux (24 h)	% P_G contribution to respiration requirement
Spongia sp.	-0.0056 ± 0.0018	0.0127 ± 0.0097	-0.0538 ± 0.0348	-0.49 ± 0.43	11.8 ± 9.7
Spheciospongia sp.	0.0285 ± 0.0109	0.0363 ± 0.0255	-0.0158 ± 0.0061	0.25 ± 0.31	114.6 ± 4.5
Phyllospongia foliascens	0.0518 ± 0.0122	0.0751 ± 0.0149	-0.0232 ± 0.0024	0.62 ± 0.18	162.0 ± 1.0
Haliclona koremella	0.3039 ± 0.0273	0.3929 ± 0.0302	-0.0890 ± 0.0180	3.65 ± 0.42	220.7 ± 15.7
Amphimedon sp.	-0.0275 ± 0.0102	0.0089 ± 0.0090	-0.0373 ± 0.0082	-0.34 ± 0.15	11.9 ± 2.1
Dactylospongia elegans	0.0293 ± 0.0118	0.0442 ± 0.0157	-0.0149 ± 0.0043	0.35 ± 0.20	148.4 ± 2.0
Axinella sp.	0.0134 ± 0.0036	0.0205 ± 0.0044	-0.0066 ± 0.0018	0.17 ± 0.06	154.3 ± 0.2
Clathria reinwardti	0.0278 ± 0.0067	0.0609 ± 0.0112	-0.0289 ± 0.0068	0.39 ± 0.16	105.6 ± 2.2

P:R ratio, daily flux and contribution of photosynthesis to the daily oxygen budget

Haliclona koremella had the highest P:R ratio among all six sponges with a positive ratio (i.e. autotrophic sponge; Figure 3.1) of 3.91 ± 0.68. Four sponges (*Spheciospongia* sp., *Phyllospongia foliascens*, *Dactylospongia elegans*, and *Axinella* sp.) had a similar ratio at ~2.

Clathria reinwardti was the sponge with the lowest P:R ratio among the six autotrophic sponges, with a ratio of 0.92 ± 0.25. Meanwhile, the two sponges with negative P_N – *Spongia* sp. and *Amphimedon* sp. had a P:R ratio of -0.53 ± 0.38 and -0.91 ± 0.27, respectively. From the one-way ANOVA, there were statistically significant differences in the P:R ratio between the sponges ($F(7,16) = 22.906$, $p < .001$). There was not a statistically significant different P:R ratio between the two sponges with negative ratio (i.e. heterotrophic sponges; *Spongia* sp. and *Amphimedon* sp.), but they were significantly different with almost the rest of the sponges. Meanwhile, among the sponges with positive ratio (i.e. autotrophic sponges), *Haliclona koremella* had a P:R ratio that significantly different with *Spheciospongia* sp., *Axinella* sp., and *Clathria reinwardti*; but not with *Phyllospongia foliascens* and *Dactylospongia elegans*. See Figure 1 for the sponges P:R ratios accompanied with a note for which sponges that significant difference, and Table S.3.4 (App. B) for the full result of the Tukey post-hoc test for the multiple comparisons of P:R ratio means.

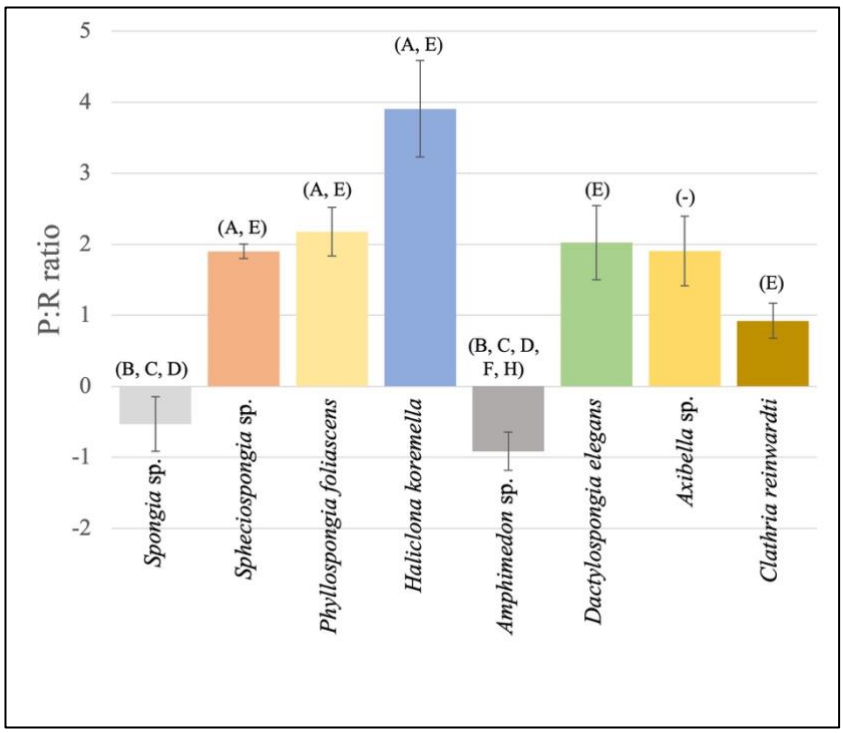

Figure 3.1. The net primary production to dark respiration (P:R) ratio for nine sponges that inhabited a seagrass meadow in the Wakatobi National Park, Indonesia. A positive P:R ratio (P:R > 0) means the sponge gross primary production (P_G) is higher to its respiration (R_D) and is the threshold for being considered autotrophic. Values are expressed in mean ± SE (n = 5). The capital letter above the column denotes which sponge species that had statistically significant difference in P:R ratio. A = *Spongia* sp; B = *Spheciospongia* sp.; C = *Phyllospongia foliascens*; D = *Haliclona koremella*; E = *Amphimedon* sp.; F = *Dactylospongia elegans*; G = *Axinella* sp.; H = *Clathria reinwardti*.

In regard to daily fluxes, *Haliclona koremella* had the biggest release of oxygen over 24 hrs with 3.65 ± 0.42 mol g_{AFDW}^{-1} d^{-1}, followed by *Phyllospongia foliascens* with 0.62 ± 0.18 mol g_{AFDW}^{-1} d^{-1}. *Dactylospongia elegans* and *Clathria reinwardti* both had a similar positive daily oxygen flux at about 0.4 mol g_{AFDW}^{-1} d^{-1}; *Spheciospongia* sp. and *Axinella* sp. with 0.25 ± 0.31 and 0.17 ± 0.06 mol g_{AFDW}^{-1} d^{-1}, respectively. Meanwhile, *Spongia* sp. and *Amphimedon* sp. were estimated to remove oxygen over 24 hrs at a rate of -0.49 ± 0.43 and -0.34 ± 0.15 mol g_{AFDW}^{-1} d^{-1}.

The extrapolation of instantaneous gross primary productivity (P_G) and respiration rates (R_D) to a daily budgets assuming 12 h photosynthesis and 24 h respiration showed that the photosynthesis of all sponges had a positive P:R ratio (*Spheciospongia* sp., *Phyllospongia foliascens*, *Haliclona koremella*, *Dactylospongia elegans*, and *Axinella* sp. and *Clathria reinwardti*) were able to provide more oxygen to the sponge holobiont than their daily oxygen requirement (>100%; Table 3.3). Meanwhile, for *Spongia* sp. and *Amphimedon* sp., it was estimated that photosynthesis provided approximately 11% of their daily oxygen requirement.

Photosynthetic pigment analysis

There was considerable variation in the concentration and composition of photosynthetic pigments among the seagrass sponges. All sponges contained chlorophyll-*a*., but not all contained chlorophyl-*b* and carotenoids. Three species did not contain chlorophyll-*b* (*Phyllospongia foliascens*, *Amphimedon* sp., and *Clathria reinwardti*), while *Spongia* sp. did not have carotenoids (Figure 3.2). Four sponges had chlorophyll-*a* concentrations below 10 μg g^{-1}, while the other four had chlorophyll-*a* concentrations between 40–70 μg g^{-1}. None of the sponges showed chlorophyll-*a* concentrations higher than 100 μg g^{-1}. However, based on the total pigment concentration (i.e. total of Chlorophyll-*a*, -*b*, and carotenoids), three sponges had pigment concentrations of more than 100 μg g^{-1} (*Phyllospongia foliascens*, *Haliclona koremella*, and *Dactylospongia elegans*), three sponges had pigment concentrations between 60 and 80 (*Spheciospongia* sp., *Haliclona koremella*, *Axinella* sp., and *Clathria reinwardti*), and two had pigment concentrations below 50 μg g^{-1} (*Spongia* sp. and *Amphimedon* sp.; Figure 3.2).

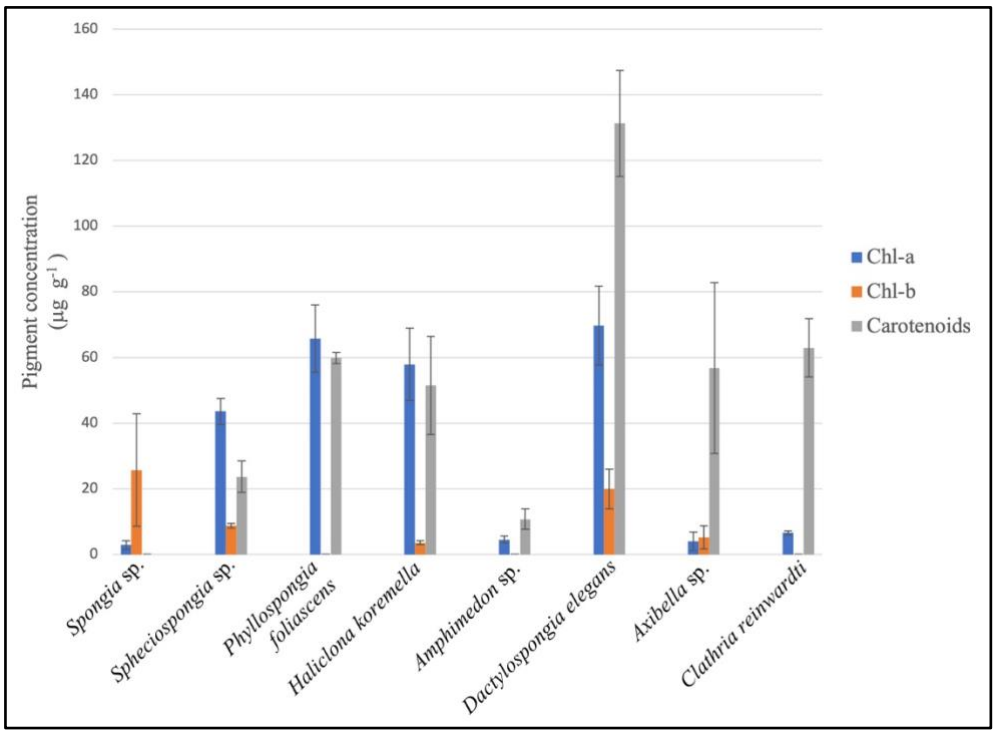

Figure 3.2. Photosynthetic pigment concentrations of the seagrass sponges (expressed as means ± SE, n = 5) in the Wakatobi National Park, Indonesia. The unit for the pigments concentrations is µg pigments g^{-1}sponge wet weight as the unit.

Even though chlorophyll-*a* was found in all sponges, it was not always the most abundant pigment. Chlorophyll-*a* was the dominant pigment for three sponges: *Spheciospongia* sp., *Phyllospongia foliascens*, and *Haliclona koremella* However, carotenoids were the dominant pigment in *Amphimedon* sp., *Dactylospongia elegans*, *Axinella* sp., and *Clathria reinwardti*. The pigment composition of *Spongia* sp. was dominated by chlorophyll-*b*.

Sponge assemblage structure: Autotrophic vs heterotrophic sponges

Both autotrophic and heterotrophic sponges are found in tidal zones of both sites in considerable abundance (see Table 3.4). The proportion of autotrophic and heterotrophic sponges in high-shore and middle-shore was similar (ranging between 40–60%), except at Hoga-2 middle-shore, where the composition of heterotrophic and autotrophic sponges was 30 to 70%. Meanwhile, at near-reef-flat zone, autotrophic sponges dominated the sponge assemblage by about 98% and 81% in Hoga-1 and -2, respectively (Table 3.4). The two-way PERMANOVA test showed that the composition of autotrophic and heterotrophic sponges was significantly different between zones (Pseudo-F = 3.9008, P(perm) = .032), but not between sites and the interactions between zones and sites (P(perm) > .05). See Table S.3.4 (Appendix B) for the full results of the PERMANOVA test.

Table 3.4. The abundance and proportion of autotrophic and heterotrophic sponge to the total sponge biomass in the seagrass meadow of Hoga Island, Wakatobi National Park, Indonesia. Heterotrophic sponges were *Spongia* sp., *Amphimedon* sp., and *Rhopaloeides* sp., while autotrophic sponges were *Spheciospongia* sp., *Phyllospongia foliascens*, *Haliclona koremella*, *Dactylospongia elegans*, *Axinella* sp., and *Clathria reinwardti*. Values of sponge abundance and proportion are expressed in mean ± SE, n = 3. The estimates of sponge abundance for each sponge species are from Chapter 2.

Site	Tidal zone	Sponge abundance (g_{AFDW} m^2)		Sponge proportion (%)	
		Heterotrophic	Autotrophic	Heterotrophic	Autotrophic
Hoga-1	High-shore	0.3467 ± 0.0126	0.5508 ± 0.4197	58 ± 23	42 ± 23
	Middle-shore	0.2521 ± 0.0723	0.5060 ± 0.2614	44 ± 19	56 ± 19
	Near-reef-flat	0.0087 ± 0.0027	0.5252 ± 0.2001	2 ± 1	98 ± 1
Hoga-2	High-shore	0.0757 ± 0.0113	0.1227 ± 0.0537	43 ± 9	57 ± 9
	Middle-shore	0.3950 ± 0.1031	0.8517 ± 0.2406	33 ± 6	67 ± 6
	Near-reef-flat	0.1654 ± 0.0128	0.9452 ± 0.3041	19 ± 8	81 ± 8

Sponge-mediated primary production in seagrass meadow

At the assemblage level, the sponge assemblage at the high-shore and middle-shore zones were estimated to be net carbon consumers, where the assemblage consumed carbon at a rate of -0.1644 ± 0.0633 and -0.0309 ± 0.0468 mol m^2 d^{-1}, respectively, at Hoga-1; and -0.0286 ± 0.0083 and -0.0798 ± 0.0654 mol m^2 d^{-1}, respectively, in Hoga-2. However, at the near-reef-flat zone, the sponge assemblage was estimated to be a net carbon producer with a release of 0.1518 ± 0.0356 and 0.2514 ± 0.0455 mol m^2 d^{-1} carbon over 24 h, in Hoga-1 and -2, respectively. See Table 3.5 for sponge-mediated primary production of each sponge species at each tidal zone and site.

Table 3.5. Sponge-mediated daily oxygen flux (24 h) at three tidal zones (high-shore, middle-shore, and near-reef-flat) of two spatially separated site (Hoga-1 and -2) in Hoga Island's seagrass meadow. The unit is in mol m^2 d^{-1} and the values are expressed in mean ± SE, n = 3. One asterisk denotes the heterotrophic sponges and two asterisk for the autotrophic sponges, based on their P:R ratios.

Sponge	Hoga-1			Hoga-2		
	HS	**M**	**NRF**	**HS**	**M**	**NRF**
Spongia sp.*	-0.2339 ± 0.0060	-0.1211 ± 0.0367	0	-0.0472 ± 0.0022	-0.1563 ± 0.0217	-0.0504 ± 0.0204
Spheciospongia sp.**	0.0837 ± 0.0629	0.0003 ± 0.0003	0.0104 ± 0.0102	0.0186 ± 0.0081	0.0894 ± 0.0510	0.0575 ± 0.0294
*Phyllospongia foliascens***	0	0.1159 ± 0.0213	0.1369 ± 0.0338	0	0.0175 ± 0.0076	0.1483 ± 0.0176
*Haliclona koremella***	0	0.0108 ± 0.0016	0	0	0.0689 ± 0.0105	0.1621 ± 0.0211
Amphimedon sp.*	-0.0142 ± 0.0029	-0.0614 ± 0.0030	-0.0051 ± 0.0005	0	-0.1218 ± 0.0246	-0.0721 ± 0.0055
*Dactylospongia elegans***	0	0.0222 ± 0.0194	0.0096 ± 0.0043	0	0.0232 ± 0.0203	0
Axinella sp.**	0	0.0024 ± 0.0014	0	0	0	0.0059 ± 0.0026
*Clathria reinwardti***	0	-0.0001 ± 0.0005	0.0000 ± 0.0003		-0.0007 ± 0.0042	0
TOTAL	-0.1644 ± 0.0633	-0.0309 ± 0.0468	0.1518 ± 0.0356	-0.0286 ± 0.0083	-0.0798 ± 0.0654	0.2514 ± 0.0455

Discussion

Many tropical shallow-water sponges harbour photosynthetic symbionts (e.g. Steindler et al. 2002; Erwin and Thacker 2007), which are thought to play a crucial role in the ability of sponges to colonize nutrient-poor habitats (Wilkinson and Cheshire 1990). However, few studies have demonstrated the contribution of photosynthetic symbionts to host nutrition, which has generally been inferred by measuring primary production to respiration (P:R) ratios (Bell et al.

2020). This information is important for our ability to predict how any changes in sponge assemblages might impact overall ecosystem functioning. I found the presence of photosynthetic pigments in all my study species. However, not all of sponges species were classified as autotrophic. I found that six sponge species were autotrophic and had positive instantaneous net primary productivity rate ($P_N > 0$), and were all were net primary producers based on their daily budget. The other two sponges were considered heterotrophic sponges with a negative instantaneous P_N, thus were net consumers based on their daily budget. At the assemblage level, the domination of autotrophic sponges means sponges are net oxygen/carbon producers. My results challenge the commonly held view that sponges are largely net consumers of carbon.

Autotrophic vs heterotrophic in the seagrass sponge assemblage

Primary production of the sponge holobiont exceeding respiration requirements (i.e. $P_N > 0$) is the key requirement to be considered an autotrophic sponge (Wilkinson 1983). However, further information is also need to fully confirm the actual contribution of symbionts the host nutrition, such as symbiont-host translocation rates. Wilkinson (1983) also added that the photosynthesis (P_G) of an autotrophic sponge needs to contribute at least 50% of its daily (24 h) oxygen needs. Based on these criteria, six out of my eight studied sponge species can be considered autotrophic. These six species had a positive P:R ratios and their primary production was able to provide more than 50% of their daily respiration requirements (see Figure 3.1 and Table 3.3). Furthermore, these species were net oxygen producers based on their daily budget. The proportion of autotrophic sponges in my seagrass sponge assemblage is similar to that reported from a coral reef sponge assemblage on the Great Barrier Reef, where six out of the ten most abundant sponges were autotrophic (Wilkinson 1983). Thus, autotrophic-sponge-dominated assemblages do not exclusively apply to coral reef sponge assemblages and may also apply to shallow water seagrass ecosystems.

Nutrient availability has been proposed as an important driver of the prevalence of autotrophic sponges in the Indo-Pacific, with Wilkinson and Cheshire (1990) reporting a pattern of increasing autotrophic sponge abundance further away from the mainland. These authors also proposed that the lack of autotrophic sponges in the Caribbean, compared with the Great Barrier Reef, was due to differences in nutrient availability, with the Great Barrier Reef being more oligotrophic than the Caribbean. These earlier findings led to the hypothesis that sponge symbioses with photosynthetic organisms is critical for their survival in oligotrophic

environments. Although this might be true for coral reefs, seagrass meadows are expected to have more nutrients than coral reefs as one of their functional roles is filtering sediment and suspended particles (Hemminga and Duarte 2000; Nagelkerken 2009), and yet most of the sponges in my studied seagrass meadow were also autotrophic sponges. Therefore, it seems likely that other conditions that just nutrient availability have led to the evolution of seagrass sponges to establish symbioses with photosynthetic symbionts.

In my studied seagrass meadow, I found variation in the dominance of heterotrophic versus autotrophic sponges between shore zones, even though there was a high number of autotrophic sponge species in the overall sponge assemblage. I found significant differences between tidal zones in the composition of autotrophic and heterotrophic sponges, while sites and the interactions between sites and zones were not different (Table S.3.4, App. B). At the high-shore and middle part of the seagrass meadow, the proportion of autotrophic and heterotrophic sponge biomass was similar. However, at the near-reef-flat zone of the seagrass meadow I found autotrophic sponges dominated the sponge biomass. The near-reef-flat zone is at the edge of the seagrass meadow, bordering the local coral reef flat, with a 1.9 m depth during hightide; autotrophic sponge biomass was up to 81% and 98% in Hoga-2 and Hoga-1 (Table 3.4). One possible reason is that the light conditions at high-shore and middle zone (which are shallower) of the seagrass meadow may trigger photoinhibition for some of the sponge symbionts and limit their distribution across intertidal zone of the meadow. Photoinhibition is known to cause coral bleaching, an event where zooxanthellae leave the corals-host or lose their pigments (Iglesias-Prieto et al. 1992; Hoegh-Guldberg 1999; Hoogenboom et al. 2006; Baird et al. 2009). With regard to sponges, Cheshire et al. (1997) reported a decrease in net primary production when sponges collected from 30 m depth were exposed to higher light intensity at 20 m depth, suggesting that photoinhibition had occurred. However, photoinhibition is unlikely the major driver of autotrophic sponge distribution in the seagrass meadow, as the middle-shore zone was observed to harbour the most sponges (based on biomass) among the three intertidal zones (Chapter 2). The middle-shore zone also had relatively equal proportion of autotrophic and heterotrophic sponges (Table 3.4). Interestingly, the abundance of autotrophic sponges (in term of biomass) was higher at the middle-shore than the near-reef-flat zone. Importantly, the domination of autotrophic sponges in the near-reef-flat zones were more the result of heterotrophic sponge biomass in this zone, rather than due to an increase in the absolute biomass of autotrophic sponges. Further research is needed to understand these patterns.

It is important to note that both autotrophic and heterotrophic sponges occurred in all tidal zones, including the high-shore zone that is exposed to air during low tide. Furthermore, the composition of these two nutritional modes in the sponge assemblage was about equal at the high-shore and middle-shore tidal zones of the seagrass meadow (see Table 3.4). I noticed that all sponges that inhabit the high-shore zone are endopsammic sponges, i.e. they buried themselves under the substrate and leave only small part of their body above the substrate. This growth form is considered morphologically adapted to cope with aerial exposure, where the sponges are exposed to a very high temperature and UV light radiation (Schönberg 2021). Yet, one of the only two sponges that inhabit this zone is autotrophic: *Spheciospongia* sp.. From my field observations, during low tide, the sponges at the high-shore were covered by seagrass blades, which may keep them cool and wet during aerial exposure. Unfortunately, I did not identify the photosynthetic symbionts and look at their exact location in the sponge tissue, which would have allowed us to further understand any adaptations of the symbionts and their sponge-host. However, this shows the importance of morphological adaptation of sponges in coping with extreme environmental conditions.

Sponge-mediated primary production in the seagrass meadow

Facilitating primary production is an important sponge functional role, as many sponges host photosynthetic symbionts (Bell 2008). Despite this, the contribution of sponge-mediated primary production to the environment has been generally overlooked. My study has revealed that seagrass sponge assemblages may be a net primary producer over a 24 h cycle. Sponge assemblages in near-reef-flat zone, the area where autotrophic sponges dominate the assemblage by more than 80%, produced more carbon than they respired (Table 3.5). Meanwhile, in the high- and middle-shore zone of the seagrass meadow, where the proportion of autotrophic sponges to the assemblage was about 40–70%, the sponge assemblage was estimated to be a net carbon consumer.

The sponge assemblage in the near-reef-flat zone of Hoga-1 and -2 produced 0.15 ± 0.04 and 0.25 ± 0.05 mol C m^2 d^{-1}, respectively. For comparison, the average primary production of seagrass meadows has been estimated to be approximately 0.45 mol C m^2 d^{-1} (e.g. Yarbro and Carlson 2008; Reynolds et al. 2018). This means that the primary productivity mediated by the sponge assemblage could reach up to 50% of seagrass bed total productivity, which may have result in an overestimation of the sponge-holobiont's productivity. For example, both photosynthesis and respiration are actually changing over the course of 24 h as a result of light

intensity and temperature changes. Another caveat is the depletion of oxygen and food availability during the incubation, which may influence sponge metabolic rates over the course of the incubation. Nevertheless, this finding highlights the importance of sponges to energy flow and the potential magnitude of its impact in the seagrass meadow. Wilkinson (1983) reported that all the autotrophic sponges on the Great Barrier Reef released 1–5% of the carbon fixed by symbionts as dissolved organic carbon to the water column, by direct carbon measurements. If the autotrophic sponges I studied also have a positive carbon flux (i.e. release more carbon than they take up), which still needs to be confirmed by direct measurement of carbon fluxes (not only from oxygen fluxes), then we need to revise our views on the role of sponges in the flow of organic matter in seagrass ecosystems.

Photosynthetic pigments in sponges

Many studies have used the concentration of chlorophyll-*a* as a predictor of sponge gross productivity (Wilkinson 1983; Erwin and Thacker 2008), since chlorophyll-*a* is the only light-absorbing pigment that is present in both photosystems (antennas and reaction centres), and is considered to be the principal photosynthetic pigment (Lodish et al. 1999). In contrast, chlorophyll-*b* and carotenoids are considered 'accessory' pigments since they are not directly involved in the energy conversion process (Bullerjahn and Post 1993; 1999). Chlorophyll-*b* and carotenoids support photosynthesis by extending the light spectrum (Smith and French 1963), and carotenoids in particular, also protect photosynthetic organisms from the photodegradation caused by any excess excitation energy (Foote 1968; Frank and Brudvig 2004). In my study, I found that all eight sponge species contained chlorophyll-*a*, but not every sponge had chlorophyll-*b* and carotenoids (see Figure 3.2). This suggests that the sponge symbionts in the different sponge species are utilizing different light wavelengths for photosynthesis. Based on the assumption that the sponge species experienced the same light conditions in the seagrass meadow, one possible reason for the different dominating photosynthetic pigments is that the sponges harbour different photosynthetic symbionts. Sponges are known to host various photosynthetic symbionts, and although most of the sponge species have cyanobacteria as their photosymbionts (Rützler 1985; Usher 2008), there are some sponge species that host dinoflagellates (i.e. *Symbiodinium* spp.; Schönberg and Loh 2005; Hill et al. 2011), as well as rhodophytes, chlorophytes, and diatoms (Rützler 1985; Taylor et al. 2007; Lemloh et al. 2009). Unfortunately, I did not identify the specific photosynthetic symbionts harboured in my studied sponges, although I recognise that the symbiont type is likely to impact P:R ratios. However,

based from literature for related or similar species, cyanobacteria are the likely photosymbionts in *Phyllospongia foliascens*, *Dactylospongia elegans*, and *Axinella corrugata*, and *Clathria styloprothesis* (Wilkinson 1988; Keesing et al. 2012; White et al. 2012; Gao et al. 2017); and dinoflagellates most likely in *Spheciospongia* sp. and *Haliclona koremella* (Alvin et al. 2000; Sacristán-Soriano et al. 2020).

Chlorophyll-*a* concentrations of the seagrass sponge assemblage in my study was within a much lower range than reported in coral reef sponges. The higher light intensity in the seagrass meadow, compared to coral reefs, as they are shallower may explain the much lower chlorophyll-*a* concentration in the seagrass sponge assemblage. Erwin and Thacker (2007) reported from a Caribbean coral reef sponge assemblage that 33% of the sponges (20 sponge species) had a chlorophyll-*a* concentration higher than 125 μg g^{-1}, and three sponges had a concentration higher than 50 μg g^{-1}. Since these earlier authors considered a chlorophyll-*a* concentration of 50 μg g^{-1} as the threshold for photosynthetically active sponges, they concluded that 37% of the sponges (22 out of 60 sponges) were photosynthetically active. However the term "photosynthetically active" might not mean the sponge species are autotrophic, as P:R ratios are needed to confirm this. From my study, the two sponges *Axinella* sp. and *Clathria reinwardti* had chlorophyll-*a* concentrations below 10 μg g^{-1}, although they were actually autotrophic sponges based on their P_N and daily budgets.

In addition to the abundance of photosynthetic symbionts, another important factor that influences the contribution of symbionts to sponge nutrition is the 'quality' of the symbioses. Freeman et al. (2013) suggested that the photosynthetic capacity of the hosted photosynthetic symbionts has a greater effect on the nutritional transfer from microbial symbionts to sponge host (i.e. P:R ratio) than the abundance of the symbionts. This might explain the stark difference in gross productivity and P:R ratio of *Haliclona koremella* compared with the other studied species, even though its pigment concentration was similar to that of the other autotrophic sponges. The chlorophyll-*a* and total pigment concentration of *Haliclona koremella* was similar to *Phyllospongia foliascens*, which had the second-highest gross productivity in the assemblage. However, the gross productivity of *Haliclona koremella* was about five times higher than *Phyllospongia foliascens*. There are several explanations for this difference. First, the symbiont phylotype of *Haliclona koremella* is different from the other species; second, there are some characteristics (physical, chemical, or biological) of the host-sponge that support symbiont photosynthetic capacity or; a combination of those two explanations. For *Haliclona*

koremella, the sponge has a ramose (bushy) growth form giving it a large surface area to body ratio (Table 3.2), which likely enhances its ability to capture sunlight for photosynthesis. Furthermore, we always found *Haliclona koremella* sitting on the top of seagrass canopy, without anything blocking the sunlight reaching the sponge.

It is important to note that the P:R ratio itself is a relative comparison between the primary production and respiration. While the photosynthetic symbionts are responsible entirely for the gross primary production, the components of the sponge holobiont are responsible for the holobiont's respiration. Thus, using respiration rate as the ratio's denominator could give different P:R ratios for two or more sponges with similar net productivity. For example, *Dactylospongia elegans* and *Clathria reinwardti* had similar net primary productivity (0.029 ± 0.012 and 0.028 ± 0.007 mol g_{AFDW}^{-1} h^{-1} respectively), but the respiration rate of *Clathria reinwardti* was approximately double the respiration rate of *Dactylospongia elegans*. As a result, the P:R ratio of *Dactylospongia elegans* was double that of *Clathria reinwardti*, whereby their P:R ratios were 2.02 ± 0.52 and 0.92 ± 0.25, respectively. From the extrapolation of gross production (P_G) and respiration (R_D) to the daily budget of the sponges, the photosynthesis by the symbionts of *Clathria reinwardti* were able to potentially provide ~100% of the sponge holobiont daily respiration requirement, while the photosynthetic symbionts in *Dactylospongia elegans* potentially provided the host with approximately 150% of its daily carbon requirement. These differences in respiration rates between species are also likely to be influenced strongly by the overall microbial community living within the sponge. Assuming that all the sponges experienced the same light intensity and temperature, it confirms that the quantity (i.e. photosymbionts concentration) and quality (i.e. photosynthetic capacity) of the photosynthetic symbionts are important for P_G (Freeman et al. 2013).

Conclusion

The seagrass sponge assemblage that I studied at Hoga Island is dominated by sponges containing photosynthetic symbionts, with six of the eight sponge species being net oxygen producers over a 24-hour period. However, based on the biomass, autotrophic sponges only dominated at the near-reef-flat zone. The domination of autotrophic sponges in the near-reef-flat zone likely results in a positive daily carbon flux by the sponge assemblage with the sponge assemblage in this habitats being a net primary producer. The contribution of sponge-mediated

primary production needs to be carefully considered when estimating overall habitat primary productivity.

CHAPTER 4.

Contribution of autotrophic and heterotrophic sponges to carbon flux in a shallow Indonesian seagrass bed

Abstract

Understanding the carbon flow through marine ecosystems is important because it drives population dynamics and overall productivity. Sponges are major components of benthic marine communities that play important roles in benthic carbon cycling. However, while sponges have generally been considered net consumers of carbon from the water column, this might not be the case where autotrophic sponges dominate sponge assemblages. In this study, I investigated the flow of carbon between sponges and the water column during the day and in darkness (simulating night-time) at three tidal zones in a seagrass sponge assemblage, which comprised both autotrophic and heterotrophic sponges. I measured the sponge-mediated net flux (uptake or release) of total organic carbon (TOC) using *in situ* incubation chambers in a seagrass meadow at Hoga Island in the Wakatobi National Park, Indonesia. I investigated the five most abundant sponges (representing 75.1–99.8 % of total sponge biomass) at my study site. The sponges included two heterotrophic sponges (*Spongia* sp. and *Amphimedon* sp.) and three autotrophic sponges (*Spheciospongia* sp., *Phyllospongia foliascens*, and *Haliclona koremella*). Consistent with previous studies, I found that all sponges removed carbon in darkness, but at species-specific rates. There was no significant difference in sponge-mediated carbon net-flux observed between the heterotrophic and autotrophic sponges during the night ($p > 0.05$). However, during the day, the autotrophic sponges were net producers of carbon, reducing the overall daily carbon removal by the sponge assemblage from the water column, while the heterotrophic sponges continued to remove organic carbon from the water column. At the assemblage level, over a 24 hour period the seagrass sponge assemblage removed more carbon than they produced. This chapter challenges the view that sponges are primarily involved in removing large amounts of carbon from the water column. In some ecosystems they may actually provide substantial carbon to the water column during day time. My results have implications for energy transfer through food webs in marine ecosystems and the way we view the roles of sponges in these systems.

Introduction

Marine ecosystems worldwide are under tremendous environmental pressure and are declining at an alarming rate (Duarte et al. 2008; Wilkinson 2008; Boyce et al. 2010; Hamilton and Casey 2016; Ramírez et al. 2017). The depletion or loss of any specific group of organisms from an ecosystem is likely to result in considerable environmental disruption as each group is likely to play a particular functional role (Chassot et al. 2010; Estes et al. 2011). Of particular concern are changes to food webs (Smith and Smith 2015), as such changes have the potential to alter the transfer of energy across multiple trophic levels (Krebs 2009). Therefore, understanding the feeding interactions for key components of different ecosystems is critical for understanding how ecosystem dynamics might be impacted by environmental change.

Sponges are a major component of benthic ecosystems worldwide, where they have many important functional roles (Bell 2008; Bell et al. 2015). Sponges are sessile suspension feeders that can pump large quantities of water and filter out small particles (Reiswig 1971a; Reiswig 1974). For example, Southwell et al. (2008) reported that a 109 L sized barrel sponge, *Xestospongia muta* could pump 24,000 L of water in an hour; and a 1.1 L sized brown tube sponge, *Agelas conifera* could pump water through its body at the rate of 280 L h^{-1}. This high rate of water flow through sponges, coupled with the high abundance of sponges in some habitats, means that they have the potential to exert major regional-scale impacts on biogeochemical cycles and food webs (Maldonado et al. 2015).

As a result of global climate change, which is causing ocean warming (OW) and ocean acidification (OA), some coral reef sponges have been proposed as potential 'winners' in near-future climate scenarios as they are generally less affected by the OW and OA than many other benthic organisms. Any such shift might result in a change to the overall benthic community structure (Bell et al. 2018a). Bell et al. (2018b) considered the potential ecological impacts resulting from a regime shift to sponge-domination, and as a result identified the need to understand how such shifts may alter sponge-mediated carbon fluxes, along with potential food limitation for sponges. This information will enable a better understanding of how sponge-dominated reefs might persist and continue to function. More recently, Lesser and Slattery (2020) have argued that a regime shift to sponge-dominated reefs is unlikely due to sponge food limitation. These researchers suggest that under the predicted climate change scenarios, the physical oceanography of coral reefs is likely to change and cause a decrease in net primary production (NPP), picoplankton abundance and phytoplankton-derived dissolved organic

matter (DOM), ultimately limiting sponge populations. However, sponges have other means of fulfilling their nutritional requirements.

In order to meet their energy requirements, sponges can feed on particulate and dissolved organic carbon (e.g. Hadas et al. 2009; Hoer et al. 2018) or rely on carbon produced by their symbionts (e.g. Wilkinson 1983; Erwin and Thacker 2008). With respect to heterotrophy on particulate organic carbon (POC), sponges have been observed to consume both live particulate organic carbon (ultraplankton; e.g. Pile et al. 2003; Yahel et al. 2006) and non-living particulate organic carbon (detritus; Hadas et al. 2009). Furthermore, Hadas and Marie (2006) also reported that the coral reef sponge *Negombata magnifica* could remove virus particles at an average efficiency of 23.3 ± 2.9 %, although the fate of these particles still remains unclear. Dissolved organic carbon (DOC) is the largest exchangeable reservoir of carbon in the marine environment (Druffel et al. 1992; Hansell and Carlson 2014), and until recently, it was thought that only bacteria were able to consume DOC (Carlson 2002). However, a number of studies have reported that DOC can account for more than 90% of the total organic carbon (TOC) removed by some sponges (e.g. Yahel et al. 2003; de Goeij et al. 2008). More recently, Hoer et al. (2018) reported that three of the seven studied tropical sponges from the Caribbean could remove between 13–24 % of the available DOC, suggesting DOC is likely removed at species-specific rates, and that not all sponges consume DOC.

Several authors have hypothesized that the microbes associated with sponges are involved in DOC uptake by sponges (e.g. Reiswig 1981; Hoer et al. 2018). Sponges have long been known to have critical associations with microbes, and these microbes can constitute up to 40–60 % of the tissue volume (Vacelet and Donadey 1977; Willenz and Hartman 1989). Thus, sponges and their associated microorganisms interact with the environment as one entity, known as a holobiont (see Simon et al. 2019). Based on the density of the hosted microbes, sponges can be categorized as either high-microbial abundance (HMA) or low-microbial abundance (LMA; Vacelet and Donadey 1977; Wilkinson 1978). There is growing evidence that HMA sponges consume more DOC than LMA sponges. For example, de Goeij et al. (2008) noted that the three studied sponges that removed significantly more DOC from the water column contained microbes, even though the study did not differentiate DOC consumption by the host sponge and its associated microbes. In a more recent study on DOC consumption by seven Caribbean reef sponges, all HMA sponges (four sponges) removed a large amount of DOC; whereas all LMA sponges (three sponges) had very low DOC removal rates (Hoer et al. 2018).

Not all sponges obtain their nutritional requirements from exclusively feeding on POC and DOC in the water column. Many sponges have also established symbioses with photosynthetic organisms and gain supplemental carbon from their symbionts (Wilkinson 1983). Wilkinson (1983) defined sponges that fulfil at least 50% of their total energetic requirement from their hosted photosymbionts as autotrophic sponges. Photosymbiont-containing sponges have been found in tropical waters across the globe (see Bell et al. 2020). For example, in an earlier study six out of the ten most common sponges on the Great Barrier reef (Indo-Pacific) were found to be net primary producers (Wilkinson 1983). Steindler et al. (2002) reported that 64 % and 85 % of the sponges that live in the intertidal and subtidal zone in Zanzibar (West Indian Ocean) respectively, hosted photosynthetic symbionts. Furthermore, on Panamanian reefs (tropical West Atlantic), Erwin and Thacker (2007) reported that one-third of the studied sponges (20 species) had high photosynthetic-symbiont abundance. The evolution of these symbioses between sponges and photosymbionts has been considered important in supporting sponge survival in oligotrophic environments (Wilkinson 1987a; Wilkinson and Cheshire 1990) and may become more important in the future if the availability of POC decreases.

Sponges are major benthic components of seagrass meadows (e.g. Ogden and Ogden 1982; Barnes 1999), although seagrass sponge diversity is generally lower than on adjacent coral reefs (Barnes and Bell 2002; Díaz 2005). For example, from my sponge surveys in the Wakatobi National Park (Southeast Sulawesi, Indonesia), only ten sponge species inhabited the seagrass bed (Chapter 2). In contrast, on adjacent coral reefs, more than 140 sponge species have been recorded (Rovellini et al. 2019). A similar ratio was also observed in the Seribu Islands National Park, north of Jakarta, where 13–15 sponges were found in the seagrass beds (Ismet et al. 2017), compared to 118 sponge species on the surrounding coral reefs (de Voogd and Cleary 2008). However, despite the lower sponge diversity in seagrass beds, many species have high biomass. My sponge surveys in Wakatobi National Park (Chapter 2) estimated that sponge biomass (in volume) was between 3000 – 7500 L in seagrass bed areas of between 0.151 km^2 and 0.213 km^2. With this level of biomass, sponges are likely to have a major impact on ecological processes in seagrass beds.

Given the different nutritional modes, autotrophic and heterotrophic sponges might be expected to have different interactions with the water column, particularly during the daytime when light for photosynthesis is available. In this study, I conducted *in situ* incubations of the sponges inhabiting a seagrass bed in Wakatobi National Park, Southeast Sulawesi, Indonesia. My aims

were to: (1) measure the sponge-mediated carbon net flux in dark and light incubations (simulating night- and daytime) by autotrophic and heterotrophic seagrass sponges to assess how sponges with different nutritional modes impact water column carbon dynamics; and (2) assess the importance of the overall seagrass sponge assemblage, that comprises both hetero- and autotrophic sponges, in daily carbon fluxes.

Methods

Study area and sponge collection

The study was conducted at Hoga Island in the Wakatobi National Park, Indonesia (5° 28' S, 123° 4" E). Sponge incubations were performed *in situ* in the middle of a seagrass meadow on the island's west side, where the tidal range was between 0.6 and 1.9 m, with a mid-tide depth of 1.2 m. The seagrass meadow of Hoga Island is a multispecies seagrass bed, inhabited by eight seagrass species. I found *Cymodecea rotundata*, *Cymodecea serrulata*, *Enhalus acoroides*, *Halophila ovalis*, *Halophila minor*, *Syringodium isoetifolium*, *Thalassia hemprichii*, and *Halodule univervis* in the seagrass meadow surrounding Hoga Island (Chapter 2). The seagrass meadow had a 1.8 ± 0.1 mm fine sand substrate (Unsworth et al. 2018).

I studied five sponge species: two heterotrophic sponges (*Spongia* sp. and *Amphimedon* sp.) and three autotrophic sponges (*Spheciospongia* sp., *Phyllospongia foliascens*, and *Haliclona koremella*). I based the assessment of whether a sponge was heterotrophic or autotrophic on earlier observations (Chapter 3). If photosynthesis provided at least 100% of the sponge's daily respiration needs, the sponge was considered autotrophic. From my earlier sponge surveys (Chapter 2), the total abundance of these five studied sponges represented 99.8%, 75.4% and 80.7% of the total sponge biomass at the high-shore, middle, and near-reef-flat zones of Hoga-1, respectively; and in Hoga-2 was 75.1%, 82.2%, and 90.1% of the total sponge biomass at the high-shore, middle, and near-reef-flat zones (see Table 4.1 for the detailed sponge abundance in ash-free dry-weight). The seagrass meadow zonation was based on the distance to shore, where the average depth during high tide at the high-shore, middle, and near-reef-flat zones was 0.5, 1.2, and 1.9 m, respectively.

Table 4.1. P:R ratio and sponge biomass of the studied sponges in the seagrass meadow of Hoga Island, Wakatobi National Park, Indonesia. The seagrass meadow was divided into three zones: high-shore zone (HS), middle zone (M) and near-reef-flat zone (NRF). Values are mean ± SE, and the unit for sponge biomass is g_{AFDW} m^{-2}. Source: Chapters 2 and 3.

Sponge	P:R ratio	Hoga-1			Hoga-2		
		HS (0.0256 Km^2)	M (0.0759 Km^2)	NRF (0.0492 Km^2)	HS (0.0385 Km^2)	M (0.1365 Km^2)	NRF (0.0378 Km^2)
Heterotrophic sponges							
Spongia sp.	0.51 ± 0.33	0.33 ± 0.01	0.17 ± 0.09	0	0.07 ± 0.01	0.22 ± 0.05	0.07 ± 0.05
Amphimedon sp.	0.42 ± 0.20	0.02 ± 0.02	0.08 ± 0.02	0.01 ± 0.004	0	0.16 ± 0.16	0.09 ± 0.04
Autotrophic sponges							
Spheciospongia sp.	2.13 ± 0.79	0.55 ± 0.42	0.0023 ± 0.0023	0.07 ± 0.07	0.12 ± 0.05	0.59 ± 0.34	0.38 ± 0.20
Phyllospongia foliascens	3.24 ± 0.34	0	0.34 ± 0.14	0.40 ± 0.23	0	0.05 ± 0.05	0.43 ± 0.12
Haliclona koremella	4.85 ± 0.64	0	0.0042 ± 0.0042	0	0	0.03 ± 0.03	0.06 ± 0.05

I collected ten separate sponges of each species in a non-destructive way to maintain the integrity of the sponge holobiont (i.e. care was taken not to cut or damage the sponge tissue) and moved them to my working station without exposing them to air. I used different size ranges for each species as the sponges had species-specific natural size ranges. The average sizes of *Spongia* sp. and *Spheciospongia* sp. that I used were 453 ± 127 ml and 355 ± 125 ml, respectively. The average size of *Amphimedon* sp. was 202 ± 73 ml, while the average sizes of *Phyllospongia foliascens* and *Haliclona koremella* were much smaller than the other species: 78 ± 7 ml and 134 ± 9 ml, respectively. The sponge volume was measured using the water displacement method at the end of the incubations (after Rützler 1978). Depending on sponge size and morphology, I put the sponge in a graduated cylinder or measuring cup and filled it to the top with seawater to its maximum scaled volume. Then, I took the sponge out and measured the volume loss as the sponge's volume.

Before I ran any incubations, sponges were acclimatized for four weeks at my working station. I then chose five sponges of each species that showed no signs of necrosis (i.e. they were healthy sponges) for the incubations.

Study design

Here I focused on the total flux of organic carbon due to sponge interactions with the water column. The total organic carbon (TOC) is composed of particulate organic carbon (POC) and dissolved organic carbon (DOC), which is operationally defined based on whether it can pass through a 0.45 μm filter or not (Rice et al. 2017). I measured the net organic carbon flux of the seagrass sponges *in situ* using six incubation chambers, where for a series of measurements, five chambers were used for sponges, and one chamber acted as a control.

I measured the TOC flux for all five sponge species (n = 5) under light and dark conditions to represent daytime and night-time. The measurement for each sponge species was conducted in three batches over three days to capture intra-day variability. The ambient concentration of TOC and DOC in the seagrass bed was also measured. I took the water samples from the middle part of the seagrass meadow, about 180 m from shore, at mid-tide during the daytime on three different days (n = 3). The ambient organic carbon concentration was considered as the food available to sponges.

To determine how much of each TOC component (i.e. POC plus DOC) was being removed by the sponges during their feeding, I also measured the flux of DOC. This was measured by evaluating the DOC flux in dark incubations only, when all sponges exclusively gain their nutritional needs from suspension feeding. The DOC flux was only measured for the two sponge species with the highest biomass at my study site, *Spheciospongia* sp. and *Phyllospongia foliascens* (n = 3).

Incubation chambers

The incubation chambers were made from 10-litre polyethylene plastic bags fixed to the seafloor of the seagrass bed (after Hansen et al. 2000). The chambers had a sampling port with a three-way valve to collect the water samples with a syringe. I used transparent plastic bags for the light incubations and black plastic bags for the dark incubations. The flexibility of the plastic bags allowed the physical force created by any movement in the water column to be

passed into the chamber and naturally agitate the water inside the chamber (Hansen et al. 2000; Camp et al. 2015).

Incubation duration and time

I ran a preliminary experiment with three sponge species, *Spongia* sp., *Spheciospongia* sp., and *Phyllospongia foliascens* (n = 5) to determine an appropriate incubation duration based on chamber size and oxygen depletion over time. It was suggested by Muscatine (1980) to have the incubation time no longer than two hours and oxygen depletion not exceeding 25% of normal oxygen levels to avoid animal stress. I found that a combination of 10-litre chambers and 72-minute incubations resulted in 10–15% oxygen depletion.

I ran the dark incubations in the morning starting at 7 AM when the sunlight intensity was still low, and between 11 AM and 2 PM for the light incubations. I installed Hobo pendant loggers inside the chambers (one logger for each chamber) as well as outside the chamber (on the seafloor at an average depth of 1.2 m) and recorded the light intensity during incubations every minute. During the dark incubation, the ambient light intensity in the seagrass bed ranged between 400–800 μmol s^{-1} m^2, while it was totally dark (i.e. zero lux) inside the black chambers. The average light intensity at the seagrass bed recorded during light incubation was about 1400 μmol s^{-1} m^2. The light intensity inside the light chambers was in the same range as outside the chambers, indicating that the chambers did not reduce the light level experienced by the sponges in the chambers.

Net TOC and DOC fluxes

The sponges were placed into the chambers (one sponge holobiont in one chamber) and acclimated for 10 minutes before taking initial water samples. I then withdrew 100 ml of water from the chamber with a syringe, transferred it into an amber glass bottle with a silicon-Teflon cap, and stored it in a cooler box until it could be processed (30 minutes max after the water samples were taken). Finally, another water sample was collected in the same way at the end of the incubation as the final water carbon concentration.

The sponge-mediated flux of TOC and DOC were measured based on the change between the initial and final water samples taken from the chambers and then normalized with the flux from the control chamber. I then calculated the removal or release efficiency (*RE*; as a percentage)

and rate (*RR*; the actual amount of carbon) by standardizing the flux to one hour with the sponge ash-free-dry-weight (AFDW). Thus, a positive final flux value represents organic carbon removal and a negative value organic carbon release. Finally, I used the controls to measure the natural organic carbon flux in the water column resulting from phytoplankton and microbial activity. The formulae to calculate the flux of organic carbon is as follow:

$$\text{Carbon flux} = (C_{Sp0} - C_{Sp1}) - (C_{ctrl\text{-}0} - C_{ctrl\text{-}1})$$

Where :

C_{Sp0} : Initial organic carbon concentration of the sponge-containing chamber

C_{Sp1} : Organic carbon concentration of the sponge-containing chamber at the end of incubation

$C_{ctrl\text{-}0}$: Initial organic carbon concentration of the control chamber

$C_{ctrl\text{-}1}$: Organic carbon concentration of the control chamber at the end of incubation

To determine the significance level of the sponge-mediated organic carbon flux, one-tailed independent t-test was performed against zero flux (i.e. no removal or release), with the assumption of unequal variance between the sponge-mediated flux and zero flux. The sponge-mediated organic flux is considered significantly different to zero flux if the P-value was lower than 0.05 ($p < .05$). For further analysis, I only used significantly different fluxes.

I also performed an independent one-tailed t-test to determine if there was a significant difference between the heterotrophic and autotrophic sponges for their mediated organic carbon flux, separately, for dark and light incubations. Before running the t-test, I performed the F-test to determine if the two sample variances was equal (homoscedastic) or unequal (heteroscedastic). The F-test result confirmed the assumptions of the t-test. The same procedures also run to determine the significance level of the sponge-mediated organic carbon flux between dark and light incubations for each sponge species.

Concentration of Total Organic Carbon, Dissolved Organic Carbon, and Particulate Organic Carbon

Water samples for determining total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations were processed immediately after collection before being transported to Jakarta, Indonesia, for analysis. Samples were processed following Rice et al. (2017) for sample handling, processing, and analysis of TOC and DOC concentrations. For DOC samples, the water was filtered through a PTFE 0.45 μm syringe filter. The TOC and DOC samples were subsequently acidified with 38% HCl (analytical HCl) to reduce the pH to below 2 (pH < 2). The samples were kept at 4^{o}C in a dark container until analyses. I determined the TOC and DOC concentration with the high-temperature combustion method using the Lotix TOC Combustion analyzer (Teledyne Tekmar). The concentration of particulate organic carbon (POC) was calculated by subtracting the TOC concentration from the DOC concentration, assuming that TOC is composed of DOC and POC (TOC = DOC + POC).

Contribution of POC and DOC to the TOC removed

Since the TOC is composed of DOC and POC (i.e. TOC = DOC + POC), the removed POC and DOC portion were calculated with the following formulae:

$$DOC\ portion = \frac{Ingestion\ rate\ of\ DOC}{Ingestion\ rate\ of\ TOC} \times 100\%$$

$$POC\ portion = \left(1 - \frac{Ingestion\ rate\ of\ DOC}{Ingestion\ rate\ of\ TOC}\right) \times 100\%$$

Sponge influence on the carbon cycling in the seagrass meadow

The contribution of the sponge assemblage to carbon cycling in the seagrass meadow was determined based on how much organic carbon in the water column was removed or released by the sponges (in mol C). The water column volume was calculated based on the maximum depth during high tide at each tidal zone, multiplied by the total seagrass meadow zonal area from my previous study (Chapter 2). I calculated the contribution of each sponge species using the sponge biomass estimates from my previous study (summarized in Table 4.1) and based on

the assumption of a 12h light - dark period. I used the following formulae to calculate the contribution of each sponge species to the removal or release of carbon to the water column:

$$Total\ carbon\ removed = Removal\ or\ Release\ Efficiency\ \times Ambient\ TOC\ \times Water\ volume \times\ Sponge\ biomass\ density\ \times 12h$$

$$Sponge\ contribution\ (\%) = \left(\frac{Total\ carbon\ removed\ or\ released}{Ambient\ Carbon\ concentration\ \times\ Water\ volume}\right)$$

Results

Natural state of organic carbon in the water column

At my study site, TOC and DOC ambient concentrations were 141.4 ± 21.9 and 119.8 ± 20.4 µmol C L^{-1}, respectively. Using these figures, I calculated that the POC concentration was about 6 µmol C L^{-1}, and the TOC comprised 85% DOC and 15% POC. I observed carbon flux in the water column from the control incubations during the light and dark. Under light incubations, the TOC concentration decreased at an average rate of 3.49 ± 0.55 % C h^{-1}, while the rate of TOC depletion was more than double under dark incubations at a rate of 8.28 ± 1.42 % C h^{-1}. I also found that about 90% of the depleted TOC in dark incubation was DOC (Table 4.2).

Table 4.2. Water column carbon flux in the seagrass meadow of Hoga Island, Wakatobi National Park, Indonesia from the control chambers. The volume of the incubation chambers was 10-litres. Values are mean ± SE. NA denotes data is not available.

	Day		Night	
	TOC	DOC	TOC	DOC
Removal rate (µmol C h^{-1})	55.3 ± 7.2	NA	97.6 ± 6.2	87.4 ± 26.3
Removal efficiency (% C h^{-1})	3.49 ± 0.55	NA	8.28 ± 1.42	8.9 ± 1.7

Organic carbon removal and/or release rate and efficiencies

With the exception of *Haliclona koremella* ($t(4) = 2.6$, $p = .061$), all sponges significantly reduced the TOC concentration in the chambers in the dark incubations ($p < .05$) but at species-specific rates and efficiencies. No significant difference was observed in sponge-mediated TOC flux between the heterotrophic and autotrophic sponges ($t(14) = 0.13$, $p = .449$) in dark incubations. The autotrophic sponge *Phyllospongia foliascens* had the highest removal efficiency among the five sponges with a removal efficiency of 3.26 ± 0.31 % C g_{AFDW}^{-1} h^{-1}, while the lowest removal efficiency was found for *Spheciospongia* sp., another autotrophic sponge, with a removal efficiency of 0.58 ± 0.28 % C g_{AFDW}^{-1} h^{-1} (Table 4.3).

Table 4.3. Organic carbon removal or release rate (*RR*) and efficiency (*RE*) for seagrass sponges at Hoga Island in the Wakatobi National Park - Indonesia. A positive value represents carbon removal, while a negative value indicates the release of carbon. Values are mean ± SE, and the unit for *RR* is µmol C g_{AFDW}^{-1} h^{-1}, while the *RE* is in % C g_{AFDW}^{-1} h^{-1}. NA denotes data is not available; and NS denotes flux is not significant.

Sponge	Day		Night			
	TOC		TOC		DOC	
	RR	*RE*	*RR*	*RE*	*RR*	*RE*
Heterotrophic						
Spongia sp.	6.5 ± 2.1	0.70 ± 0.23	17.8 ± 5.2	1.83 ± 0.78	NA	NA
Amphimedon sp.	NS	NS	24.5 ± 15.0	1.58 ± 0.73	NA	NA
Autotrophic						
Spheciospongia sp.	-14.4 ± 6.6	-0.91 ± 0.42	9.4 ± 4.3	0.58 ± 0.28	5.3 ± 3.6	0.37 ± 0.26
Phyllospongia foliascens	-42.3 ± 22.5	-2.52 ± 1.26	34.6 ± 19.9	3.26 ± 0.31	3.2 ± 2.5	0.22 ± 0.17
Haliclona koremella	-17.6 ± 7.3	-2.01 ± 0.62	NS	NS	NA	NA

In light incubations, I observed a significant sponge-mediated TOC flux for all sponges ($p < 0.05$), except for *Amphimedon* sp. ($t(4) = 5.3$, $p = 0.059$). In contrast with the observations in the dark incubations, I observed a significantly different pattern in TOC flux between autotrophic and heterotrophic sponge species in the light incubations ($t(23) = 4.6$, $p < 0.001$). While the heterotrophic sponges showed organic carbon removal, the autotrophic sponges (*Spheciospongia* sp., *Phyllospongia foliascens*, and *Haliclona koremella*) increased the TOC concentrations. The TOC removal efficiency of *Spongia* sp. was 0.70 ± 0.23 % C g_{AFDW}^{-1} h^{-1}.

Meanwhile, the TOC production efficiency of *Spheciospongia* sp., *Phyllospongia foliascens*, and *Haliclona koremella* were -0.91 ± 0.42, -2.52 ± 1.26, and -2.01 ± 0.62 % C g_{AFDW}^{-1} h^{-1}, respectively.

Even though the heterotrophic sponges were observed to remove more TOC from the water column during dark incubations than light incubations, the removal rates and efficiencies were not significantly different for *Spongia* sp. ($t(8) = -1.4$, $p = 0.103$) and *Amphimedon* sp. ($t(8) = -1.0$, $p = 0.202$). See Table 4.3 for the sponge removal rates and efficiencies.

POC and DOC contribution to the total removed TOC

I examined the DOC removal by the autotrophic sponges *Spheciospongia* sp. and *Phyllospongia foliascens* only in dark incubations to determine the composition of the removed TOC without the influence of photosynthesis. The DOC removal efficiency of the two sponges were 0.37 ± 0.26 and 0.22 ± 0.17 % C g_{AFDW}^{-1} h^{-1}, respectively. Based on the actual amount of the DOC removed (i.e. removal rate), the DOC removal accounted for 9% and 56% of the total TOC removed by *Phyllospongia foliascens* and *Spheciospongia* sp., respectively.

Assemblage level sponge-mediated carbon flux

At the assemblage level, the studied seagrass sponge assemblage comprised of both heterotrophic and autotrophic sponges were estimated to remove carbon during night-time (represented by dark incubations), but released carbon in day time (represented by light incubations) across all tidal zones at both sites. In all studied areas, the amount organic carbon removed during night-time (12 h) was greater than the release in day-time (12 h), resulting in net carbon removal by sponges (over 24 h). See Table 4.4 for full results on the estimation of the sponge-mediated carbon flux in the studied seagrass meadow.

Table 4.4. The amount of organic carbon removed from the water column by the seagrass sponges per 1 m^2 at three tidal zones of the seagrass bed, expressed as the actual amount (mol C m^{-2}) and percentage (in the brackets) relative to the available organic carbon. Values are in means ± SE (n = 5).

Sponge	Hoga-1					
	HS		M		NRF	
	Day	Night	Day	Night	Day	Night
Heterotrophic sponges						
Spongia sp.	25.0 ± 1.1 (2.8 ± 0.1 %)	65.3 ± 2.8 (7.2 ± 0.3 %)	130.3 ± 67.2 (1.4 ± 0.7 %)	340.7 ± 175.7 (3.7 ± 1.9 %)	0	0
Amphimedon sp.	0	3.1 ± 3.1 (0.3 ± 0.3 %)	0	136.8 ± 33.0 (1.5 ± 0.4 %)	0	4.9 ± 2.6 (0.2 ± 0.1 %)
Autotrophic sponges						
Spheciospongia sp.	-54.5 ± 41.5 (-6.0 ± 4.6)	34.7 ± 26.5 (3.8 ± 2.9 %)	-2.3 ± 2.3 (~0 %)	1.4 ± 1.4 (~0 %)	-22.7 ± 22.5 (-0.8 ± 0.7 %)	18.8 ± 18.8 (0.6 ± 0.6)
Phyllospongia foliascens	0	0	-931.0 ± 393.5 (-10.2 ± 4.3 %)	1204.4 ± 509.1 (13.2 ± 5.6 %)	-365.2 ± 207.7 (-12.0 ± 6.9)	614.1 ± 349.3 (20.3 ± 11.5)
Haliclona koremella	0	0	-9.2 ± 9.2 (-0.1 ± 0.1 %)	0	0	0
Total	-29.5 ± 41.6 *(-3.3* ± 4.6 %)	103.2 ± 26.8 *(11.4* ± 3.0)	-812.2 ± 399.3 *(-8.9* ± 4.4 %)	1683.3 ± 539.5 *(18.4* ± 5.9 %)	-387.9 ± 208.9 *(-12.8 ± 6.9 %)*	637.8 ± 349.8 *(21.0 ± 11.5 %)*
TOTAL DAILY (24 h)	**73.7 ± 49.5** ***(8.1 ± 5.5 %)***		**871.1 ± 671.2** ***(9.5 ± 7.4 %)***		**249.9 ± 407.4** ***(8.2 ± 13.4 %)***	

Sponge	Hoga-2					
	HS		M		NRF	
	Day	Night	Day	Night	Day	Night
Heterotrophic sponges						
Spongia sp.	7.6 ± 0.6 *(0.6 ± 0.0 %)*	19.8 ± 1.6 *(1.5 ± 0.1 %)*	302.4 ± 71.6 *(1.8 ± 0.4 %)*	790.6 ± 187.2 *(4.8 ± 1.1 %)*	84.5 ± 58.1 *(0.6 ± 0.4 %)*	221.0 ± 152.0 *(1.6 ± 1.1 %)*
Amphimedon sp.	0	0	0	487.7 ± 487.7 *(3.0 ± 3.0 %)*	0	250.3 ± 94.6 *(1.8 ± 0.7 %)*
Autotrophic sponges						
Spheciospongia sp.	-18.2 ± 8.0 *(-1.3 ± 0.6 %)*	11.6 ± 5.1 *(0..9 ± 0.4 %)*	-1054.2 ± 609.1 *(-6.4 ± 3.7 %)*	671.9 ± 388.2 *(4.1 ± 2.4 %)*	-587.7 ± 304.8 *(-4.1 ± 2.1 %)*	374.6 ± 194.3 *(2.6 ± 1.4 %)*
Phyllospongia foliascens	0	0	-252.5 ± 252.5 *(1.5 ± 1.5 %)*	326.6 ± 326.6 *(2.0 ± 2.0 %)*	-1858.1 ± 507.1 *(-13.1 ± 3.6 %)*	2403 ± 656.1 *(16.9 ± 4.6 %)*
Haliclona koremella	0	0	-105.8 ± 105.8 *(-0.6 ± 0.6 %)*	0	-215.9 ± 184.4 *(-1.5 ± 1.3 %)*	0
Total	-10.6 ± 8.0 *(-0.8 ± 0.4 %)*	31.4 ± 5.3 *(2.3 ± 0.4 %)*	-1110.1 ± 671.6 *(-6.8 ± 4.1 %)*	2276.8 ± 728.2 *(13.9 ± 4.4 %)*	-2577.1 ± 622.5 *(-18.1 ± 4.4 %)*	3249.5 ± 707.3 *(22.8 ± 5.0 %)*
TOTAL DAILY (24 h)	**20.8 ± 9.6** ***(1.5 ± 0.7 %)***		**1166.7 ± 990.6** ***(7.1 ± 6.0 %)***		**672.4 ± 942.2** ***(4.7 ± 6.6 %)***	

The area with the most carbon removed by the sponge assemblage was the near-reef-flat zone at Hoga-2, where the assemblage was estimated to remove 3249.5 ± 707.3 mol C m^{-2} (or 22.8 ± 5.0 % of the available carbon) during night-time. However, during day-time, the assemblage also released -2577.1 ± 622.5 mol C m^{-2} (-18.1 ± 4.4 % of the total available carbon), reducing the carbon removal to just 672.4 ± 942.2 mol C m^{-2} or about 4.7 ± 6.6 % of the total available carbon in the water column over a typical 24 hour period. Meanwhile, the area with the least removed carbon was the high-shore zone at Hoga-2. There, the sponge assemblage was estimated to remove 31.4 ± 5.3 mol C m^{-2} (2.3 ± 0.4 % of the total available carbon); with a release of carbon in day time as much as -10.6 ± 8.0 mol C m^{-2} (-0.8 ± 0.4 % of the total available carbon), the sponge-mediated daily carbon flux became 20.8 ± 9.6 mol C m^{-2} (1.5 ± 0.7 % of total available carbon) in carbon removal.

Although autotrophic sponges were releasing carbon during the daytime, they accounted for a considerable amount of the total TOC removed daily (24 h) at all zones at both sites. At the high-shore zone, the only autotrophic sponge in the zone (*Spheciospongia* sp.) was responsible for approximately 33.3% and 39.1% of the total carbon removed during night-time at Hoga-1 and -2, respectively. The autotrophic sponges in the middle zone were responsible for about 71.7% and 43.9% of the total carbon removed during night-time at Hoga-1 and -2, respectively. The contribution of autotrophic sponges to the carbon removed during night-time at the near-reef-flat zone was about 99.5% and 85.5% at Hoga-1 and -2, respectively.

Discussion

This study is the first to examine sponge-mediated carbon flux in a seagrass bed by considering the different impacts of autotrophic and heterotrophic species, and how these fluxes might vary over the complete 24-hour cycle. My results showed that sponges influence the water column carbon flux differently during the day and night. As expected, all autotrophic and heterotrophic sponges removed organic carbon from the water column in the dark. However, carbon removal rates were species-specific. In contrast, autotrophic sponges and heterotrophic sponges influenced the water column differently during the daytime. The autotrophic sponges released organic carbon into the water column, again at species-specific rates, while heterotrophic sponges continued to remove organic carbon from the water column, but at a lower rate than during the dark. At the assemblage level, the existence of autotrophic sponges in the seagrass meadow reduced the total daily sponge assemblage-mediated TOC flux, showing that the

removal of carbon from the water column might be less for some ecosystems, like seagrass beds, than previously thought.

Influence of sponges on water column dynamics: autotrophic vs heterotrophic

My results show that autotrophic sponges release more carbon during the daytime than they consume, offsetting carbon consumed by heterotrophic sponges at the assemblage level. Photosynthesis by marine organisms is known to release DOC into the water column (e.g. Brylinsky 1977; Bertilsson et al. 2005; Wetz and Wheeler 2007; López-Sandoval et al. 2013). With respect to sponges, from a ^{14}C-labelling experiment, Wilkinson (1983) showed that both autotrophic and heterotrophic sponges released DOC, in the range of 0.5–5 % under consecutive light and dark incubations (1-hour incubations). In the same study, the heterotrophic sponge *Spongia* sp., released 4.39% of fixed ^{14}C as DOC after one hour of consecutive light and dark incubations. However, in my study, while I did not measure the DOC flux for the related species *Spongia* sp., I did observe a considerable difference in sponge-mediated TOC reduction, where the rate at the light and dark incubation was 0.70 ± 0.23 and 1.83 ± 0.78 % C $g^{-}_{AFDW}{}^{-1}$ h^{-1}, respectively. From my previous study (Chapter 3), *Spongia* sp. hosts photosynthetic symbionts, indicated by a chlorophyll concentration of 28.59 ± 17.18 μg g^{-1}-sponge and was estimated to obtain 25.3 ± 16.4 % of its respiration requirement from its symbiont photosynthesis. As photosynthesis of marine organisms releases DOC to the water column, the host symbiont photosynthetic-derived carbon release might be the reason for the observed TOC flux discrepancy (about 60%) between light and dark incubations.

To my knowledge there are only two studies that have reported a release of DOC by sponges. Apart from the study by Wilkinson (1983), Hoer et al. (2018) reported that *Spheciospongia vesparium* and *Nipathes digitalis* were releasing DOC. However, the rates were not significantly different from zero (i.e. high standard error values), suggesting they are neither taking-up nor releasing DOC to the water column. It should be noted that the study by Hoer et al. (2018) utilized the InEx method, which is probably not the best approach to capture photosynthetically-derived DOC release. InEx methods, a technique introduced by Yahel et al. (2003), measures the difference in organic carbon concentration between paired inhalant and exhalant samples (Yahel et al. 2005). The underlying assumption for this approach is that sponges are filter feeders that pump a high volume of water and filter out all the organic carbon (i.e. they always remove organic carbon from the water column). Whereas we know that

photosymbionts are usually spread across the entire sponge body surface to capture the available sunlight for photosynthesis (Sarà 1971). Therefore, DOC is likely to be exuded across the body surface rather than in the exhalent water (e.g. Moebus and Johnson 1974; López-Sandoval et al. 2013). Thus, it is likely that the InEx technique does not capture the signals of the photosynthesis-produced DOC as part of the sponge-derived carbon flux. But using the incubation approach, *Spheciospongia* sp. in my study showed a DOC uptake of 0.37 ± 0.26 % C g_{AFDW}^{-1} h^{-1} in dark incubations and a release of TOC -0.91 ± 0.42 % C g_{AFDW}^{-1} h^{-1} in the light incubations. I did not measure the DOC flux under light incubation in my study. However, it is likely that the photosynthesis-derived DOC production was responsible for the TOC release in light incubations since my previous study (Chapter 3) showed that *Spheciospongia* sp. was an autotrophic sponge with a total photosynthetic pigment concentration of 76.02 ± 6.29 μg g^{-1}-sponge, where the symbionts' photosynthesis was found to provide 145.5 ± 4.7 % of the respiration requirement.

All autotrophic sponges removed carbon (expressed by the TOC flux and DOC flux by the two measured sponges) from the water column during dark incubations, showing that they still need to acquire food at night. Sponges have been reported to have pumping cessations across 24 h periods, but none have shown a diel pattern or a cessation period that lasts for the entire day or night (Reiswig 1971a; McMurray et al. 2014). Therefore, I propose that the autotrophic sponges were still pumping and filtering carbon from the water column during the daytime, at least for some of the time. This might mean that the photosynthetically-fixed carbon in the form of DOC from the photosymbionts was even higher than observed from the daytime net flux since sponges will also have been consuming some of the DOC during the daytime. This excess of DOC from sponges during the daytime is likely to contribute to the biogeochemical cycling and microbial processes in the surrounding environment (Azam and Malfatti 2007). For example, Haas et al. (2011) reported that a significant amount of labile DOC exuded by five types of algae on a coral reef stimulated a rapid bacterioplankton growth in the water column.

The proportion of POC and DOC removed

High-microbial abundance sponges are known to consume more DOC than the LMA sponges (e.g. Hoer et al. 2018), leading to higher DOC flux (uptake) detected from HMA than from LMA sponges. My study found that POC and DOC accounted for different proportions of the total TOC removed for *Spheciospongia* sp. and *Phyllospongia foliascens*, despite both species

being autotrophic. One possible explanation for this is a difference in the microbial abundance in the sponge species, where *Spheciospongia* sp. is known to be a high-microbial abundance (HMA) sponge (Vicente 1990; Weisz et al. 2008), while *Phyllospongia foliascens* is a low-microbial abundance (LMA) sponge (Moitinho-Silva et al. 2017). Assuming that the two sponges' photosynthetic symbionts produced the same amount of DOC (i.e. the same photosynthetic capacity), the abundance of microbes hosted by the sponges may explain the difference in DOC flux between them as the overall DOC consumption by the microbes would be different.

I also found that *Spheciospongia* sp. and *Phyllospongia foliascens* consumed much less DOC as part of their diet than reported from other studies. For example, Hoer et al. (2018) reported that *Xestospongia muta* (at 18 m) in the Caribbean removed 13–15 % of the ambient DOC, accounting for 96% of the total carbon removed by the sponge. Another study from the Red Sea reported that DOC represented 97% of the TOC removed by *Theonella swinhoei* (Yahel et al. 2003). However, in my study, I observed that only 9% and 56% of the DOC was removed by *Phyllospongia foliascens* and *Spheciospongia* sp., respectively. Some studies have suggested that the sponge diet composition depends on the composition of the plankton community. For example, Pile et al. (1996) reported a lack of feeding selectively for any picoplankton groups from sponges in the Gulf of Maine (Northwest Atlantic Ocean). Furthermore, Ribes et al. (1999) reported that the diet composition of the coral reef sponge *Dysidea avara* varied between seasons, based on the water column's planktonic composition, and supporting the idea that sponges are opportunistic particulate feeders. In regards to my site, the seagrass meadow had 55% higher TOC, with nine times higher POC concentrations than what has been reported by other coral reef studies (Yahel et al. 2003; de Goeij et al. 2008; Hoer et al. 2018). Therefore, it is likely that the much lower DOC proportion in the seagrass sponge diet was due to the higher availability of other food, particularly in the form of POC.

The significance of the seagrass sponge assemblage

From my studies of the daily (across 24 h) total carbon flux by the sponges in the seagrass beds (see Table 4.4), considering the sponge abundance in each seagrass bed zone and their removal or release efficiency, I found that the autotrophic sponges had different interactions with the water column. *Spheciospongia* sp. released (during daytime) more carbon than it removed (at night) in all three seagrass-bed zones. Meanwhile, *Phyllospongia foliascens* removed more carbon at night than it released during the daytime in middle and near-reef-flat zones (it did not

live in the high-shore zone). With respect to *Haliclona koremella*, I could not make these comparisons as I did not capture the sponge-mediated carbon flux in dark incubations. But all the heterotrophic sponges removed carbon over the entire day (24 h period). Thus, my results challenge the traditional view that sponges are net consumers of carbon from the water column (Bergquist 1978; Maldonado et al. 2012). Instead, I have demonstrated that some sponges may be a net producer of carbon to the water column when the carbon flux during night and daytime is considered.

The release of organic carbon to the water column by the autotrophic sponges during the daytime, presumably in the form of photosynthetically-derived DOC, has the potential to fuel the microbial loop in the water column and return the energy to the conventional planktonic food chain (Robertson et al. 1982; Azam et al. 1983). Furthermore, since some sponges also consume both bulk DOC (e.g. de Goeij et al. 2008; Hoer et al. 2018) and picoplankton (e.g. Yahel 2003; Perea-Blázquez et al. 2010), heterotrophic sponges could also consume the DOC released by autotrophic sponges. However, at the assemblage level, the seagrass sponge assemblage was still removing more organic carbon from the water column than it produced.

In the context of a regime shift to sponge-dominated reefs as a response to climate change, the existence of autotrophic sponges may reduce the bottom-up forcing to the assemblage by increasing sponge food sources through niche partitioning. This might play a crucial role in the success of any regime shift in sponge-dominated reefs. Lesser and Slattery (2020) argued that picoplankton would be limited in future oceans, which would place limitations on food supply (also to DOC). The food limitation will then limit the growth of sponge populations and prevent any regime shift into sponge-dominated reefs. However, my findings challenge that view since autotrophic sponges have the potential to act as carbon producers instead of consumers during daytime and therefore food may not be as limited as suggested by Lesser and Slattery (2020).

Conclusions

This chapter revealed the potential of autotrophic sponges to be net carbon producers, particularly during the daytime. The studied autotrophic sponges released organic carbon at a similar rate at which they removed carbon during dark periods, although the carbon type consumed at night was different from that produced during the day. My results suggest that the pressure of food limitation for shallow-water sponge assemblages due to ocean warming and ocean acidification may not be that strong, especially where autotrophic sponges dominate.

CHAPTER 5.

Autotrophic vs heterotrophic sponge-mediated picoplankton flux in an Indo-Pacific seagrass meadow

Abstract

Sponges form an important link in the flow of carbon between pelagic and benthic environments and they have the ability to remove a considerable amount of organic carbon from the water column. However, sponges have different nutritional modes. Sponges can be autotrophic, where the hosted photosynthetic symbionts provide more than half of the sponge's nutritional needs, or heterotrophic, where the sponge relies entirely on the captured food to fulfil its nutritional needs. Sponges with these different modes are likely to differ in their effect on the water column picoplankton community. Furthermore, since photosynthesis only occurs in the light, autotrophic sponges might show diel patterns in their impact on the picoplankton community. In this chapter, I measured the ambient picoplankton flux in the water column and the sponge-mediated picoplankton flux for six sponge species in dark (representing night-time) and light (representing daytime) incubations, representing 75.1–99.8 % of the total sponge biomass that inhabited a seagrass meadow in the Wakatobi National Park, Southeast Sulawesi. Then, I assessed the overall removal of picoplankton at the ecosystem level to determine how important sponge assemblages are in the benthic-pelagic carbon flow. I found diel variability in the abundance of all the picoplankton groups observed and also in the sponge-mediated fluxes. Heterotrophic sponges removed significantly more picoplankton-derived organic carbon than the autotrophic sponges ($P_{perm} < 0.01$). The autotrophic sponges did not consume heterotrophic bacteria during the day or night. Meanwhile, the heterotrophic sponges removed all picoplankton groups at a time- and species-specific rates. This resulted in a large difference in the picoplankton-derived carbon that autotrophic and heterotrophic sponges removed, with heterotrophic sponges removing 40–60 times more picoplankton derived carbon than the autotrophic sponges. At the sponge-assemblage level, heterotrophic sponges dominated the removal of picoplankton-derived carbon, except in the area where autotrophic sponges dominated the sponge assemblage biomass (near-reef-flat zone). Over 24 h, sponge assemblages in the high-shore, middle-shore, and near-reef-flat zone of the studied seagrass meadow was estimated to remove up to ~11%, ~14%, and ~10% of the ambient picoplankton-derived organic carbon, respectively. My study highlights the importance of sponge assemblages in linking pelagic and benthic environments and that differences in nutritional modes (autotrophic versus heterotrophic) impact the role of sponges in carbon flow from the water column to the benthos.

Introduction

Understanding trophic interactions is crucial to understand how ecosystems function and may respond to environmental changes. One of the major food components in aquatic ecosystems across the world are the picoplankton (Stockner and Antia 1986). Picoplankton are single-celled planktonic organisms whose size ranges between 0.2–2 or 3 µm in diameter (Sieburth and Lenz 1978; Vaulot et al. 2008), and are the most abundant living particulate organic carbon source in the oceans (Waterbury et al. 1979; Chisholm et al. 1988). Picoplankton comprises heterotrophic bacteria and autotrophic picoplankton (i.e. picophytoplankton), which both have a worldwide distribution and form the basis of global ocean productivity (Fogg 1995; Buitenhuis et al. 2012). Combined with heterotrophic bacteria, picoplankton play major roles in global carbon cycling as they are at the base of the aquatic microbial food web and fuel the microbial loop (Azam et al. 1983; Azam and Malfatti 2007).

Picoplankton biomass comprises about 46% picophytoplankton and 54% heterotrophic bacteria (Linacre et al. 2015), where picophytoplankton includes both prokaryotes and eukaryotes (Fogg 1986). The prokaryotic picophytoplankton is divided into two major groups: the genera *Prochlorococcus* (~0.6 µm) and *Synechococcus* (~1 µm); while the picoeukaryotes (0.8–3 µm) are far more diverse with four main algal phyla: Chlorophyta, Haptophyta, Cryptophyta, and Heterokontophyta (Vaulot et al. 2008). The abundance of heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, and picoeukaryotes in oligotrophic waters has been reported as about 10^6, 10^5, 10^4, and 10^3 cells mL^{-1}, respectively (Fogg 1986; Kudoh et al. 1990; Caron et al. 1991; Campbell and Vaulot 1993), and therefore they represent some of the most abundant organisms on the planet.

Predation and viral lysis have been identified as the main factors causing picoplankton mortality (e.g. Kudoh et al. 1990; Caron et al. 1991; Weinbauer 2004). Protozoans like flagellates and ciliates are well documented as the major grazers of *Prochlorococcus* and *Synechococcus* (e.g. Christaki et al. 1999; Guillou et al. 2001), but there are several metazoan filter feeders such as ascidians, bryozoans, bivalves and sponges, that are also known to feed on picoplankton (e.g. Ribes et al. 2005; Yahel et al. 2005). While flagellates and ciliates have growth rates that can balance that of picoplankton and are thought to be the main reason for stable picoplankton abundance (Berninger et al. 1991; Sheldon et al. 1992), little is known of the role that metazoans play in keeping the picoplankton abundance in an equilibrium state in most coastal ecosystems.

Benthic suspension feeders can capture large quantities of particles from the water column, which may give them the potential to directly regulate primary production and therefore secondary production indirectly (Jørgensen 1990; Gili and Coma 1998) through so called bottom up effects (White 1978). Sponges are a major component of benthic communities in many marine ecosystems across the world (Van Soest et al. 2012; Bell et al. 2020), which are known to have many functional roles in (Bell 2008). Sponges are able to pump and filter large quantities of water (e.g. Reiswig 1971a; Reiswig 1974), and by doing so, they remove a considerable amount of the organic carbon from the water column (e.g. Hadas et al. 2009). For example, in my previous chapter (Chapter 4), I found that the seagrass sponge assemblage was responsible for removing up to 10% of the ambient total organic carbon from the water column over a 24-hour period.

Sponges consume both particulate and dissolved organic matter (e.g. Pile et al. 1996; Yahel 2003; Wooster et al. 2019), but at species-specific rates and preferences (McMurray et al. 2018; Gantt et al. 2019). While many studies have reported dissolved organic carbon (DOC) comprises a major portion of the sponge diet composition (e.g. de Goeij et al. 2008; Hoer et al. 2018), sponges might actually prefer particulate organic carbon (POC) over DOC as their food (McMurray et al. 2016). McMurray et al. (2016) proposed that although their studied sponges got ~70% of their diet from DOC, under the assumption that all food types are equally abundant, the sponges showed a clear preference for living POC (i.e. picoplankton) as their food over DOC, probably to fulfil their nitrogen needs (Pile et al. 2003).

In addition to gaining nutrition from suspension feeding, many sponges have also established symbioses with photosynthetic organisms and can fulfil their nutritional needs from the symbionts' photosynthetic products; these are called autotrophic sponges (Wilkinson 1983; Erwin and Thacker 2008). My previous chapter (Chapter 4) showed that autotrophic sponges interact differently with the water column, compared to heterotrophic sponges, particularly during the daytime when the symbionts are photosynthesising. While the heterotrophic sponges were consistently net carbon consumers in the night- and daytime, autotrophic sponges were net carbon producers during daytime – releasing more organic carbon than they take up from the water column. Assuming the released carbon was photosynthetically-derived carbon, it suggests that the host sponges obtained extra nutrition from the photosymbionts. Due to the different degree of reliance on heterotrophy of these two nutritional modes, sponges might

influence the picoplankton abundance in the water column differently, especially during the day time – the period when photosynthesis is occurring.

In this chapter, I conducted *in situ* incubations to measure the effect of hetero- and autotrophic sponge feeding on different picoplankton groups that inhabited a seagrass meadow in the Wakatobi National Park, Southeast Sulawesi, Indonesia. My aims were to: (1) investigate the abundance, composition, and the natural flux (i.e. growth and loss rate) of picoplankton in the water column of a seagrass meadow that provides a food source for sponges; (2) measure any differences in the patterns in sponge-mediated picoplankton flux in the water column between hetero- and autotrophic sponges during the day and night; and (3) assess the importance of the seagrass sponge assemblage to picoplankton abundance based on my previously collected sponge abundance data.

Material and methods

Study site and in situ experiment

This study was conducted in a seagrass meadow at the west side of Hoga Island in the Wakatobi National Park, Southeast Sulawesi, Indonesia (5^{o} 28' S, 123^{o} 4" E). The meadow was a multispecies seagrass meadow, comprised of eight species: *Enhalus acoroides* (Linnaeus f.) Royle, *Thalassia hemprichii* (Ehrenberg) Ascherson in Petermann, *Cymodocea rotundata* Ehrenberg et Hemprich ex Ascherson, *Cymodocea serrulata* (R.Brown) Ascherson et Magnus, *Halodule uninervis* (Forsskål) Ascherson, *Syringodium isoetifolium* (Ascherson) Dandy, *Halophila minor* (Zollinger) den Hartog, and *Halophila ovalis* (R.Brown) J. D. Hooker (Chapter 2). The average seagrass cover at my study site was 53 ± 3 % (Chapter 2), with coral-sand sized 1.8 ± 0.1 mm as the meadow's substrate (Unsworth et al. 2008). From my previous chapter (Chapter 4), the water column of the seagrass bed had an average total organic carbon (TOC) concentration of 141.4 ± 21.9 µmol C L^{-1} and dissolved organic carbon (DOC) concentration of 119.8 ± 20.4 µmol C L^{-1}, which enabled the concentration of particulate organic carbon (POC) to be calculated at about 21.6 µmol C L^{-1} (Chapter 4). Thus, the proportion of the TOC in the water column consisted of 85% DOC and 15% POC.

From my earlier survey (Chapter 2), I identified ten sponge species inhabiting the seagrass meadow: *Spongia* sp., *Spheciospongia* sp., *Phyllospongia foliascens*, *Haliclona koremella*, *Amphimedon* sp., *Dactylospongia elegans*, *Axinella* sp., *Clathria reinwardti*, *Rhopaloeides* sp.,

and *Siphonodictyon mucosum*. For this study, I examined two heterotrophic sponges: *Spongia* sp. and *Amphimedon* sp.; and four autotrophic sponges: *Spheciospongia* sp., *Phyllospongia foliascens*, *Haliclona koremella* and *Dactylospongia elegans* (see Chapter 3 for the determination of autotrophic/heterotrophic status). Together, the six studied sponge species contributed 99.8, 91.8, and 90.2 % to the total sponge biomass in the high-shore, middle-shore, and near-reef-flat zones, respectively, in Hoga-1; and 75.1, 92.8, and 90.1 % in Hoga-2 (see Table 5.1 for data on sponge abundance in the meadow).

Table 5.1. Data on sponge abundance (g_{AFDW}) at each zone in the studied seagrass meadow in Hoga Island, Wakatobi National Park, Southeast Sulawesi – Indonesia (Chapter 2). Hoga-1 and -2 were two spatially separated areas of seagrass bed, which were divided into three tidal zones: high-shore (HS), middle (M), and near-reef-flat (NRF) zones. Heterotrophic sponges in red font, Autotrophic sponges in green font, and sponge with unknown nutritional mode in black font. Asterisk marked the sponges that were not studied in this chapter.

Sponge	**Hoga-1**			**Hoga-2**		
	HS (0.026 Km^2)	**M** (0.076 Km^2)	**NRF** (0.010 Km^2)	**HS** (0.038 Km^2)	**M** (0.137 Km^2)	**NRF** (0.065 Km^2)
Amphimedon sp.	0 – 938	4553 – 7450	151 – 491	0	0 – 42802	2180 – 4833
Spongia sp.	8050 – 8783	6250 – 19566	0	2348 – 2752	22860 – 37046	835 – 3413
Axinella sp.*	0	0 – 4517	0	0	0	687 – 4785
*Clathria reinwardti**	0	0 – 626	0 – 270	0	1962 – 12107	0
Dactylospongia elegans	0	0 – 19497	1316 – 4125	0	0 – 36658	0
Haliclona koremella	0	0 – 637	0	0	0 – 7297	346 – 4409
Phyllospongia foliascens	0	14788 – 36442	8441 – 30714	0	0 – 13894	11869 – 20782
*Siphonodictyon mucosum**	0	0	948 – 3408	1044 – 3708	1785 – 4083	0 – 1625
Spheciospongia sp.	3362 – 24883	0 – 345	42 – 6712	2651 – 6783	33915 – 126726	6883 – 21716

Ten sponges of each species were collected non-destructively to maintain the integrity of the sponge holobiont (i.e. not cutting the sponges) and moved to my working station without exposing them to air. The sponges had a four week acclimation period before I chose five sponges of each species that showed no sign of necrosis (i.e. were healthy) to work with. Due to the different morphologies of each sponge species, the average size of the studied sponges was different. The average sizes (in volume) for *Spongia* sp., *Spheciospongia* sp., *Dactylospongia elegans*, *Amphimedon* sp., *Haliclona koremella* and *Phyllospongia foliascens*

were 453 ± 127, 355 ± 125, 208 ± 63, 202 ± 73, 134 ± 9 and 78 ± 7 mL, respectively. The water displacement method was used to measure the sponge volume (after Rützler 1978) at the end of the incubation process. I put the sponge into a graduated cylinder or measuring cup (depending on the shape and size of the sponge), topped it up with seawater until the maximum volume scale, and took out the sponge and recorded the volume loss as the sponge volume.

Study design

This study focused on the sponge-mediated flux of picoplankton in the seagrass meadow, using *in situ* incubations. My working station for the *in situ* incubations was in the middle shore zone of the meadow, about 180 m from shore, with an average depth of 1.2 m during the incubations. Six incubation chambers were deployed in each measurement batch, of which five chambers were used for the sponges and one chamber for the control (i.e. without any sponge). The chambers were made from 10-litre polyethylene plastic bags fixed to the seafloor of the seagrass bed (after Hansen et al. 2000), with a three-way valve as the port to withdraw water samples using syringes. I used transparent chambers for light incubations and black chambers for dark incubations.

I measured five specimens of each sponge species (n = 5), separated into three batches to capture variability between days. Each sponge was measured under dark and light conditions, representing night and day. The dark incubations started at 7 am, while the light incubations were run between 11 am and 2 pm. From my Hobo pendant loggers that I installed inside the chambers and on the seafloor (outside of the chambers), the average light intensity during dark incubation was 74 ± 24 μmol photons m^{-2} s^{-1} on the seafloor and total dark (i.e. 0 μmol photons m^{-2} s^{-1}) in the chambers. Meanwhile, during light incubations, the average light intensity on the seafloor and inside the chambers was 1067 ± 82 μmol photons m^{-2} s^{-1}, about 14 times higher than the intensity during the early morning when the dark incubations were run.

I placed one sponge specimen into a chamber, closed it, and allowed 10 minutes acclimation before I withdrew my first water sample. The second water sample was withdrawn after 72 minutes. From my preliminary measurements, a suitable incubation duration for 10-litre sized chambers was 72 minutes (see Chapter 3). The water sample was withdrawn with a syringe, transferred into an amber glass bottle, and stored in a cooler box until I could process it in my field laboratory (250 m from my working station). I made triplicates samples using 1.5 ml sterile cryovials, preserved them with glutaraldehyde (0.1% final concentration), which were flash-

frozen in liquid nitrogen and stored at –80°C until I could perform a flow cytometry analysis (after Marie et al. 1995).

To standardize the picoplankton flux mediated by the sponges, I measured the ash-free-dry-weight (AFDW) of the sponges. At my field laboratory in Hoga Island, I measured the sponge dry weight (DW) by oven drying them at 60°C until the sponges did not show any further weight loss. Then I measured the AFDW of the sponges at IPB University in Bogor, Indonesia, using a muffle furnace at 550°C for 4.5 hours. The weight loss during the process was considered to be the AFDW. The average ash-free dry-weight of *Spongia* sp., *Spheciospongia* sp., *Phyllospongia foliascens*, *Haliclona koremella*, *Amphimedon* sp., and *Dactylospongia elegans* that I measured were 17.5 ± 4.2, 25.5 ± 3.1, 8.5 ± 0.8, 5.3 ± 0.6, 19.4 ± 3.4, and 16.6 ± 6.8 g, respectively.

Flow cytometry analyses

The Cytek® Aurora flow cytometry system (from Cytek Biosciences) at the Malaghan Institute of Medical Research in Wellington, New Zealand, was used to identify and quantify the picoplankton populations in my seawater samples. The system uses five lasers (20 mW 355 nm UV, 100 mW 405 nm Violet, 50 mW 488 nm Blue, 50 mW 561 nm Yellow-Green, and 80 mW 640 nm Red) and 64 fluorescence detectors that enable it to capture the entire emission spectrum from each fluorochrome. From a trial with seawater, no staining dye was needed to capture the picoplankton populations with this flow cytometry system.

DNA positive events in the flow cytometry analysis were identified as the picoplankton populations, which then were gated and viewed in a Forward Scattered Light (FSC) – Side Scattered Light (SSC) dot plot. V12-A and YG1-A fluorescence were used as the y- and x-axis, respectively, to view and identify the cells of interest. Then, the boundaries for the distinct populations were drawn. The identification of the picoplankton populations was based on the emitted fluorescence. *Synechococcus* was identified based on orange and red fluorescence emission, as the group contains phycobiliproteins and chlorophyll, which emit strong orange and red fluorescence. *Prochlorococcus* was identified based on the presence of red fluorescence, but it lacks orange fluorescence emission as this group only contains chlorophyll. Picoeukaryotes were distinguished based on their scatter characteristics, chlorophyll and phycoerythrin, and DNA signals. Meanwhile, the DNA positive events that do not emit both orange and red fluorescence was identified as heterotrophic bacteria populations. The sample

list-mode files were analysed using the FlowJo (version 10.8.0) software package (see Three Star Inc., www.flowjo.com).

Data analysis

Picoplankton flux

The flux of each picoplankton group in every chamber was calculated relative to its initial abundance, following this equation:

$$Picoplankton\ flux\ (\%) = \left(\frac{(C_1 - C_2)}{C_1}\right) \times 100\%$$

where,

C_1 = Cell abundance (cell mL^{-1}) of the first water sample (at the beginning of the incubation)

C_2 = Cell abundance (cell mL^{-1}) of the second water sample (at the end of the incubation)

Since picoplankton has a high growth and loss rate, I normalized the sponge-mediated flux with the controls (i.e. subtracting the flux in the sponge chamber from the control's flux) and then calculated the removal efficiency (RE) for each picoplankton group during a one-hour period by standardizing it to the sponge's AFDW, using this equation:

$$RE = \left(\frac{flux_{Sp} - flux_{ctrl}}{AFDW_{Sp}}\right) \times \left(\frac{60}{72}\right)$$

where,

RE = Removal efficiency (% cell-abundance g_{AFDW}^{-1} h^{-1})

$flux_{Sp}$ = Sponge-mediated picoplankton flux (% cell-abundance)

$flux_{ctrl}$ = Control's picoplankton flux (% cell-abundance)

$AFDW_{Sp}$ = Sponge's ash-free-dry-weight (g)

To determine the significance level of the picoplankton removal by the sponges, a t-test was conducted against zero removal (i.e. no removal). If the P-value was higher than 0.05 ($p \geq .05$), then the flux was not significantly different with zero removal. Meanwhile, if the P-value was

lower or equal to 0.05 ($p \leq 0.05$) the removal was significant different to zero removal. For further analysis, I only calculated the flux for those species that had a P-value < 0.05.

After picoplankton fluxes based on the number of cells retained were determined, I then calculated the amount of the removed carbon. Cell abundance (cells L^{-1}) of each picoplankton group was converted to biomass (μgC L^{-1}) using constant conversion factors based on previous studies from tropical marine environments. The conversion constant for heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, and picoeukaryotes were 20, 56, 112, and 1010 fgC $cell^{-1}$, respectively (Lee and Fuhrman 1987; Garrison et al. 2000; DuRand et al. 2001).

Permutational multivariate analysis of variance (PERMANOVA) was conducted to determine any differences between nutritional mode (auto- and heterotrophic), diurnal cycle (i.e. day and night, represented by light and dark incubations), and their interactions, for the picoplankton-derived carbon removal by the sponges. The removed carbon data were fourth root transformed, and then the data resemblance was established based on Bray Curtis similarity, before the PERMANOVA was run. I used the software PRIMER v6 (with the PERMANOVA+ add-on; Anderson et al. 2008) .

Ambient picoplankton abundance, biomass and flux

Each picoplankton group's ambient abundance, biomass, and natural flux were determined from the initial water samples from the control chambers, following the same protocols for the sponge-mediated picoplankton flux (as above). In total, I had six replicates of control for light (n = 6) and dark (n = 6) incubations that were taken across six different days to capture intra-day variability.

Assessments of the removal of picoplankton-derived organic carbon

In my earlier sponge survey at the Hoga Island's seagrass meadow (Chapter 2), I surveyed two spatially separated sites, named with Hoga-1 and -2, which were divided into three tidal zones: high-shore (HS), middle (M), and near-reef-flat (NRF) zones. The total area coverage by Hoga-1 and -2 were 0.151 km^2 and 0.213 km^2, respectively. The maximum depth of high-shore (HS), middle (M), and near-reef-flat (NRF) zones during high tide were approximately 0.5, 1.2, and 1.9 m depth, respectively. The data on the sponge abundance at the seagrass meadow are summarized in Table 5.1; this information was used to calculate the living organic carbon

removed by the sponge assemblage. The organic carbon removal rate of each sponge was calculated by multiplying the average removal efficiency with the ambient concentration and the biomass of each picoplankton group, both in dark and light incubations. The results were then extrapolated to the scale of the seagrass meadow using the sponge's biomass from my previous sponge survey (Chapter 2; Table 5.1) over a 24 h cycle (12 h dark – 12 h light) to estimate the living organic carbon removed by the seagrass sponge assemblage. The calculation formulae for the removed carbon by the sponges in each diel cycle (day and night) at each seagrass area (sites and zones) is as follow:

$$Carbon\ removed\ = (RE \times ambient\ picoplankton\ concentration \times picoplankton\ carbon\ weight) \times 12\ h \times Sponge\ biomass$$

where the sponge's removal efficiency (RE) and the picoplankton ambient concentration were specified for day and night-time, and I summed the carbon removed in the day and at night to calculate the total daily carbon removed.

Results

Picoplankton ambient abundance, biomass, and flux

From the flow cytometry analysis, I was able to capture and identify four groups of picoplankton from my water samples: heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, and picoeukaryotes (see Supplementary Figure S.5.1 and S.5.2 for dark and light incubations, respectively). Heterotrophic bacteria greatly outnumbered the other three groups, based on the number of cells. The concentration of heterotrophic bacteria was two orders of magnitude greater than the *Prochlorococcus* and *Synechococcus* concentrations, and three orders of magnitude greater than the picoeukaryotes concentration (Table 5.2). However, based on the biomass (i.e. the weight of the carbon), the differences were not as dramatic as the number of cells. Even though the heterotrophic bacteria dominated the picoplankton composition in the water samples, the other groups showed a much higher biomass contribution. The contribution of *Prochlorococcus*, *Synechococcus*, and the picoeukaryotes to the total picoplankton biomass were 1–2 %, 3 %, and 4–6 %, respectively, while the rest (~90%) was the heterotrophic bacteria (Figure S.5.3, App. C).

Table 5.2. The ambient concentration, biomass, and flux of each picoplankton group at the study site. The negative values in the picoplankton flux mean more cells were found at the end of the incubations than at the start. The values are means ± SE (n = 6).

Picoplankton group	Ambient concentration (cells mL^{-1})		Ambient biomass (μgC L^{-1})		Natural flux (% cells mL^{-1} h^{-1})	
	Dark	Light	Dark	Light	Dark	Light
Heterotrophic bacteria	4.76 ± 1.03 x 10^6	3.80 ± 0.53 x 10^6	95.1 ± 20.5	75.9 ± 10.5	32.3 ± 12.5	-25.9 ± 20.4
Prochlorococcus	17.7 ± 2.7 x 10^3	25.6 ± 4.1 x 10^3	1.0 ± 0. 2	1.4 ± 0.2	-25.9 ± 4.5	21.1 ± 10.9
Synechococcus	26.8 ± 3.4 x 10^3	20.9 ± 4.8 x 10^3	3.0 ± 0.4	2.3 ± 0.5	2.4 ± 0.8	-31.5 ± 9.1
Picoeukaryotes	6.0 ± 0.5 x 10^3	3.7 ± 0.6 x 10^3	6.1 ± 0.5	3.8 ± 0.6	5.3 ± 2.8	-15.0 ± 7.8

The ambient concentrations of each picoplankton group during the dark and light incubations were different. The ambient concentration of heterotrophic bacteria, *Synechococcus* and picoeukaryotes in the dark were higher than in the light incubations. Meanwhile, *Prochlorococcus*' ambient abundance in the water column showed the reverse pattern (Table 5.2). In regard to the ambient fluxes, each picoplankton group had a specific rate and diel pattern (see Table 5.2). *Synechococcus* and picoeukaryotes had lower cell numbers during dark incubations than light. Heterotrophic bacteria also showed a decrease in cell concentration in the dark incubations, but at a much higher rate than *Synechococcus* and picoeukaryotes (32.3 ± 12.5 % cells mL^{-1} h^{-1} compared to 2.4 ± 0.8 and 5.3 ± 2.8 % cells mL^{-1} h^{-1} for *Synechococcus* and picoeukaryotes, respectively). Meanwhile, during light incubations, heterotrophic bacteria had a relatively higher cell abundance (-25.9 ± 20.4 % cells mL^{-1} h^{-1}) at the end of incubations. In contrast to the other three picoplankton groups that had an increase in cells concentration in light and decrease in dark incubations, *Prochlorococcus* showed an increase during dark incubations (-25.9 ± 4.5 % cells mL^{-1} h^{-1}) but a decrease of 21.1 ± 10.9 % cells mL^{-1} h^{-1} in light incubations.

Sponge-mediated picoplankton flux

I could not determine any *Prochlorococcus* removal by the sponges as the detected flux consistently showed an increase in cell numbers at the end of dark and light incubations across all sponge species. Therefore, I did not include *Prochlorococcus* in any further sponge-mediated picoplankton flux analysis.

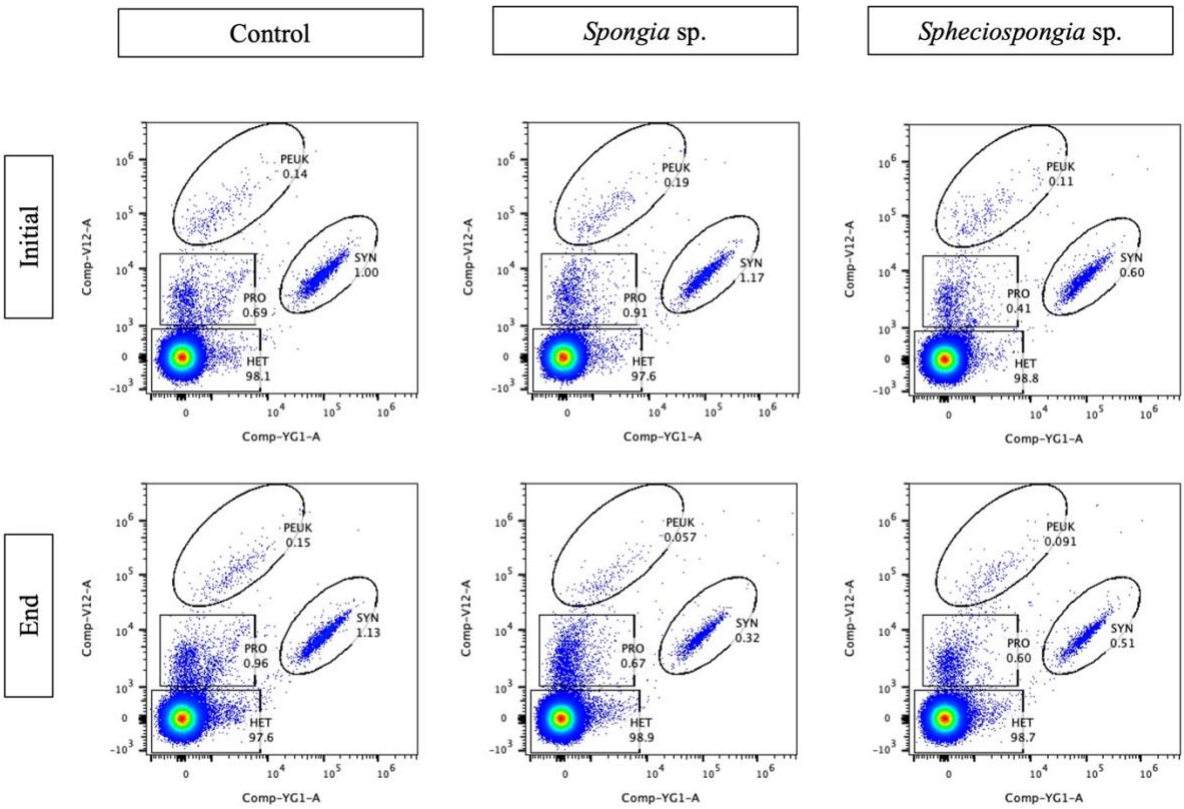

Figure 5.1. An example of flow cytometric analyses of sponge-mediated picoplankton flux from one of the dark incubations. One measurement batch consisted of one empty incubation bag that acted as a control and up to five incubation bags with sponges (one sponge in each bag). Picoplankton cells were determined from the water samples withdrawn at the beginning (Initial) and end (End) of the incubation. The gates for each picoplankton group were determined based on the best fit with all the water samples from that particular measurement batch. HET, PRO, SYN, and PEUK denote heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, and picoeukaryotes, respectively.

In the light incubations, the autotrophic sponges (all four sponges) only significantly removed *Synechococcus* ($p < .05$) from the water column, but with a species-specific removal efficiency. Meanwhile, the heterotrophic sponges were observed to remove heterotrophic bacteria ($p < .05$) and *Synechococcus* ($p < .05$), also with a species-specific removal efficiency. The removal of

picoeukaryotes in light incubations was not observed for any sponge species (both auto- and heterotrophic).

All heterotrophic and autotrophic sponges removed *Synechococcus* at species-specific rates in the dark incubations ($p < .05$). However, the removal efficiencies were lower in the light incubations, except for *Spongia* sp. as it had a similar *Synechococcus* removal efficiency during dark and light incubations (see Figure 5.1). In the dark incubations, picoeukaryotes were removed from the water column by *Spongia* sp., *Spheciospongia* sp., and *Dactylospongia elegans* with a removal efficiency of 3.5 ± 1.1, 0.5 ± 0.3, and 1.0 ± 0.1 % cells mL^{-1} g_{AFDW}^{-1} h^{-1}, respectively. None of the sponges removed heterotrophic bacteria during dark incubations. See Table S.5.1 (Appendix C) for the summary of t-test results for the picoplankton removal by the sponges in light and dark incubations.

Estimated picoplankton biomass removed by the seagrass sponges

The two heterotrophic sponge species removal rates of picoplankton biomass far exceeded the removal rate by autotrophic sponges, particularly in light incubations (Figure 5.2). *Spongia* sp. and *Amphimedon* sp. removed 2.1 and 3.1 µg C $L_{seawater}^{-1}$ g_{AFDW}^{-1} h^{-1} in light incubations respectively, compared to 0.05–0.14 µg C $L_{seawater}^{-1}$ g_{AFDW}^{-1} h^{-1} by the autotrophic sponges. From these results, the heterotrophic bacteria biomass contributed 96 and 93% to the carbon removed by *Spongia* sp. and *Amphimedon* sp. in light incubations, respectively.

In dark incubations, the two heterotrophic sponges *Spongia* sp. and *Amphimedon* sp. removed much lower living organic carbon compared to light incubations at 0.3 and 0.01 µg C $L_{seawater}^{-1}$ g_{AFDW}^{-1} h^{-1}, respectively, as these two heterotrophic sponges did not remove heterotrophic bacteria. With regard to the autotrophic sponges, all sponges showed similar removal rates of picoplankton biomass between dark and light incubations (Figure 5.2).

The two-way PERMANOVA test confirmed that the removal of picoplankton-derived carbon was significantly different between the autotrophic and heterotrophic sponges (Pseudo-F = 4.4512, $p < .01$), and showed that the removal rates were also different between day and night (represented by light and dark incubations; Pseudo-F = 2.9912, $p < .05$). There was no significant interaction between nutritional mode and diel cycle (i.e. day and night-time) (Pseudo-F = 0.53275, $p = .652$). See Table S.5.2 (Appendix C) for full PERMANOVA results.

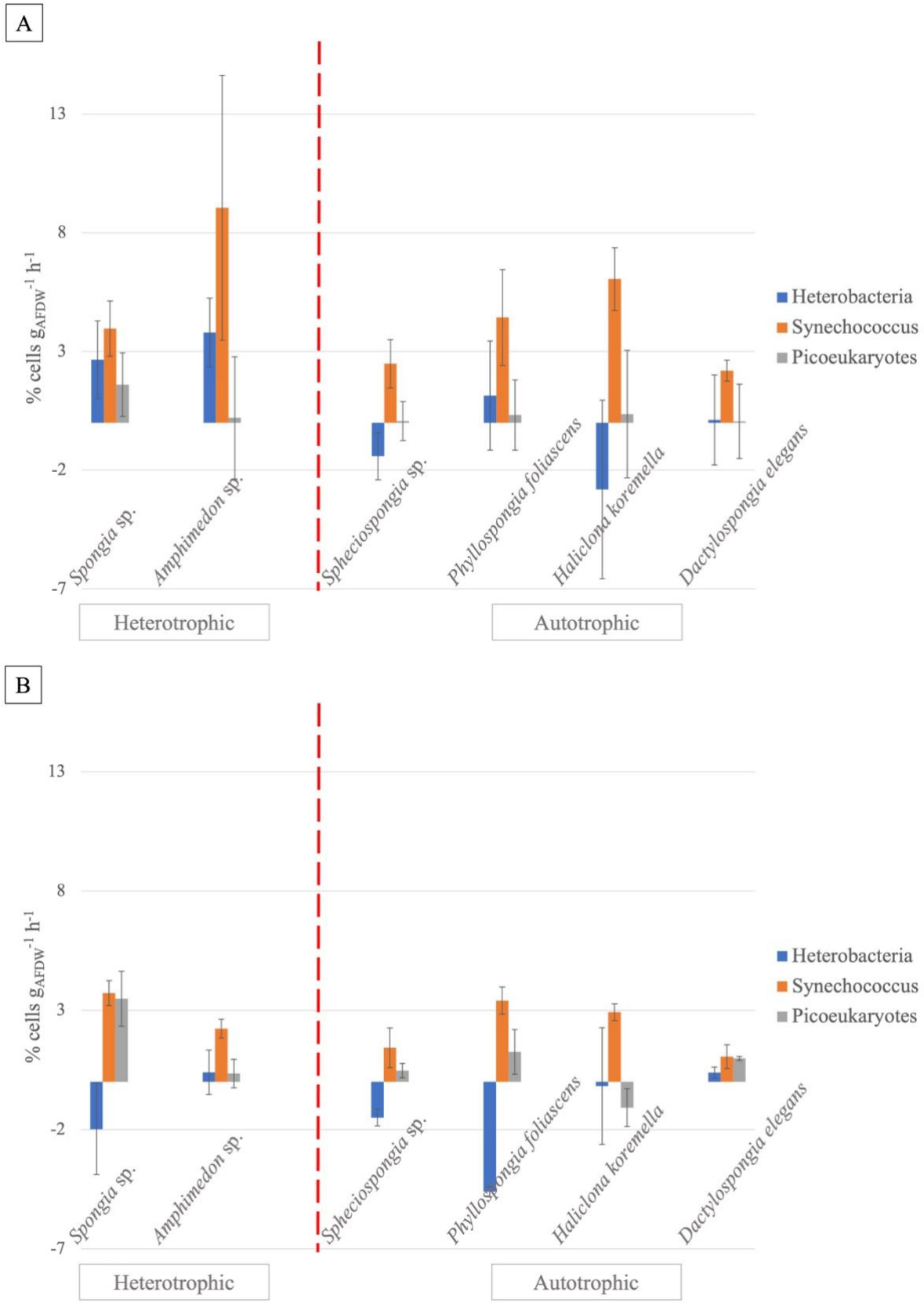

Figure 5.2. Sponge removal efficiency on each picoplankton group during light (A) and dark (B) incubations. The unit is % cells g_{AFDW}^{-1} h^{-1}. A positive value means a decrease in picoplankton cells (i.e. a removal), while negative value means an increase in the abundance of picoplankton cells.

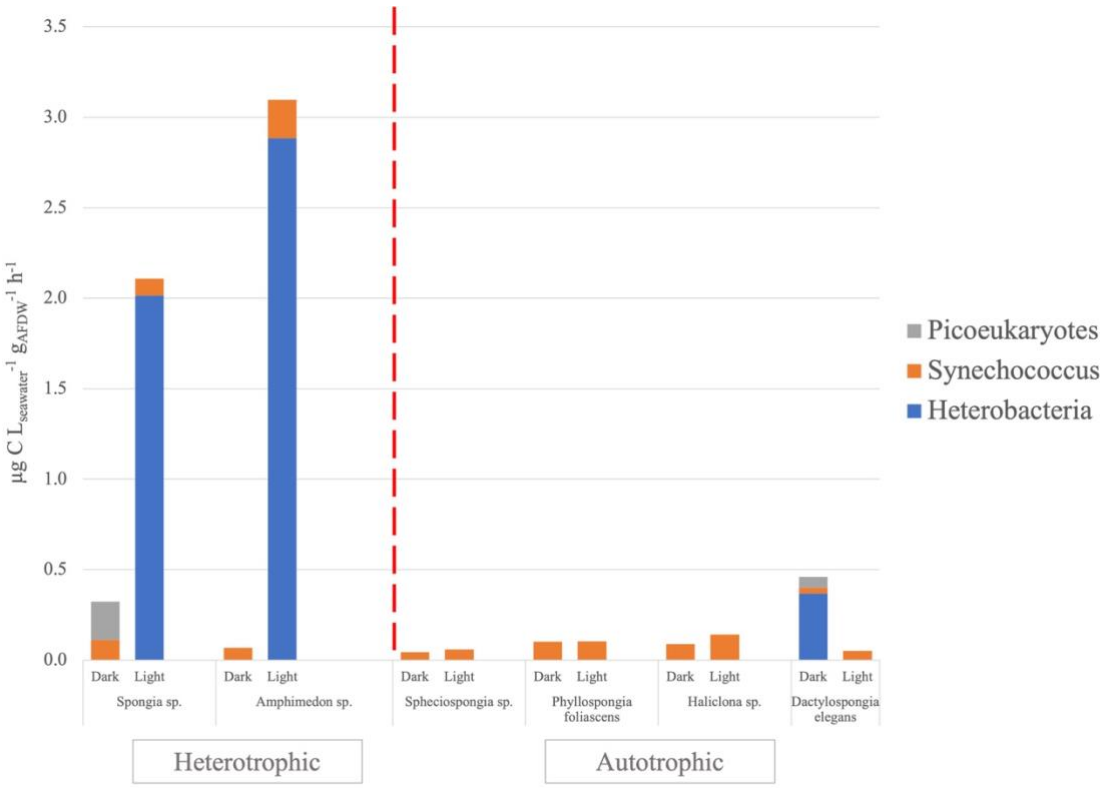

Figure 5.3. The accumulation of seagrass sponge removal rate of picoplankton biomass (carbon) in dark and light incubations, utilising the average value of the sponge-mediated removal rates for each picoplankton group. The unit is µg C $L_{seawater}^{-1}$ g_{AFDW}^{-1} h^{-1}.

My assessment of total picoplankton-sourced organic carbon removal by all six sponges that inhabited the high-shore zone of Hoga-1 and -2, were about 61.5–84.1 and 18.7–23.3 g C m^{-2} d^{-1}, or about 11% and 2% of the ambient concentration, respectively. At both sites (Hoga-1 and -2), the heterotrophic sponges were removing more than six times what the autotrophic sponges removed. In the middle zone of the seagrass bed, the six sponges removed organic carbon 348.3–946.3 and 625.6–2737.3 g C m^{-2} d^{-1}, or about 10% and 14% of the ambient concentration at Hoga-1 and -2, respectively. In the middle zone, heterotrophic sponges were removing organic carbon 1.5 to 17 times the amount the autotrophic sponges at Hoga-1 removed, reaching 50 times more at Hoga-2. Meanwhile, in the near-reef-flat zone, a total of 95.6–356.7 and 247.5–673.9 g C m^{-2} d^{-1}, or approximately 10% and 4% of the ambient picoplankton biomass were removed by the six sponges at Hoga-1 and -2, respectively. In the near-reef-flat zone of Hoga-1, the autotrophic sponges removed at least double the carbon compared to the heterotrophic sponges. However, at Hoga-2, the heterotrophic sponges removed more organic carbon; six times more than the autotrophic sponges removed.

Table 5.3. Estimates of the picoplankton biomass removed from the water column during high tide by seagrass sponges two sites over a 24 h cycle (g C m^{-2} d^{-1}). Hoga-1 and -2 were two spatially separated areas of the seagrass meadow at the studied site; the areas were divided into three tidal zones: high-shore (HS), middle (M), and near-reef-flat (NRF) zones.

Sponge	Phase	Hoga-1			Hoga-2		
		HS	M	NRF	HS	M	NRF
Heterotrophic sponges							
Spongia sp.	Dark	7.8 – 8.5	20.6 – 64.6	0	2.3 – 2.7	75.4 – 122.2	5.0 – 27.2
	Light	52.4 – 57.1	138.2 – 432.6	0	15.3 – 17.9	505.4 – 819.1	33.7 – 182.0
Amphimedon sp.	Dark	0 – 0.2	3.1 – 5.1	0.3 – 0.9	0	0 – 29.4	2.7 – 6.0
	Light	0 – 8.7	143.8 – 235.3	12.6 – 41.0	0	0 – 1351.6	125.6 – 278.3
Autotrophic sponges							
Spheciospongia sp.	Dark	0.7 – 5.3	0 – 0.3	0.1 – 13.0	0.6 – 1.5	24.7 – 92.4	9.2 – 28.9
	Light	0.6 – 4.3	0 – 0.2	0.1 – 10.5	0.5 – 1.2	20.1 – 75.1	7.4 – 23.5
Phyllospongia foliascens	Dark	0	27.0 – 66.5	40.8 – 148.3	0	0 – 25.3	39.5 – 69.1
	Light	0	15.6 – 38.5	23.6 – 86.0	0	0 – 14.7	22.9 – 40.1
Haliclona koremella	Dark	0	0 – 0.6	0	0	0 – 6.5	0.6 – 7.2
	Light	0	0 – 0.9	0	0	0 – 10.5	0.9 – 11.6
Dactylospongia elegans	Dark	0	0 – 18.2	3.3 – 10.2	0	0 – 34.3	0
	Light	0	0 – 10.2	1.8 – 5.7	0	0 – 19.2	0

Discussion

Sponges create an important link between benthic and pelagic environments (Bell 2008), filtering a large quantities of water and removing organic carbon (Reiswig 1971a; Reiswig 1974) that enables carbon flow to higher trophic levels (Wulff 2006). However, two different sponge nutritional modes, autotrophy and heterotrophy, could be expected to influence carbon removal differently, particularly living particulate organic carbon (POC). I found that the heterotrophic sponges removed far more picoplankton-derived organic carbon over a 24 h cycle than the autotrophic sponges. In the study areas, the seagrass sponge assemblages removed a large amount of living POC, with the heterotrophic sponges dominating in this role despite their lower abundance, compared with the autotrophic sponges. My results confirm that sponge nutritional mode influences the degree of sponge-mediated picoplankton flux in the water

column, and that heterotrophic and autotrophic sponges might play a different role in the flow of carbon in seagrass beds.

Picoplankton in the seagrass meadow

The abundance of heterotrophic bacteria, *Synechococcus*, and picoeukaryotes at my study sites was within the range reported from other studies (e.g. Fogg 1986; Campbell and Vaulot 1993). For example, the picoplankton abundance at my study sites was within the range reported from the Gulf of Mexico (Linacre et al. 2015), although higher than the average. This was expected, since picoplankton abundance has been reported to increase from oligotrophic to moderately eutrophic waters (El Hag and Fogg 1986; Fogg 1995), and seagrass beds are significantly enriched with particulate organic carbon (Duarte et al. 1999).

Prochlorococcus was detected, but the abundance was very low. I only detected 17–25 x 10^3 cells mL^{-1} of *Prochlorococcus*, compared to 2 x 10^5 cells mL^{-1} reported from subtropical Pacific waters (Campbell and Vaulot 1993). *Prochlorococcus* has a relative abundance of more than 60% of the total autotrophic picoplankton in some locations (Linacre et al. 2015). However, the detected *Prochlorococcus* cells were only about 35% of the total autotrophic groups at my study sites. The cellular chlorophyll content of *Prochlorococcus* which lives in near-surface waters is very low (Dusenberry and Frankel 1994), which has often been considered to cause an underestimation of the *Prochlorococcus* cell concentration (Partensky et al. 1999). The measured natural flux of *Prochlorococcus* in dark and light incubations was also not consistent with the group's known cell cycle. My flow cytometry analyses showed that *Prochlorococcus* growth was happening in dark incubation and declined in the light incubations. More cells being detected at the end of the dark incubations was probably not the result of cell division, but due to the growth rate of the cells, gaining in chlorophyll concentration, thus becoming more detectable. The natural flux of *Prochlorococcus* that I observed contrasts with common knowledge that picophytoplankton cells only divide during daytime (Sheldon et al. 1992; Vaulot and Marie 1999). Meanwhile, the decline of *Prochlorococcus* cells at the end of light incubation was probably due to the decreased chlorophyll content as a response to very high light intensity, thus fewer cells were detectable. Because of these considerations, I concluded that my experimental approach was not appropriate for estimating *Prochlorococcus* fluxes and thus I decided not to analyse it for sponge-mediated flux. For this picoplankton group, I suggest that the *InEx* method – a method that measures the difference in the cell concentrations between

the inhalant and exhalant point of the sponge to calculate the sponge's filtration rate (see Yahel et al. 2005 for details on the method), would be a better approach.

It is important to note that cell division and loss are responsible for daily picoplankton cycles (Vaulot and Marie 1999; Binder and DuRand 2002). Thus, the flux of picoplankton abundance from the controls represents the balance from the cell division, lyses and grazing by microheterotrophs (i.e. flagellates and ciliates) that is taking place in the water column. I found that the heterotrophic bacterial population had a similar rate of growth (25.9 ± 20.4 % cells mL^{-1} h^{-1} during light incubations) and mortality (32.3 ± 12.5 % cells mL^{-1} h^{-1} during dark incubations). Although my study did not differentiate the nature of the cell loss, whether due to viral lysis or grazing by protozoans, the balance between growth and mortality rates might explain the stable heterotrophic bacteria abundance in the water column. However, the picophytoplankton, *Synechococcus* and picoeukaryotes showed significant population growth during daytime (31.5 ± 9.1 and 15.0 ± 7.8 % cells mL^{-1} h^{-1}, respectively), but a low mortality rate during the dark incubations (2.4 ± 0.8 and 5.3 ± 2.8 % cells mL^{-1} h^{-1}, respectively). My observations of autotrophic picoplankton confirm the findings of Becker et al. (2020), who also observed variability in these groups related to diurnal cycles in seagrass beds. However, while Becker et al. (2020) found heterotrophic bacteria had a consistent abundance throughout the day (24 h) at their study sites, I found that the abundance of heterotrophic bacteria at my studied seagrass beds showed variation related to diurnal cycles.

Sponge-mediated picoplankton flux in the seagrass meadow

There is growing evidence that sponges are not indiscriminate suspension feeders but rather actively and selectively feed on available planktonic foods (Yahel et al. 2006; Hanson et al. 2009; Maldonado et al. 2010; Maldonado et al. 2012). Therefore it is expected that sponges will cause different sponge-mediated fluxes for each picoplankton group in their habitat. In my study, the seagrass sponge assemblage also showed this behaviour. Some sponges excluded one or two picoplankton groups from their diet (Figure 5.1), although it was species- and time-specific. Interestingly, I found clear evidence that sponge nutritional mode influences food preferences and carbon removal. While heterotrophic sponges clearly removed three picoplankton groups (heterotrophic bacteria, *Synechococcus*, and picoeukaryotes) from the water column, all autotrophic sponges did not remove heterotrophic bacteria – the most abundant picoplankton group from the water column, during both dark and light incubations.

When the removed cell number was converted into biomass (i.e. carbon weight), a stark difference in the carbon removed between the heterotrophic and autotrophic sponges was evident. Heterotrophic sponges removed about 40–60 times more carbon than the autotrophic sponges during the light incubations (representing daytime; Figure 5.2). But during dark incubations (representing night-time), the period when all sponges are in heterotrophic mode (i.e. no photosynthesis occurs), there was no distinct pattern in carbon removal between heterotrophic and autotrophic sponges. This finding supports the hypothesis that autotrophic sponges gain supplementary nutrition from the host photosymbionts, which eases the pressure to obtain their nutritional needs from suspension feeding.

It is not clear why autotrophic sponges discriminated against heterotrophic bacteria as their food, even though it was the most abundant picoplankton (i.e. food source) in the water column, resulting in much less carbon removal than achieved by heterotrophic sponges. A model of filter-feeders' food selectivity developed by Lehman (1976), predicted that filter feeders achieve their optimal harvest when their ingestion rates are maximal for particles slightly larger than those most numerous in the mixture of food sources. That is what heterotrophic bacteria were: the most numerous and the lightest picoplankton among all the groups; and *Synechococcus* is slightly heavier than heterotrophic bacteria. This result needs further investigation.

Sponges were observed to have high retention of picoplankton consistent with previous studies (e.g. Pile et al. 1996), thus food selection is thought to occur at the post-capture phase – not at the filtering stage (Ribes et al. 1999; Yahel et al. 2006). Yahel et al. (2006) hypothesized that pre-ingestion sorting occurs within the trabecular recticulum, whereby the indigestible and less preferred food will be secreted directly through the exhaled water, while the preferred food will be transferred into food vacuoles. Due to the difference in time needed to digest different microbial types (Maldonado et al. 2010), it is possible that digesting heterotrophic bacteria might create a lost opportunity for the sponges in gaining better nutrition from other food sources. Based on the foraging theory, organisms prefer the food that provides them with the most nutrition, and that requires less time and energy to obtain and process (Stephens and Krebs 1986). So, sponges may prefer to skip the less preferred food to make ways in their digestion system for the preferred ones, known as "the principle of the lost opportunity" in the foraging theory (Stephens and Krebs 1986). In the context of sponges, this means they only retain the preferred foods at the microvilli collar until they got phagocytosed by the choanocyte.

I also found diel variation in sponge-mediated picoplankton flux at my studied seagrass meadow. The removal of picoeukaryotes was only observed during dark incubations (representing night-time) across both sponge nutritional modes, most likely due to the group's availability level as the picoeukaryotes ambient concentration in the dark was almost twice the concentration in light incubations (Table 5.2). This result aligns with the general assumption that food availability drives sponge feeding rate (e.g. Coma et al. 2001; Perea-Blázquez et al. 2013). Meanwhile, the removal of *Synechococcus* and heterotrophic bacteria by the sponges was more pronounced when these picoplankton groups were having cell division indicated by higher cells number at the end of incubation. These two picoplankton groups had a similar ambient concentration between day and night (represented by light and dark incubations), diminished food availability as the driver of this phenomena. All sponges (both heterotrophic and autotrophic sponges) were observed to have slightly higher removal efficiency for *Synechococcus* in the light incubations, which coincided with the period when the group showed an increase at the end of incubation. Heterotrophic bacteria also exhibited this pattern as the removal of heterotrophic bacteria by heterotrophic sponges was only observed during light incubations, which was also when it was observed to be more abundant at the end of the incubation. Again, the reason for these synchronized events is not apparent. While large-scale spatial and temporal variation in the sponge feeding rate and preferences have been well studied (Ribes et al. 1999; Hanson et al. 2009; Perea-Blázquez et al. 2013), and have been explained mainly by the abundance of the available food type, little is known of the daily or diel variation of sponge feeding. My results highlight the importance of further studying the linkage between the picoplankton's life cycle with sponge feeding rates and preferences, as it will give a better understanding of the dynamic of the benthic-pelagic relationship.

Seagrass sponge assemblages and benthic-pelagic carbon flow

In my study, the seagrass sponge assemblage was estimated to remove picoplankton-derived carbon at a rate of 84.1, 2737.3, and 673.9 g C m^{-2} d^{-1} during high tide at high-shore, middle, and near-reef-flat zones of the seagrass bed, respectively. The different amounts of carbon consumed between zones were due to the differences in sponge abundance (biomass), the relative composition of heterotrophic and autotrophic sponges at each zone, and the removal efficiency of each picoplankton group. The available picoplankton abundance at each zone also contributed to the removed carbon calculations as every area (sites and zones) holds a different water volume. The amount of carbon being removed in my study areas is generally larger when

compared to other studies. For example, a study in a temperate zone by Perea-Blazquez et al. (2012) estimated that sponge assemblages remove only up to 3.5 g C m^{-2} d^{-1} from feeding on picoplankton. The much lower removed carbon reported by this earlier studies, compared to my study areas, was most likely due to much less sponge biomass at their study site, as the study assumed that the site had sponge coverage of 5% m^{-2} with 1 cm sponge thickness to estimate the amount of sponge abundance (based on volume) that was involved in carbon removal, compared to my study site (see Table 5.1). The amount of carbon flowing through sponges in my study reaffirms the important role of sponges in benthic-pelagic carbon flow.

In the tropical oligotrophic waters, picoplankton abundance varies very little over various time scales (Campbell et al. 1997), and protozoans are the main predators of these picoplankton groups. However, with the high sponge-mediated flux for picoplankton that my results showed, the role of sponge assemblages in keeping the marine microbial food web in equilibrium cannot be ignored, especially in habitats where sponges are very abundant and the water shallow. *Synechococcus* appeared to be the common food across all studied sponges in dark and light incubations (representing day and night-time). At night-time, when the natural flux of *Synechococcus* showed a decline of 2.4 ± 0.8 % cells mL^{-1} h^{-1} rate, presumably caused by cell lysis and protozoan grazing, the sponge-mediated flux showed a reduction of 1–4 % cells mL^{-1} g_{AFDW}^{-1} h^{-1}. Coupled with high sponge abundance (biomass) in the habitat, sponge assemblage potentially plays a major role in keeping the daily abundance of *Synechococcus* in the water column stable as it had a high population growth rate during light incubations. The same contribution was also shown by some of the sponges, but not all. *Spongia* sp. and *Dactylospongia elegans* reduced picoeukaryotes concentration during night-time at the rate of 3.5 ± 1.1 and 1.0 ± 0.1 % cells mL^{-1} h^{-1}, respectively. While this group's natural flux (caused by cell lysis and protozoan predation) only caused a reduction of 5.3 ± 2.8 % cells mL^{-1} h^{-1} at night, the contribution of these sponges to balance the abundance of picoeukaryotes was significant. However, not all sponges were observed to remove heterotrophic bacteria from the water column in dark incubations, in the period where this picoplankton group showed a significantly high mortality rate (32.3 ± 12.5 % cells mL^{-1} h^{-1}; see Table 5.2). The heterotrophic sponges were removing heterotrophic bacteria during the time when heterotrophic bacteria were observed to have a cell division phase (light incubations). This suggests that the sponges can balance the daily abundance of heterotrophic bacteria in the water column, although not as much as the combination of their own cell lysis and the grazing by protozoans.

Since the amount of picoplankton-derived carbon removed by heterotrophic and autotrophic sponges was notably different, the importance of the sponge assemblage in removing living particulate organic carbon from the water column depends partly on the sponge nutritional mode. In the area where the sponge assemblage has an equal proportion of heterotrophic and autotrophic sponges (high-shore and middle tidal zones), the removed picoplankton-derived carbon by heterotrophic sponges far exceeded that of the autotrophic sponges. Only in the area where autotrophic sponges contributed 97–98 % of the total sponge biomass – the near-reef-flat zone of Hoga-1 (Chapter 3), autotrophic sponges were estimated to remove more picoplankton-derived carbon than the heterotrophic sponges. Even in the area where autotrophic sponges contributed up to 78–80 % of the total sponge biomass (the near-reef-flat zone of Hoga-2; Chapter 3), it was the heterotrophic sponges that were found to remove more carbon. Considering that the autotrophic sponges are net oxygen producers (although not all; see Chapter 3), releasing organic carbon during daytime (Chapter 4), and now in this study, they obtained much less organic carbon from suspension-feeding, we need to reconsider the role of sponges in the flow of matter and energy in their habitats.

Conclusion

This study reaffirms the importance of sponge assemblages in linking pelagic and benthic environments in the flow of matter and energy, as the sponges were found to remove a significant amount of picoplankton-derived organic carbon from the water column. The role of sponges in keeping the abundance of picoplankton in equilibrium, which ensures the stability of microbial food web, cannot be ignored. Sponge nutritional mode heavily influenced the amount of the removed carbon, supporting the hypothesis that autotrophic sponges do not have to obtain their nutritional needs from suspension feeding since they are already sufficiently supplied by the host photosymbionts. At the habitat level, heterotrophic sponges contributed a large portion of the removed carbon from the sponge assemblage, even though their biomass portion was much smaller than that of the autotrophic sponges. This study clarifies the significant role of sponges in the environment’s carbon flow.

CHAPTER 6.

General discussion

Thesis overview

The overall aim of my thesis was to increase our understanding of the interactions between sponge assemblages and the water column in shallow-water seagrass ecosystems within the Indo-Pacific bioregion. In particular, I investigated how sponges with different nutritional modes might influence water column dynamics. I studied the sponge assemblages at three tidal zones that inhabit a seagrass meadow in the Wakatobi National Park, Indonesia.

I conducted sponge and seagrass surveys to investigate community structure, distribution patterns, and potential drivers of the patterns I observed. Then, I measured the P:R ratio of the most abundant sponge species to determine sponge nutritional mode (heterotrophic or autotrophic). Using this information, I then measured the sponge-mediated organic carbon flux of the heterotrophic and autotrophic sponges during the day and night-time (represented by light and dark incubations) and calculated the daily sponge-mediated net carbon flux at the assemblage level in my studied seagrass meadow. Lastly, I investigated the influence of sponges on the picoplankton community in the water column by measuring the sponge-mediated picoplankton flux by heterotrophic and autotrophic sponges during the day and night, and scaled this up to the assemblage level impact.

I found that sponges inhabited all tidal zones of the seagrass meadow, even the high-shore zone – an area that regularly experiences aerial exposure during low tide. From my observations, sponge morphology may have aided their adaptation to the prevailing environmental conditions in the different tidal zones. Based on the P:R ratios, I found that six out of eight studied sponges (representing 80% of the total sponge species found in the seagrass meadow) were autotrophic, with five of them being net oxygen producers over 24 hours. Autotrophic sponges were found in all tidal zones, including the high-shore zone, and contributed considerably to the seagrass sponge assemblage biomass at all tidal zones. In the near-reef-flat zone, the autotrophic sponges contributed up to 98% of the total sponge biomass.

With respect to effects of sponge on the water column carbon balance, I found that heterotrophic and autotrophic sponges influenced the water column differently during the daytime. The autotrophic sponges showed net release of carbon while the heterotrophic sponges only removed it. However, both heterotrophic and autotrophic sponges were observed to be net removers of organic carbon during the night time with no significant difference in removal rates between the two nutritional modes. This suggests that their different interactions with the water

column during daytime were related to photosynthetic activity by the symbionts hosted by the autotrophic sponges. However, at the sponge assemblage level, sponge feeding still resulted in net carbon removal from the water column over 24-hours (one day-night cycle). With respect to the influence of sponges on picoplankton abundance, I found that heterotrophic sponges removed 40–60 times more picoplankton-derived organic carbon than autotrophic sponges over 24 hours, supporting the hypothesis that autotrophic sponges gain supplementary nutrition from their photosynthetic symbionts, which likely eases the pressure to fulfil their nutritional needs from suspension feeding.

The role of sponges in seagrass energy flow

To understand ecosystem dynamics and predict the impact of environmental change, we need to know how each organism contributes to food web dynamics (Krebs 2009). Traditionally, sponges have been viewed as consumers of carbon in benthic environments. Researchers were mainly focused on how much organic matter sponges were removing from the water column as the result of their feeding (e.g. Reiswig 1975; Gili and Coma 1998; Hadas and Marie 2006). Furthermore, the ability of sponges to efficiently filter dissolved (e.g. Yahel et al. 2003; de Goeij et al. 2008) and particulate organic carbon (Hadas et al. 2009) have supported and solidified this view on sponge-water column interactions. However, not all sponges are able to remove dissolved organic carbon from the water column. Wilkinson (1983) reported that some sponges actually release dissolved organic carbon as well as being net oxygen producers (i.e. autotrophic). However, little effort has been made to consider further sponge contributions to oxygen and organic carbon to the water column since this early work. My thesis has attempted to fill some of this knowledge gap.

Sponges do not photosynthesize, but they can facilitate the photosynthetic symbionts (Bell 2008). As sponges and their hosted photosynthetic symbionts interact with the environment as one entity (i.e. as a holobiont; Pita et al. 2018), autotrophic sponges have similar traits to true autotrophs with respect to their water column interactions. First, in transforming solar energy into chemical energy, autotrophs release oxygen. When oxygen production exceeds the oxygen required for respiration, the autotrophs contribute excess oxygen to the environment, which other organisms can then use (Krebs 2009). When an autotroph lives inside another organism (i.e. endosymbiosis), the mechanism of releasing oxygen into the environment might be more complex than for “standalone” autotrophs. However, little is known about the intercellular

oxygen transfer from the photosynthetic symbionts to the sponge host. Most of the studies on symbioses in the aquatic environment have measured the oxygen flux in the water column, including my study, to determine the net water column oxygen balance through the holobiont. In the case of sponge holobionts, the contribution of autotrophic sponges to the water column oxygen concentration depends on the balance of their daily production (which only occurs in the daytime) and respiration (24 h). Chapter 3 showed that five autotrophic sponges were net oxygen producers over each full day-night cycle (24 h). Photosynthesis by the photosynthetic symbionts that the sponge hosted produced more oxygen than the entire holobiont respired, thus providing the environment with excess oxygen. Photosynthesis by aquatic autotrophs is crucial since it is responsible for about half of our planet's oxygen production (Field et al. 1998b). Considering there is a growing concern over declining oxygen concentrations in the global oceans and coastal waters due to global warming and pollution (Breitburg et al. 2018), the role of sponges in facilitating primary production might become critical. All this time, the contribution of sponge symbioses-derived oxygen production to the environment has been generally overlooked. For example, the autotrophic sponge *Spheciospongia* sp. was the most abundant sponge in my studied seagrass bed (in the middle-shore) with 0.588 ± 0.339 g_{AFDW} m^{-2} (Chapter 2). This sponge had a net flux of oxygen production of ~0.44 mol g_{AFDW}^{-1} d^{-1} (Chapter 3), which resulted in the release of ~0.25 mol of oxygen for every one m^2 of seagrass in my study area. For comparison, the average oxygen production for every one m^2 of seagrass bed has been estimated at 0.45 mol a day (e.g. Yarbro and Carlson 2008; Reynolds et al. 2018). That means autotrophic sponge-derived oxygen production coupled with high abundance could make an important contribution to the environment's oxygen supply.

Another feature of aquatic autotrophs is the potential for organic carbon release into the water column, as observed in macroalgae (e.g. Moebus and Johnson 1974; Brylinsky 1977) and phytoplankton (López-Sandoval et al. 2013). With respect to sponge–photosynthetic symbioses, some photosynthate is translocated from the symbiont to the sponge host intracellularly, while the rest is released to the surrounding waters (Wilkinson 1980; Wilkinson 1983). Wilkinson (1980) reported that 9–17% of the total photosynthate of the photosynthetic symbionts was translocated to the sponge host in the form of sucrose and glucose. In addition, the sponges could also utilise the released photosynthate in the form of dissolved organic carbon through a suspension-feeding mechanism. My study (Chapter 4) measured the sponge-mediated organic carbon flux in the water column, representing the balance of the release and organic carbon removal by sponges. My results showed that all autotrophic sponges released more

organic carbon than they removed during light incubations (representing daytime). I suggest that this released carbon was photosynthesis-related, which means it was likely in the form of dissolved organic carbon (DOC). Besides being consumed again by the sponges, this excess of photosynthetically-fixed DOC could also fuel the microbial loop (Azam et al. 1983), passing energy to higher trophic levels.

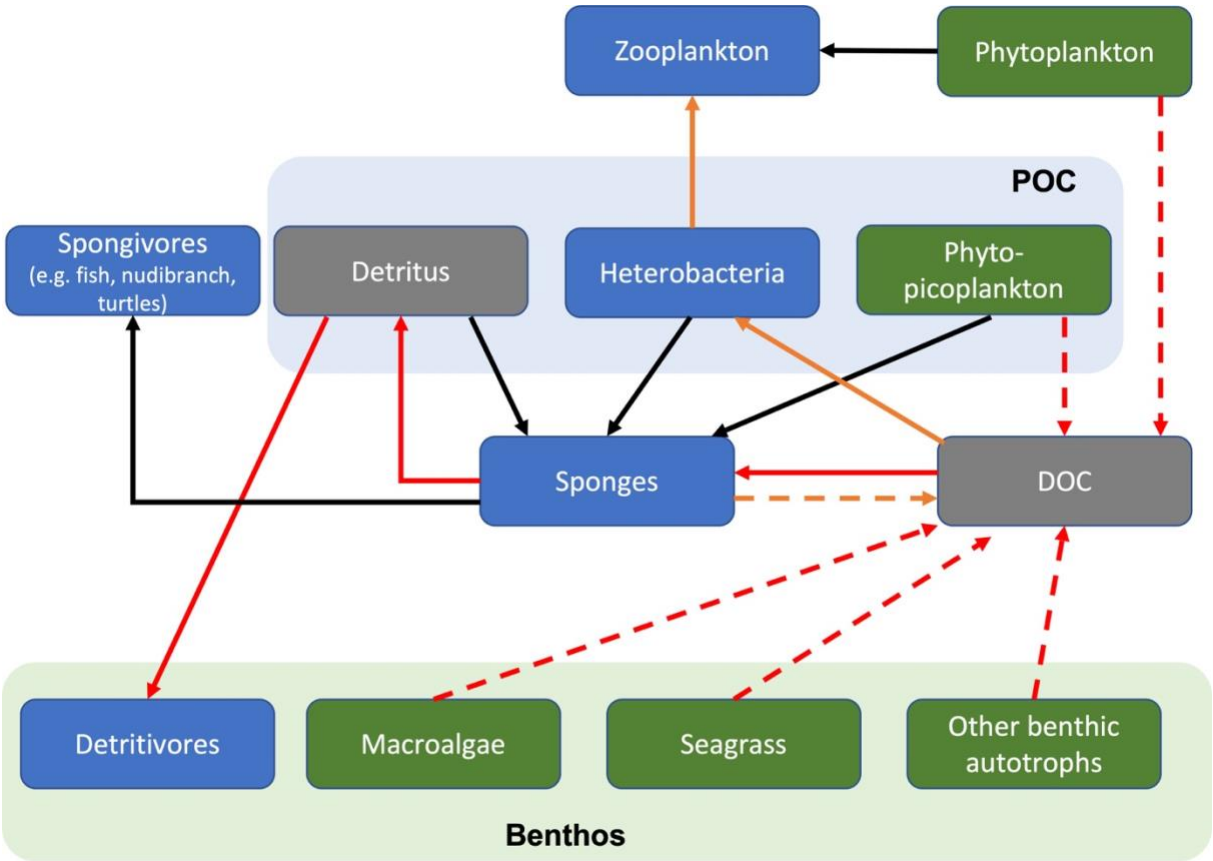

Figure 6.1. A conceptual diagram of carbon flow through sponges in seagrass beds incorporating the microbial and sponge loops. Green compartments represent primary producers, blue consumers, and grey represent non-living organic carbon pools (DOC and POC-detritus). Dashed red arrows indicate DOC release to the water column by the autotrophs. Solid red arrows indicate the flow of carbon in the sponge loop, whereas solid orange arrows show the microbial loop carbon flow. The dashed orange arrow indicates the release of DOC, exclusively by autotrophic sponges, to the water column. The solid black arrows represent the flow of carbon through feeding mechanisms outside of microbial and sponge loops.

The bulk pool of DOC in the oceans is divided into labile, semi-labile, and refractory fractions based on their turnover time (see review by Ogawa and Tanoue 2003). The labile fraction of the DOC, which is only about 2% of the total DOC, is rapidly turned over (hours to daily), while semi-labile DOC takes weeks to months to be cycled by bacteria. The largest portion of

the DOC pool – the refractory fraction, needs centuries to millennia to be biologically cycled. The photosynthetically-fixed carbon by aquatic autotrophs is generally the labile form so that it will stimulate the rapid growth of bacterioplankton in the water column (Azam and Malfatti 2007; Haas et al. 2011). Sponges are assumed to consume labile and semi-labile DOC (i.e. labile and semi-labile DOC; e.g. de Goeij et al. 2008), the same DOC fractions utilised by microbes (heterotrophic bacteria), which means that they may be competing to use the exudated DOC. But it is also important to note that other mineral nutrients might limit the growth of heterotrophic bacteria, such as nitrogen (N), phosphorus (P), and iron (Fe), which the heterotrophic bacteria might have to compete with the phytoplankton for (Kirchman 1994; Tortell et al. 1999; Thingstad 2008). Therefore, even though the microbes that use DOC have a rapid growth rate and lifecycle (Pomeroy 1974), that does not necessarily mean the sponges are outcompeted. Nevertheless, there is a chance for the autotrophic sponge-exudated organic carbon to fuel the microbial loop. Since this DOC release only happens during the daytime, thus this might contribute to microbial diel patterns.

Chapter 5 showed that sponges with both nutritional modes removed picoplankton over 24 hours (day-night cycle). Therefore irrespective of nutritional mode, sponges are still consumers of picoplankton in my studied seagrass bed. This feature of sponges is vital to maintaining the food web equilibrium. For example, the decimation of the sponge assemblages was reported as the main factor behind plankton blooms in Florida Bay, demonstrating the critical role of sponges in controlling picoplankton abundance (Peterson et al. 2006). From my results, the difference between the heterotrophic and autotrophic sponges was in their removal rates. By removing 40–60 times more picoplankton-derived organic carbon than the autotrophic sponges (Chapter 5), heterotrophic sponges potentially play a more critical role in controlling the picoplankton population, particularly in controlling the heterotrophic bacteria in the water column.

The implications of sponge nutritional mode in a changing environment

Our world faces rapid ocean warming and acidification that affects many marine organisms and disrupts ecosystem functioning (e.g. Hoegh-Guldberg et al. 2007; Ramírez et al. 2017; Hughes et al. 2018). Some sponges are predicted to become "winners" on coral reefs in the face of climate change in the near-future scenarios, and in some cases, sponges may take over from corals as the dominant organisms (Bell et al. 2013; Bell et al. 2018a; Bell et al. 2018b). Non-calcifying sponges, particularly bioeroder sponges, are also thought to benefit from ocean acidification (Duckworth and Peterson 2013; Wisshak et al. 2013). Photosymbiont-containing sponges also seem more resistant to rising sea-surface temperature than corals. Temperature rise has also been reported to increase the productivity of *Synechococcus* – a common symbiont hosted by sponges (Fu et al. 2007). Since seagrass beds are considered extreme environments (e.g. already experiencing high temperatures and light exposure during low tide and water turbidity), we can expect the seagrass sponges to be among the first to be impacted by climate change.

Recently there has been considerable discussion on how sponges might or might not persist under near-future climate scenarios (Bell et al. 2018a; Lesser and Slattery 2020). Instead of favouring sponge-dominated regime shifts, climate change might actually limit the growth of sponge populations due to decreased food availability (Lesser and Slattery 2020). Lesser and Slattery (2020) highlighted that new climate-change-derived oceanographic conditions might cause a decline in picoplankton abundance, the sponges' main POC diet, and the producers of about 50% of the ocean's dissolved organic matter – another component of sponge diet, causing a bottom-up forcing. I would argue that the existence of autotrophic sponges in the sponge assemblage might overcome this situation for at least two reasons. First, autotrophic sponges do not consume much picoplankton compared to heterotrophic sponges. My results showed that the autotrophic sponges consumed only one-sixtieth to one-fortieth of picoplankton-derived organic carbon compared to the consumption of heterotrophic sponges. The bottom-up forcing is eased as the autotrophic sponges receive supplementary nutrition from their photosynthetic symbionts. Second, the exudation of photosynthesis-fixed organic carbon by the autotrophic sponges makes them part of the microbial loop. The heterotrophic bacteria can utilise the exudated DOC by the autotrophic sponges to grow (Pomeroy 1974), and then the sponges can consume these microbes. From my results (Chapter 5), it was the heterotrophic sponges that consumed heterotrophic bacteria, as autotrophic sponges were not observed to remove

heterotrophic bacteria from the water column. To conclude, the combination of autotrophic and heterotrophic sponges could overcome some of the potential food limitation issue (Lesser and Slattery 2020) and keep the picoplankton abundance equilibrium.

Future directions

My thesis has revealed differences in how heterotrophic and autotrophic sponges interact with the water column carbon flow in a seagrass ecosystem. Symbioses with photosynthetic symbionts are commonly seen as a strategy to overcome the low water nutrient concentrations in many tropical environments (Wilkinson and Cheshire 1990). However, the water column of seagrass meadows has more nutrients than coral reefs; therefore low nutrients might not be the environmental conditions that have led to the evolution of symbioses in seagrass sponges. It would be interesting to see if the sponge assemblages in the other tropical marine ecosystems, including coral reefs and mangroves, share the same patterns as I found for seagrass sponges, to understand better the ecosystem dynamics and sponge assemblage role in those ecosystems. It will also be important to consider the reliance of sponges on symbionts from other bioregions to assess the wider applicability of the patterns I have observed. This information will shed light on the underlying conditions that have led to the evolution of the sponge–photosynthetic symbiont relationships and provide a better understanding of how changes might affect the environments where sponges occur.

Autotrophic sponges at my studied seagrass meadow were observed to inhabit all tidal zones of the seagrass meadow, including the area regularly exposed to air – the high-shore zone. While sponges seem to have some morphological adaptations to cope with the physical conditions of each zone (Chapter 2 and 3), my thesis did not look at the photosynthetic symbionts and their adaptations. Therefore, I suggest identifying the sponge symbionts and investigating their adaptation to various environmental conditions as the next steps. This information will further our understanding of how the sponges and their symbionts respond to different environmental conditions and how environmental change will affect sponge assemblages.

I also recommend studying the flow of sponge-mediated carbon in the environment, both to the planktonic microbes (i.e. microbial loop; Azam et al. 1983) and via detritus pathways (i.e. sponge loop; de Goeij et al. 2013), by autotrophic and heterotrophic sponges. This information can then be used to develop more reliable models for the ecosystem dynamics where sponges

occur. Furthermore, I found synchronised diel patterns between picoplankton groups and the sponge consumption preferences and rates, we need to determine the mechanism behind this phenomenon. The knowledge of this phenomenon will further our understanding of the flow of matter and energy in marine ecosystems.

Concluding remarks

My thesis has shown differences between heterotrophic and autotrophic sponges in their interactions with the water column, where autotrophic sponges have some of the characteristics of other aquatic autotrophs. I propose that autotrophic sponges are also part of the microbial loop, whereby the exudated DOC is contributing to the DOC pool in the water column (Figure 6.1). Furthermore, the coexistence of the two nutritional modes in sponge assemblages could help each other persist in changing environments and might be the key to future sponge success.

References

Achlatis M, Pernice M, Green K, Guagliardo P, Kilburn MR, Hoegh-Guldberg O, Dove S (2018) Single-cell measurement of ammonium and bicarbonate uptake within a photosymbiotic bioeroding sponge. The ISME Journal 12: 1308-1318. https://doi.org/10.1038/s41396-017-0044-2

Achlatis M, Schönberg CHL, van der Zande RM, LaJeunesse TC, Hoegh-Guldberg O, Dove S (2019) Photosynthesis by symbiotic sponges enhances their ability to erode calcium carbonate. Journal of Experimental Marine Biology and Ecology 516: 140-149. https://doi.org/10.1016/j.jembe.2019.04.010

Achlatis M, van der Zande RM, Webb AE, de Bakker DM, de Nooijer LJ, de Goeij JM (2021) Photosynthetically stimulated bioerosion in symbiotic sponges: the role of glycerol and oxygen. Coral Reefs 40: 881-891. https://doi.org/10.1007/s00338-021-02091-0

Adlard RD, Lester RJG (1995) Development of a diagnostic test for *Mikrocytos roughleyi*, the aetiological agent of Australian winter mortality of the commercial rock oyster, *Saccostrea commercialis* (Iredale & Roughley). Journal of Fish Diseases 18: 609-614. https://doi.org/10.1111/j.1365-2761.1995.tb00365.x

Allan JD (1995) Autotrophs Stream Ecology: Structure and function of running waters. Springer Netherlands, Dordrecht, pp 83-108

Allen GR (2000) Indo-Pacific coral-reef fishes as indicators of conservation hotspots 9th International Coral Reef Symposium, Bali, Indonesia.

Alvin AC, Brett KB, Tadashi M (2000) Diversity of dinoflagellate symbionts (zooxanthellae) in a host individual. Marine Ecology Progress Series 195: 93-100. https://doi.org/10.3354/meps195093

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK

Archer SK, Hensel E, Layman CA (2018) Ambient nutrient availability drives the outcome of an interaction between a sponge (*Halichondria melanadocia*) and seagrass (*Thalassia testudinum*). Journal of Experimental Marine Biology and Ecology 503: 86-91. https://doi.org/10.1016/j.jembe.2018.02.005

Arillo A, Bavestrello G, Burlando B, Sarà M (1993) Metabolic integration between symbiotic cyanobacteria and sponges: a possible mechanism. Marine Biology 117: 159-162. https://doi.org/10.1007/BF00346438

Attrill MJ, Strong JA, Rowden AA (2000) Are macroinvertebrate communities influenced by seagrass structural complexity? Ecography 23: 114-121. https://doi.org/10.1111/j.1600-0587.2000.tb00266.x

Ávila E, Ávila-García AK, Cruz-Barraza JA (2015) Temporal and small-scale spatial variations in abundance and biomass of seagrass-dwelling sponges in a tropical estuarine system. Marine Ecology 36: 623-636. https://doi.org/10.1111/maec.12171

Ayling AL (1980) Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine Demospongiae. The Biological Bulletin 158: 271-282. https://doi.org/10.2307/1540854

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10: 257-263. https://doi.org/10.3354/meps010257

Azam F, Malfatti F (2007) Microbial structuring of marine ecosystems. Nature Reviews Microbiology 5: 782-791. https://doi.org/10.1038/nrmicro1747

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: the role of the host. Trends in Ecology Evolution 24: 16-20. https://doi.org/10.1016/j.tree.2008.09.005

Balmford A, Gaston KJ (1999) Why biodiversity surveys are good value. Nature 398: 204-205. https://doi.org/10.1038/18339

Barnes DKA (1999) High diversity of tropical intertidal zone sponges in temperature, salinity and current extremes. African Journal of Ecology 37: 424-434. https://doi.org/10.1046/j.1365-2028.1999.00197.x

Barnes DKA, Bell JJ (2002) Coastal sponge communities of the West Indian Ocean: taxonomic affinities, richness and diversity. African Journal of Ecology 40: 337-349. https://doi.org/10.1046/j.1365-2028.2002.00387.x

Battershill C, Bergquist P (2010) Phylum Porifera. In: Cook SdC (ed) New Zealand Coastal Marine Invertebrates 1. Canterbury University Press, Christchurch, pp 58-135

Becker CC, Weber L, Suca JJ, Llopiz JK, Mooney TA, Apprill A (2020) Microbial and nutrient dynamics in mangrove, reef, and seagrass waters over tidal and diurnal time scales. Aquatic Microbial Ecology 85: 101-119. https://doi.org/10.3354/ame01944

Becking LE, Cleary DFR, de Voogd NJ (2013) Sponge species composition, abundance, and cover in marine lakes and coastal mangroves in Berau, Indonesia. Marine Ecology Progress Series 481: 105-120. https://doi.org/10.3354/meps10155

Beepat SS, Woods L, Davy SK, Jhugroo K, Bell JJ (2020) Temporal variability in tropical lagoon sponges from Mauritius (Western Indian Ocean). Marine Biodiversity 50: 57. https://doi.org/10.1007/s12526-020-01079-3

Beer S, Ilan M (1998) In situ measurements of photosynthetic irradiance responses of two Red Sea sponges growing under dim light conditions. Marine Biology 131: 613-617. https://doi.org/10.1007/s002270050353

Bell JJ (2004) Evidence for morphology-induced sediment settlement prevention on the tubular sponge *Haliclona urceolus*. Marine Biology 146: 29-38. https://doi.org/10.1007/s00227-004-1429-0

Bell JJ (2008) The functional roles of marine sponges. Estuarine, Coastal and Shelf Science 79: 341-353. https://doi.org/10.1016/j.ecss.2008.05.002

Bell JJ, Barnes DKA (2000) The influences of bathymetry and flow regime upon the morphology of sublittoral sponge communities. Journal of the Marine Biological Association of the United Kingdom 80: 707-718. https://doi.org/10.1017/s0025315400002538

Bell JJ, Bennett HM, Rovellini A, Webster NS (2018a) Sponges to be winners under near-future climate scenarios. BioScience 68: 955-968. https://doi.org/10.1093/biosci/biy142

Bell JJ, Biggerstaff A, Bates T, Bennett H, Marlow J, McGrath E, Shaffer M (2017) Sponge monitoring: Moving beyond diversity and abundance measures. Ecological Indicators 78: 470-488. https://doi.org/10.1016/j.ecolind.2017.03.001

Bell JJ, Davy SK, Jones T, Taylor MW, Webster NS (2013) Could some coral reefs become sponge reefs as our climate changes? Global Change Biology 19: 2613-2624. https://doi.org/10.1111/gcb.12212

Bell JJ, McGrath E, Biggerstaff A, Bates T, Cardenas CA, Bennett H (2015) Global conservation status of sponges. Conservation Biology 29: 42-53. https://doi.org/10.1111/cobi.12447

Bell JJ, McGrath E, Kandler NM, Marlow J, Beepat SS, Bachtiar R, Shaffer MR, Mortimer C, Micaroni V, Mobilia V, Rovellini A, Harris B, Farnham E, Strano F, Carballo JL (2020) Interocean patterns in shallow water sponge assemblage structure and function. Biological Review 95: 1720-1758. https://doi.org/10.1111/brv.12637

Bell JJ, Rovellini A, Davy SK, Taylor MW, Fulton EA, Dunn MR, Bennett HM, Kandler NM, Luter HM, Webster N (2018b) Climate change alterations to ecosystem dominance: how might sponge-dominated reefs function? Ecology 99: 1920-1931. https://doi.org/10.1002/ecy.2446

Bell JJ, Smith D (2004) Ecology of sponge assemblages (Porifera) in the Wakatobi region, south-east Sulawesi, Indonesia: richness and abundance. Journal of the Marine Biological Association of the United Kingdom 84: 581-591. https://doi.org/10.1017/S0025315404009580h

Bellwood DR, Wainwright PC (2002) The history and biogeography of fishes on coral reefs. In: Sale PF (ed) Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem. Elsevier Science & Technology, pp 5-32

Bergquist P (1995) Dictyoceratida and Verongida from the New Caledonian lagoon (Porifera: Demospongiae). Memoirs of the Queensland Museum 38: 1-51

Bergquist PR (1978) Sponges. University of California Press, Berkeley and Los Angeles

Bergquist PR, Ayling AM, Wilkinson CR (1988) Foliose *Dictyoceratida* of the Australian Great Barrier Reef. Marine Ecology 9: 291-319. https://doi.org/10.1111/j.1439-0485.1988.tb00209.x

Berninger U-G, Finlay BJ, Kuuppo-Leinikki P (1991) Protozoan control of bacterial abundances in freshwater. Limnology and Oceanography 36: 139-147. https://doi.org/10.4319/lo.1991.36.1.0139

Bertilsson S, Berglund O, Pullin MJ, Chisholm SW (2005) Release of dissolved organic matter by *Prochlorococcus*. Vie Et Milieu 55: 225-231

Biggerstaff A, Smith DJ, Jompa J, Bell JJ (2015) Photoacclimation supports environmental tolerance of a sponge to turbid low-light conditions. Coral Reefs 34: 1049-1061. https://doi.org/10.1007/s00338-015-1340-9

Biggerstaff A, Smith DJ, Jompa J, Bell JJ (2017) Metabolic responses of a phototrophic sponge to sedimentation supports transitions to sponge-dominated reefs. Scientific Reports 7: 2725. https://doi.org/10.1038/s41598-017-03018-y

Binder BJ, DuRand MD (2002) Diel cycles in surface waters of the equatorial Pacific. Deep Sea Research Part II: Topical Studies in Oceanography 49: 2601-2617. https://doi.org/10.1016/S0967-0645(02)00050-4

Blackman FF (1905) Optima and limiting factors. Annals of Botany 19: 281-296. https://doi.org/10.1093/oxfordjournals.aob.a089000

Blackman FF, Matthaei GLC (1905) Experimental researches in vegetable assimilation and respiration. IV.—A quantitative study of carbon dioxide assimilation and leaf-temperature in natural illumination. Proceedings of the Royal Society of London Series B 76: 402-460. https://doi.org/10.1098/rspb.1905.0037

Bordenstein SR, Theis KR (2015) Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. PLoS Biol 13: e1002226. https://doi.org/10.1371/journal.pbio.1002226

Bosch TC, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. Zoology 114: 185-190. https://doi.org/10.1016/j.zool.2011.04.001

Boström C, Pittman SJ, Simenstad C, Kneib RT (2011) Seascape ecology of coastal biogenic habitats: advances, gaps, and challenges. Marine Ecology Progress Series 427: 191-217. https://doi.org/10.3354/meps09051

Bowen BW, Rocha LA, Toonen RJ, Karl SA, ToBo L (2013) The origins of tropical marine biodiversity. Trends in Ecology & Evolution 28: 359-366. https://doi.org/10.1016/j.tree.2013.01.018

Bowerbank J (1877) Description of five new species of sponges discoverd by AB Meyer on the Philippine Islands and New Guinea. Proceedings of the Zoological Society of London 1877: 456-464

Boyce DG, Lewis MR, Worm B (2010) Global phytoplankton decline over the past century. Nature 466: 591-596. https://doi.org/10.1038/nature09268

BPS-Statistics (2019) Wakatobi Regency in Figures 2019. BPS-Statistics of Wakatobi Regency, Wakatobi

Breitburg D, Levin LA, Oschlies A, Gregoire M, Chavez FP, Conley DJ, Garcon V, Gilbert D, Gutierrez D, Isensee K, Jacinto GS, Limburg KE, Montes I, Naqvi SWA, Pitcher GC, Rabalais NN, Roman MR, Rose KA, Seibel BA, Telszewski M, Yasuhara M, Zhang J

(2018) Declining oxygen in the global ocean and coastal waters. Science 359. https://doi.org/10.1126/science.aam7240

Brylinsky M (1977) Release of dissolved organic matter by some marine macrophytes. Marine Biology 39: 213-220. https://doi.org/10.1007/BF00390995

Buitenhuis ET, Li WKW, Vaulot D, Lomas MW, Landry MR, Partensky F, Karl DM, Ulloa O, Campbell L, Jacquet S, Lantoine F, Chavez F, Macias D, Gosselin M, McManus GB (2012) Picophytoplankton biomass distribution in the global ocean. Earth System Science Data 4: 37-46. https://doi.org/10.5194/essd-4-37-2012

Bullerjahn GS, Post AF (1993) The prochlorophytes: are they more than just chlorophyll a/b-containing cyanobacteria? Critical Reviews in Microbiology 19: 43-59. https://doi.org/10.3109/10408419309113522

Burgsdorf I, Sizikov S, Squatrito V, Britstein M, Slaby BM, Cerrano C, Handley KM, Steindler L (2021) Lineage-specific energy and carbon metabolism of sponge symbionts and contributions to the host carbon pool. The ISME Journal. https://doi.org/10.1038/s41396-021-01165-9

Camp EF, Krause SL, Santos LM, Naumann MS, Kikuchi RK, Smith DJ, Wild C, Suggett DJ (2015) The "Flexi-Chamber": A novel cost-effective In situ respirometry chamber for coral physiological measurements. PLoS One 10: e0138800. https://doi.org/10.1371/journal.pone.0138800

Campbell L, Liu H, Nolla HA, Vaulot D (1997) Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event. Deep Sea Research Part I: Oceanographic Research Papers 44: 167-192. https://doi.org/10.1016/S0967-0637(96)00102-1

Campbell L, Vaulot D (1993) Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep Sea Research Part I: Oceanographic Research Papers 40: 2043-2060. https://doi.org/10.1016/0967-0637(93)90044-4

Carballo JL, Ávila E (2004) Population dynamics of a mutualistic interaction between the sponge *Haliclona caerulea* and the red alga *Jania adherens*. Marine Ecology Progress Series 279: 93-104. https://doi.org/10.3354/meps279093

Carballo JL, Sanchez-Moyano JE, Garcia-Gomez JC (1994) Taxonomic and ecological remarks on boring sponges (Clionidae) from the Straits of Gibraltar (southern Spain): tentative bioindicators? Zoological Journal of the Linnean Society 112: 407-424. https://doi.org/10.1111/j.1096-3642.1994.tb00329.x

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. Nature 486: 59-67. https://doi.org/10.1038/nature11148

Carlson C (2002) Chapter 4 - Production and removal processes. Biogeochemistry of Marine Dissolved Organic Matter. Elsevier, pp 91-151

Caron DA, Lim EL, Miceli G, Waterbury JB, Valois FW (1991) Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. Marine Ecology Progress Series 76: 205-217. https://doi.org/10.3354/meps076205

Carpenter KE, Springer VG (2005) The center of the center of marine shore fish diversity: the Philippine Islands. Environmental Biology of Fishes 72: 467-480. https://doi.org/10.1007/s10641-004-3154-4

Carruthers TJB, Dennison WC, Kendrick GA, Waycott M, Walker DI, Cambridge ML (2007) Seagrasses of south–west Australia: A conceptual synthesis of the world's most diverse and extensive seagrass meadows. Journal of Experimental Marine Biology and Ecology 350: 21-45. https://doi.org/10.1016/j.jembe.2007.05.036

Carruthers TJB, Dennison WC, Longstaff BJ, Waycott M, Abal EG, McKenzie LJ, Long WJL (2002) Seagrass habitats of Northeast Australia: Models of key processes and controls. Bulletin of Marine Science 71: 1153-1167

Chassot E, Bonhommeau S, Dulvy NK, Melin F, Watson R, Gascuel D, Le Pape O (2010) Global marine primary production constrains fisheries catches. Ecol Lett 13: 495-505. https://doi.org/10.1111/j.1461-0248.2010.01443.x

Cheshire AC, Wilkinson CR, Seddon S, Westphalen G (1997) Bathymetric and seasonal changes in photosynthesis and respiration of the phototrophic sponge *Phyllospongia lamellosa* in comparison with respiration by the heterotrophic sponge *Ianthella basta* on Davies Reef, Great Barrier Reef. Marine and Freshwater Research 48: 589-599. https://doi.org/10.1071/MF96070

Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB, Welschmeyer NA (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature 334: 340-343. https://doi.org/10.1038/334340a0

Chombard C, Boury-Esnault N, Tillier S (1998) Reassessment of homology of morphological characters in Tetractinellid sponges based on molecular data. Systematic Biology 47: 351-366. https://doi.org/10.1080/106351598260761

Christaki U, Jacquet S, Dolan JR, Vaulot D, Rassoulzadegan F (1999) Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. Limnology and Oceanography 44: 52-61. https://doi.org/10.4319/lo.1999.44.1.0052

Cleary DFR, de Voogd NJ (2007) Environmental associations of sponges in the Spermonde Archipelago, Indonesia. Journal of the Marine Biological Association of the United Kingdom 87: 1669-1676. https://doi.org/10.1017/s0025315407052770

Coma R, Ribes M, Gili JM, Hughes RN (2001) The ultimate opportunists: consumers of seston. Marine Ecology Progress Series 219: 305-308. https://doi.org/10.3354/meps219305

Cowman PF (2014) Historical factors that have shaped the evolution of tropical reef fishes: a review of phylogenies, biogeography, and remaining questions. Frontiers in Genetics 5. https://doi.org/10.3389/fgene.2014.00394

Crutzen PJ (2002) Geology of Mankind. Nature 415: 23. https://doi.org/10.1038/415023a

Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis. Microbiology and Molecular Biology Reviews 76: 229-261. https://doi.org/10.1128/MMBR.05014-11

de Goeij JM, Lesser MP, Pawlik JR (2017) Nutrient Fluxes and Ecological Functions of Coral Reef Sponges in a Changing Ocean. In: Carballo JL, Bell JJ (eds) Climate Change, Ocean Acidification and Sponges: Impacts Across Multiple Levels of Organization. Springer International Publishing, Cham, pp 373-410

de Goeij JM, Oevelen Dv, Vermeij MJA, Osinga R, Middleburg JJ, Goeij AFPMd, Admiraal W (2013) Surviving in a marine desert: The sponge loop retains resources within coral reefs. Science 342: 108-110. https://doi.org/10.1126/science.1241981

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. https://doi.org/10.3354/meps07403

De Laubenfels MW (1954) The sponges of the west-central Pacific. Oregon State College

de Voogd NJ, Becking LE, Cleary DFR (2009) Sponge community composition in the Derawan Islands, NE Kalimantan, Indonesia. Marine Ecology Progress Series 396: 169-180. https://doi.org/10.3354/meps08349

de Voogd NJ, Cleary DFR (2008) An analysis of sponge diversity and distribution at three taxonomic levels in the Thousand Islands/Jakarta Bay reef complex, West-Java, Indonesia. Marine Ecology 29: 205-215. https://doi.org/10.1111/j.1439-0485.2008.00238.x

de Voogd NJ, Cleary DFR, Hoeksema BW, Noor A, Soest RWMv (2006) Sponge beta diversity in the Spermonde Archipelago. Marine Ecology Progress Series 309: 131-142. https://doi.org/10.3354/meps309131

de Voogd NJA, B.; Boury-Esnault, N.; Carballo, J.L.; Cárdenas, P.; Díaz, M.-C.; Dohrmann, M.; Downey, R.; Hajdu, E.; Hooper, J.N.A.; Kelly, M.; Klautau, M.; Manconi, R.; Morrow, C.C. Pisera, A.B.; Ríos, P.; Rützler, K.; Schönberg, C.; Vacelet, J.; van Soest, R.W.M. (2022) World Porifera Database. Accessed at https://www.marinespecies.org/porifera on 2022-05-25. doi:10.14284/359

Demers M-CA, Knott NA, Davis AR (2015) Under the radar: Sessile epifaunal invertebrates in the seagrass *Posidonia australis*. Journal of the Marine Biological Association of the United Kingdom 96: 363-377. https://doi.org/10.1017/s0025315415000612

Dendy A (1887) XVI.—The sponge-fauna of Madras. A report on a collection of sponges obtained in the neighbourhood of Madras by Edgar Thurston, Esq. Annals and Magazine of Natural History 20: 153-165. https://doi.org/10.1080/00222938709460032

Díaz MC (2005) Common Sponges from Shallow Marine Habitats from Bocas del Toro Region, Panama. Caribbean Journal of Science 41: 465-475

Diaz MC, Rützler K (2001) Sponge: An essential component of Caribbean coral reefs. Bulletin of Marine Science 69: 535-546

Diaz MC, Thacker RW, Rützler K, Piantoni Dietrich C (2007) Two new haplosclerid sponges from Caribbean Panama with symbiotic filamentous cyanobacteria, and an overview of sponge-cyanobacteria associations. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: Biodiversity, innovation and sustainability. Série Livros 28, Museu Nacional, Rio de Janeiro, pp 31-39

Druffel ERM, Williams PM, Bauer JE, Ertel JR (1992) Cycling of dissolved and particulate organic matter in the open ocean. Journal of Geophysical Research 97. https://doi.org/10.1029/92JC01511

Duarte CM, Benavent E, Sánchez MdC (1999) The microcosm of particles within seagrass *Posidonia oceanica* canopies. Marine Ecology Progress Series 181: 289-295. https://doi.org/10.3354/meps181289

Duarte CM, Borum J, Short FT, Walker DI, Polunin NVC (2008) Seagrass ecosystems: their global status and prospects. Aquatic Ecosystems, pp 281-294

Duarte CM, Cebrián J (1996) The fate of marine autotrophic production. Limnology and Oceanography 41: 1758-1766. https://doi.org/10.4319/lo.1996.41.8.1758

Duarte CM, Fourqurean JW, Krause-Jensen D, Olesen B (2006) Dynamics of seagrass stability and change. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, ecology and conservation. Springer, Dordrecht, The Netherlands, pp 271-294

Duarte CM, Losada IJ, Hendriks IE, Mazarrasa I, Marbà N (2013) The role of coastal plant communities for climate change mitigation and adaptation. Nature Climate Change 3: 961-968. https://doi.org/10.1038/nclimate1970

Duarte CM, Middleburg JJ, Caraco N (2005) Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 2: 1-8. https://doi.org/10.5194/bg-2-1-2005

Duckworth AR, Peterson BJ (2013) Effects of seawater temperature and pH on the boring rates of the sponge *Cliona celata* in scallop shells. Marine Biology 160: 27-35. https://doi.org/10.1007/s00227-012-2053-z

Duke NC, Schmitt K (2015) Mangroves: Unusual forests at the seas edge. In: Köhl M, Pancel L (eds) Tropical Forestry Handbook. Springer, Berlin, Heidelberg.

Duran S, Pascual M, Turon X (2004) Low levels of genetic variation in mtDNA sequences over the western Mediterranean and Atlantic range of the sponge *Crambe crambe* (Poecilosclerida). Marine Biology 144: 31-35. https://doi.org/10.1007/s00227-003-1178-5

DuRand MD, Olson RJ, Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda AtlanticTime-series station in the Sargasso Sea. Deep-Sea Research II 48: 1983-2003. https://doi.org/10.1016/S0967-0645(00)00166-1

Dusenberry JA, Frankel SL (1994) Increasing the sensitivity of a FACScan flow cytometer to study oceanic picoplankton. Limnology and Oceanography 39: 206-209. https://doi.org/10.4319/lo.1994.39.1.0206

El Hag AGD, Fogg GE (1986) The distribution of coccoid blue-green algae (Cyanobacteria) in the Menai Straits and the Irish Sea. British Phycological Journal 21: 45-54. https://doi.org/10.1080/00071618600650061

Ereskovsky AV, Tokina DB (2007) Asexual reproduction in homoscleromorph sponges (Porifera; Homoscleromorpha). Marine Biology 151: 425-434. https://doi.org/10.1007/s00227-006-0439-5

Erwin PM, Thacker RW (2007) Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages. Journal of the Marine Biological Association of the United Kingdom 87: 1683-1692. https://doi.org/10.1017/s0025315407058213

Erwin PM, Thacker RW (2008) Phototrophic nutrition and symbiont diversity of two Caribbean sponge–cyanobacteria symbioses. Marine Ecology Progress Series 362: 139-147. https://doi.org/10.3354/meps07464

Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, Bond WJ, Carpenter SR, Essington TE, Holt RD, Jackson JBC, Marquis RJ, Oksanen L, Oksanen T, Paine RT, Pikitch EK, Ripple WJ, Sandin SA, Scheffer M, Schoener TW, Shurin JB, Sinclair ARE, Soulé ME, Virtanen R, Wardle DA (2011) Trophic downgrading of Planet Earth. Science 333. https://doi.org/10.1126/science.1205106

Faridah-Hanum I, Yusoff FM, Fitrianto A, Ainuddin NA, Gandaseca S, Zaiton S, Norizah K, Nurhidayu S, Roslan MK, Hakeem KR, Shamsuddin I, Adnan I, Awang Noor AG, Balqis ARS, Rhyma PP, Siti Aminah I, Hilaluddin F, Fatin R, Harun NZN (2019) Development of a comprehensive mangrove quality index (MQI) in Matang Mangrove: Assessing mangrove ecosystem health. Ecological Indicators 102: 103-117. https://doi.org/10.1016/j.ecolind.2019.02.030

Field C, Osborn J, Hoffman L, Polsenberg J, Ackerly D, Berry J, Björkman O, Held A, Matson P, Mooney H (1998a) Mangrove biodiversity and ecosystem function. Global Ecology & Biogeography Letters 7: 3-14. https://doi.org/10.1111/j.1466-8238.1998.00278.x

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998b) Primary production of the biosphere: Integrating terrestrial and oceanic components. Science 281: 237-240. https://doi.org/10.1126/science.281.5374.237

Firmansyah F, Mustofa A, Estradivari D, Adrian H, Christian A, Gabby G, Harris J (2016) Satu Dekade Pengelolaan Taman Nasional Wakatobi Keberhasilan dan Tantangan Konservasi Laut. WWF Indonesia, Jakarta

Fogg GE (1986) Review Lecture - Picoplankton. Proceedings of the Royal Society London B 228: 1-30. https://doi.org/10.1098/rspb.1986.0037

Fogg GE (1995) Some comments on picoplankton and its importance in the pelagic ecosystem. Aquatic Microbial Ecology 9: 33-39. https://doi.org/10.3354/ame009033

Fonseca M, Bell SS (1998) Influence of physical setting on seagrass landscapes near Beaufort, North Carolina, USA. Marine Ecology Progress Series 171: 109-121. https://doi.org/10.3354/meps171109

Foote CS (1968) Mechanisms of photosensitized oxidation. Science 162: 963-970. https://doi.org/10.1126/science.162.3857.963

Frank HA, Brudvig GW (2004) Redox functions of carotenoids in photosynthesis. Biochemistry 43: 8607-8615. https://doi.org/10.1021/bi0492096

Freeman CJ, Easson CG, Fiore CL, Thacker RW (2021) Sponge–Microbe Interactions on Coral Reefs: Multiple Evolutionary Solutions to a Complex Environment. Frontiers in Marine Science 8. https://doi.org/10.3389/fmars.2021.705053

Freeman CJ, Thacker RW (2011) Complex interactions between marine sponges and their symbiotic microbial communities. Limnology and Oceanography 56: 1577-1586. https://doi.org/10.4319/lo.2011.56.5.1577

Freeman CJ, Thacker RW, Baker DM, Fogel ML (2013) Quality or quantity: is nutrient transfer driven more by symbiont identity and productivity than by symbiont abundance? The ISME Journal 7: 1116-1125. https://doi.org/10.1038/ismej.2013.7

Fu F-X, Warner ME, Zhang Y, Feng Y, Hutchins DA (2007) Effects of increased temperature and CO2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). Journal of Phycology 43: 485-496. https://doi.org/10.1111/j.1529-8817.2007.00355.x

Gantt SE, McMurray SE, Stubler AD, Finelli CM, Pawlik JR, Erwin PM (2019) Testing the relationship between microbiome composition and flux of carbon and nutrients in Caribbean coral reef sponges. Microbiome 7: 124. https://doi.org/10.1186/s40168-019-0739-x

Gao Z-M, Zhou G-W, Huang H, Wang Y (2017) The Cyanobacteria-Dominated Sponge Dactylospongia elegans in the South China Sea: Prokaryotic Community and Metagenomic Insights. Frontiers in Microbiology 8. https://doi.org/10.3389/fmicb.2017.01387

Garrison DL, Gowing MM, Hughes MP, Campbell L, Caron DA, Dennett MR, Shalapyonok A, Olson RJ, Landry MR, Brown SL, Liu H-B, Azam F, Steward GF, Ducklow HW, Smith DC (2000) Microbial food web structure in the Arabian Sea: a US JGOFS study. Deep-Sea Research Part II 47: 1387-1422. https://doi.org/10.1016/S0967-0645(99)00148-4

Gazave E, Lapébie P, Ereskovsky AV, Vacelet J, Renard E, Cárdenas P, Borchiellini C (2011) No longer Demospongiae: Homoscleromorpha formal nomination as a fourth class of Porifera. Hydrobiologia 687: 3-10. https://doi.org/10.1007/s10750-011-0842-x

Gerrodette T, Flechsig AO (1979) Sediment-induced reduction in the pumping rate of the tropical sponge *Verongia lacunosa*. Marine Biology 55: 103-110. https://doi.org/10.1007/BF00397305

Gieskes WWC, Kraay GW, Nontji A, Setiapermana D, Sutomo (1990) Monsoonal differences in primary productivity in the eastern Banda Sea. Netherland Journal of Sea Research 25: 473-483. https://doi.org/10.1016/0077-7579(90)90071-N

Gili J-M, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology & Evolution 13: 316-321. https://doi.org/10.1016/S0169-5347(98)01365-2

Glynn PW, Manzello DP (2015) Bioerosion and coral reef growth: A dynamic balance Coral Reefs in the Anthropocene, pp 67-97

Goudie L, Norman MD, Finn J (2013) Sponges: A Museum Victoria Field Guide. Museum Victoria, Melbourne, Australia

Guannel G, Arkema K, Ruggiero P, Verutes G (2016) The Power of three: Coral reefs, seagrasses and mangroves protect coastal regions and increase their resilience. PLoS One 11: e0158094. https://doi.org/10.1371/journal.pone.0158094

Guillou L, Jacquet S, Chrétiennot-Dinet M-J, Vaulot D (2001) Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. Aquatic Microbial Ecology 26: 201-207. https://doi.org/10.3354/ame026201

Haas AF, Nelson CE, Wegley Kelly L, Carlson CA, Rohwer F, Leichter JJ, Wyatt A, Smith JE (2011) Effects of coral reef benthic primary producers on dissolved organic carbon and microbial activity. PLoS One 6: e27973. https://doi.org/10.1371/journal.pone.0027973

Hadas E, Marie D (2006) Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. Limnology and Oceanography 51: 1548-1550. https://doi.org/10.4319/lo.2006.51.3.1548

Hadas E, Shpigel M, Ilan M (2009) Particulate organic matter as a food source for a coral reef sponge. The Journal of Experimental Biology 212: 3643-3650. https://doi.org/10.1242/jeb.027953

Hadi TA, Hafizt M, Hadiyanto H, Budiyanto A, Siringoringo RM (2018) Shallow water sponges along the south coast of Java, Indonesia. Biodiversitas Journal of Biological Diversity 19: 485-493. https://doi.org/10.13057/biodiv/d190223

Hamilton SE, Casey D (2016) Creation of a high spatio-temporal resolution global database of continuous mangrove forest cover for the 21st century (CGMFC-21). Global Ecology and Biogeography 25: 729-738. https://doi.org/10.1111/geb.12449

Hansell DA, Carlson CA (2014) Biogeochemistry of Marine Dissolved Organic Matter. Elsevier Science & Technology, San Diego

Hansen JW, Thamdrup B, Jørgensen BB (2000) Anoxic incubation of sediment in gas-tight plastic bags: a method for biogeochemical process studies. Marine Ecology Progress Series 208: 273-282. https://doi.org/10.3354/meps208273

Hanson CE, McLaughlin MJ, Hyndes GA, Strzelecki J (2009) Selective uptake of prokaryotic picoplankton by a marine sponge (*Callyspongia* sp.) within an oligotrophic coastal system. Estuarine, Coastal and Shelf Science 84: 289-297. https://doi.org/10.1016/j.ecss.2009.05.019

Hautier Y, Tilman D, Isbell F, Seabloom EW, Borer ET, Reich PB (2015) Anthropogenic environmental changes affect ecosystem stability via biodiversity. Science 348: 336-340. https://doi.org/10.1126/science.aaa1788

Heck KL, Hays G, Orth RJ (2003) Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series 253: 123-136. https://doi.org/10.3354/meps253123

Helfman GS, Collette BB, Facey DE, Bowen BW (2009) The diversity of fishes. Wiley-Blackwell, West Sussex, UK

Hemminga M, Duarte C (2000) Seagrass ecology: an introduction. Cambridge University Press, Cambridge

Hernawan UE, Rahmawati S, Ambo-Rappe R, Sjafrie NDM, Hadiyanto H, Yusup DS, Nugraha AH, La Nafie YA, Adi W, Prayudha B, Irawan A, Rahayu YP, Ningsih E, Riniatsih I, Supriyadi IH, McMahon K (2021) The first nation-wide assessment identifies valuable blue-carbon seagrass habitat in Indonesia is in moderate condition. Science of The Total Environment 782. https://doi.org/10.1016/j.scitotenv.2021.146818

Hill M, Allenby A, Ramsby B, Schonberg C, Hill A (2011) Symbiodinium diversity among host clionaid sponges from Caribbean and Pacific reefs: Evidence of heteroplasmy and putative host-specific symbiont lineages. Molecular Phylogenetic and Evolution 59: 81-88. https://doi.org/10.1016/j.ympev.2011.01.006

Hirose E (2015) Ascidian photosymbiosis: Diversity of cyanobacterial transmission during embryogenesis. genesis 53: 121-131. https://doi.org/10.1002/dvg.22778

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research 50: 839-866. https://doi.org/10.1071/MF99078

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318: 1737-1742. https://doi.org/10.1126/science.1152509

Hoeksema BW (2007) Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity: The Coral Triangle. In: Renema W (ed) Biogeography, Time, and Place: Distributions, Barriers, and Islands. Springer Netherlands, Dordrecht, Netherlands, pp 117-178

Hoer DR, Gibson PJ, Tommerdahl JP, Lindquist NL, Martens CS (2018) Consumption of dissolved organic carbon by Caribbean reef sponges. Limnology and Oceanography 63: 337-351. https://doi.org/10.1002/lno.10634

Hoffmann F, Radax R, Woebken D, Holtappels M, Lavik G, Rapp HT, Schlappy ML, Schleper C, Kuypers MM (2009) Complex nitrogen cycling in the sponge *Geodia barretti*. Environmental Microbiology 11: 2228-2243. https://doi.org/10.1111/j.1462-2920.2009.01944.x

Hoogenboom MO, Anthony KRN, Connolly SR (2006) Energetic cost of photoinhibition in corals. Marine Ecology Progress Series 313: 1-12. https://doi.org/10.3354/meps313001

Hooper DU, F.S. Chapin I, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setälä H, Symstad AJ, Vandermeer J, Wardle DA (2005)

Effects of biodiversity on ecosystem functioning: A concencus of current knowledge. Ecological Monographs 75: 3-35. https://doi.org/10.1890/04-0922

Hooper JN, Van Soest RW (2006) A new species of Amphimedon (Porifera, Demospongiae, Haplosclerida, Niphatidae) from the Capricorn-Bunker Group of Islands, Great Barrier Reef, Australia: target species for the 'sponge genome project'. Zootaxa 1314: 31-39. https://doi.org/10.11646/zootaxa.1314.1.2

Hooper JNA (2000) Sponguide - Guide to sponge collection and identification. Queensland Museum, South Brisbane, Australia

Hooper JNA, van Soest RWM, Willenz P (2004) Systema Porifera: A Guide to the Classification of Sponges. Springer, New York, NY, UNITED STATES

Hughes TP, Barnes ML, Bellwood DR, Cinner JE, Cumming GS, Jackson JBC, Kleypas J, van de Leemput IA, Lough JM, Morrison TH, Palumbi SR, van Nes EH, Scheffer M (2017) Coral reefs in the Anthropocene. Nature 546: 82-90. https://doi/10.1038/nature22901

Hughes TP, Kerry JT, Baird AH, Connolly SR, Dietzel A, Eakin CM, Heron SF, Hoey AS, Hoogenboom MO, Liu G, McWilliam MJ, Pears RJ, Pratchett MS, Skirving WJ, Stella JS, Torda G (2018) Global warming transforms coral reef assemblages. Nature 556: 492-496. https://doi.org/10.1038/s41586-018-0041-2

Hunting ER, Franken O, Knopperts F, Kraak MHS, Vargas R, Röling WFM, van der Geest HG (2013) Substrate as a driver of sponge distributions in mangrove ecosystems. Marine Ecology Progress Series 486: 133-141. https://doi.org/10.3354/meps10376

Iglesias-Prieto R, Matta JL, Robins WA, Trench RK (1992) Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. Proceedings of the National Academy of Sciences of the United States of America 89: 10302-10305. https://doi.org/10.1073/pnas.89.21.10302

IPCC (2019) Summary for Policymakers. In: H.-O. Pörtner DCR, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, A. Alegría,, M. Nicolai AO, J. Petzold, B. Rama, N.M. Weyer (eds) IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp 3-35

Ismet MS, Bengen DG, Radjasa OK, Kawaroe M (2017) Composition and antibacterial activities of marine sponges from different seagrass ecosystems in Kepulauan Seribu waters, Jakarta. Jurnal Ilmu dan Teknologi Kelautan Tropis 8: 729-745. https://doi.org/10.29244/jitkt.v8i2.15838

Jiménez E, Ribes M (2007) Sponges as a source of dissolved inorganic nitrogen: Nitrification mediated by temperate sponges. Limnology and Oceanography 53: 948-958. https://doi.org/10.4319/lo.2007.52.3.0948

Jørgensen CB (1990) Bivalve filter feeding: hydrodynamics, bioenergetics, physiology and ecology. Olsen & Olsen, Fredensborg, Denmark.

Kaufman L, Obura D, Rohwer F, Sala E, Sandin S, Tschirky J (2011) Coral Health Index (CHI): measuring coral community health. Science and Knowledge Division, Conservation International, Arlington, VA, USA

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649. https://doi.org/10.1093/bioinformatics/bts199

Keesing JK, Usher KM, Fromont J (2012) First record of photosynthetic cyanobacterial symbionts from mesophotic temperate sponges. Marine and Freshwater Research 63: 403-408. https://doi.org/10.1071/MF11216

Kelmo F, Attrill MJ (2013) Severe impact and subsequent recovery of a coral assemblage following the 1997-8 El Nino event: a 17-year study from Bahia, Brazil. PLoS One 8: e65073. https://doi.org/10.1371/journal.pone.0065073

Kilminster K, McMahon K, Waycott M, Kendrick GA, Scanes P, McKenzie L, O'Brien KR, Lyons M, Ferguson A, Maxwell P, Glasby T, Udy J (2015) Unravelling complexity in seagrass systems for management: Australia as a microcosm. Science of the Total Environment 534: 97-109. https://doi.org/10.1016/j.scitotenv.2015.04.061

Kirchman DL (1994) The uptake of inorganic nutrients by heterotrophic bacteria. Microbial Ecology 28: 255-271. https://doi.org/10.1007/BF00166816

Knowlton N, Rohwer F (2003) Multispecies Microbial Mutualisms on Coral Reefs: The Host as a Habitat. The American Naturalist 162: S51-S62. https://doi.org/10.1086/378684

Kok B (1956) On the inhibition of photosynthesis by intense light. Biochimica et biophysica acta International journal of biochemistry and biophysics 21: 234-244. https://doi.org/10.1016/0006-3002(56)90003-8

Koukouras A, Russo A, Voultsiadou-Koukoura E, Arvanitidis C, Stefanidou D (1996) Macrofauna associated with sponge species of different morphology. Marine Ecology 17: 569-582. https://doi.org/10.1111/j.1439-0485.1996.tb00418.x

Krautter M (1998) Ecology of siliceous sponges - Application to the environmental interpretation of the Upper Jurassic sponge facies (Oxfordian) from Spain. Cuadernos de Geología Ibérica 24: 223-239

Krebs CJ (2009) Ecology : the experimental analysis of distribution and abundance. Pearson Benjamin Cummings, San Francisco, CA

Kudoh S, Kanda J, Takahashi M (1990) Specific growth rates and grazing mortality of chroococcoid cyanobacteria *Synechococcus* spp. in pelagic surface waters in the sea. Journal of Experimental Marine Biology and Ecology 142: 201-212. https://doi.org/10.1016/0022-0981(90)90091-P

Kuo J, Hartog Cd (2001) Seagrass taxonomy and identification key. In: Short FT, Coles RG (eds) Global Seagrass Research Methods. Elsevier Science, Amsterdam, Netherlands, pp 31-58

Lazcano A (2016) Alexandr I. Oparin and the origin of life: A historical reassessment of the heterotrophic theory. Journal of Molecular Evolution 83: 214-222. https://doi.org/10.1007/s00239-016-9773-5

Lee S, Fuhrman JA (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Applied and Environmental Microbiology 53: 1298-1303. https://doi.org/10.1128/aem.53.6.1298-1303.1987

Lefcheck JS, Byrnes JEK, Isbell F, Gamfeldt L, Griffin JN, Eisenhauer N, Hensel MJS, Hector A, Cardinale BJ, Duffy JE (2015) Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nature Communications 6: 6936. https://doi.org/10.1038/ncomms7936

Lehman JT (1976) The filter-feeder as an optimal forager and the predicted shapes of feeding curves. Limnology and Oceanography 21: 501-516. https://doi.org/10.4319/lo.1976.21.4.0501

Lemloh ML, Fromont J, Brummer F, Usher KM (2009) Diversity and abundance of photosynthetic sponges in temperate Western Australia. BMC Ecology 9: 4. https://doi.org/10.1186/1472-6785-9-4

Lesser MP, Slattery M (2020) Will coral reef sponges be winners in the Anthropocene? Global Change Biology 26: 3202-3211. https://doi.org/10.1111/gcb.15039

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic Biomembranes. Methods in Enzymology 148: 350-382. https://doi.org/10.1016/0076-6879(87)48036-1

Linacre L, Lara-Lara R, Camacho-Ibar V, Herguera JC, Bazán-Guzmán C, Ferreira-Bartrina V (2015) Distribution pattern of picoplankton carbon biomass linked to mesoscale dynamics in the southern gulf of Mexico during winter conditions. Deep Sea Research Part I: Oceanographic Research Papers 106: 55-67. https://doi.org/10.1016/j.dsr.2015.09.009

Lodish H, Berg A, Zipursky SL (1999) Molecular cell biology. W.H. Freeman, New York

Long MH, Rheuban JE, Berg P, Zieman JC (2012) A comparison and correction of light intensity loggers to photosynthetically active radiation sensors. Limnology and Oceanography: Methods 10: 416-424. https://doi.org/10.4319/lom.2012.10.416

López-Sandoval DC, Rodríguez-Ramos T, Cermeño P, Marañón E (2013) Exudation of organic carbon by marine phytoplankton: dependence on taxon and cell size. Marine Ecology Progress Series 477: 53-60. https://doi.org/10.3354/meps10174

Madduppa H, Schupp PJ, Faisal MR, Sastria MY, Thoms C (2015) Persistent outbreaks of the "black disease" sponge *Terpios hoshinota* in Indonesian coral reefs. Marine Biodiversity 47: 149-151. https://doi.org/10.1007/s12526-015-0426-5

Malan DE, McLachlan A (1991) In situ benthic oxygen fluxes in a nearshore coastal marine system: a new approach to quantify the effect of wave action. Marine Ecology Progress Series 73: 69-81. https://doi.org/10.3354/meps073069

Maldonado M, Aguilar R, Bannister RJ, Bell JJ, Conway KW, Dayton PK, Díaz C, Gutt J, Kelly M, Kenchington ELR, Leys SP, Pomponi SA, Rapp HT, Rützler K, Tendal OS, Vacelet J, Young CM (2015) Sponge Grounds as Key Marine Habitats: A Synthetic Review of Types, Structure, Functional Roles, and Conservation Concerns Marine Animal Forests, pp 1-39

Maldonado M, Carmona MC, Velásquez Z, Puig A, Cruzado A, López A, Young CM (2005) Siliceous sponges as a silicon sink: An overlooked aspect of benthopelagic coupling in the marine silicon cycle. Limnology and Oceanography 50: 799-809. https://doi.org/10.4319/lo.2005.50.3.0799

Maldonado M, Ribes M, van Duyl FC (2012) Nutrient fluxes through sponges: biology, budgets, and ecological implications. Advances in Marine Biology 62: 113-182. https://doi.org/10.1016/B978-0-12-394283-8.00003-5

Maldonado M, Riesgo A (2009) Reproduction in the phylum Porifera: a synoptic overview. Treballs de la Societat Catalana de Biologia: 29-49. https://doi.org/10.2436/20.1501.02.56

Maldonado M, Zhang X, Cao X, Xue L, Cao H, Zhang W (2010) Selective feeding by sponges on pathogenic microbes: a reassessment of potential for abatement of microbial pollution. Marine Ecology Progress Series 403: 75-89. https://doi.org/10.3354/meps08411

Manconi R, Pronzato R (2008) Global diversity of sponges (Porifera: Spongillina) in freshwater. In: Balian EV, Lévêque C, Segers H, Martens K (eds) Freshwater Animal Diversity Assessment. Springer Netherlands, Dordrecht, pp 27-33

Marie D, Brussaard CPD, Thyrhaug R, Bratbak G, Vaulot D (1995) Enumeration of marine viruses in culture and natural samples by flow cytometry. Applied and Environmental Microbiology 65: 45-52. https://doi.org/10.1128/AEM.65.1.45-52.1999

Marin A, López Belluga MD (2005) Sponge coating decreases predation on the bivalve *Arca Noae*. Journal of Molluscan Studies 71: 1-6. https://doi.org/10.1093/mollus/eyh045

Marlow J, Schönberg CHL, Davy SK, Haris A, Jompa J, Bell JJ (2018) Bioeroding sponge assemblages: the importance of substrate availability and sediment. Journal of the Marine Biological Association of the United Kingdom 99: 343-358. https://doi.org/10.1017/s0025315418000164

McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Loso T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ (2013) Animals in a bacterial world, a new imperative for the life sciences. Proceedings of the National Academy of Sciences of the United States of America 110: 3229-3236. https://doi.org/10.1073/pnas.1218525110

McGlathery KJ, Sundbäck K, Anderson IC (2007) Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. Marine Ecology Progress Series 348: 1-18. https://doi.org/10.3354/meps07132

McGrath EC, Smith DJ, Jompa J, Bell JJ (2017) Adaptive mechanisms and physiological effects of suspended and settled sediment on barrel sponges. Journal of Experimental Marine Biology and Ecology 496: 74-83. https://doi.org/10.1016/j.jembe.2017.07.013

McIntyre FD, Drewery J, Eerkes-Medrano D, Neat FC (2016) Distribution and diversity of deep-sea sponge grounds on the Rosemary Bank Seamount, NE Atlantic. Marine Biology 163: 143. https://doi.org/10.1007/s00227-016-2913-z

McKenzie LJ, Campbell SJ, Roder CA (2001) Seagrass-Watch: Manual for mapping and monitoring seagrass resources by community (citizen) volunteers. Northern Fisheries Centre, Cairns, Australia.

McMellor S, Smith DJ (2010) Coral reefs of the Wakatobi: abundance and biodiversity. In: Clifton J, Unsworth RKF, Smith DJ (eds) Marine Research and Conservation in the Coral Triangle The Wakatobi National Park. Nova Publisher, New York

McMurray SE, Johnson ZI, Hunt DE, Pawlik JR, Finelli CM (2016) Selective feeding by the giant barrel sponge enhances foraging efficiency. Limnology and Oceanography 61: 1271-1286. https://doi.org/10.1002/lno.10287

McMurray SE, Pawlik JR, Finelli CM (2014) Trait-mediated ecosystem impacts: how morphology and size affect pumping rates of the Caribbean giant barrel sponge. Aquatic Biology 23: 1-13. https://doi.org/10.3354/ab00612

McMurray SE, Stubler AD, Erwin PM, Finelli CM, Pawlik JR (2018) A test of the sponge-loop hypothesis for emergent Caribbean reef sponges. Marine Ecology Progress Series 588: 1-14. https://doi.org/10.3354/meps12466

Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. Ecological Economics: 215-233. https://doi.org/10.1016/S0921-8009(99)00009-9

Moebus K, Johnson KM (1974) Exudation of dissolved organic carbon by Brown Algae. Marine Biology 26: 117-125. https://doi.org/10.1007/BF00388881

Moitinho-Silva L, Steinert G, Nielsen S, Hardoim CCP, Wu YC, McCormack GP, Lopez-Legentil S, Marchant R, Webster N, Thomas T, Hentschel U (2017) Predicting the HMA-LMA status in marine sponges by machine learning. Frontiers in Microbiology 8: 752. https://doi.org/10.3389/fmicb.2017.00752

Moore TS, Marra J, Alkatiri A (2003) Response of the Banda Sea to the southeast monsoon. Marine Ecology Progress Series 261: 41-49. https://doi.org/10.1073/pnas.121852511010.3354/meps261041

Mora C, Chittaro PM, Sale PF, Kritzer JP, Ludsin SA (2003) Patterns and processes in reef fish diversity. Nature 421: 933-936. https://doi.org/10.1038/nature01393

Mortimer C, Dunn M, Haris A, Jompa J, Bell J (2021) Estimates of sponge consumption rates on an Indo-Pacific reef. Marine Ecology Progress Series 672: 123-140. https://doi.org/10.3354/meps13786

Mueller B, de Goeij JM, Vermeij MJ, Mulders Y, van der Ent E, Ribes M, van Duyl FC (2014) Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC). PLoS One 9: e90152. https://doi.org/10.1371/journal.pone.0090152

Muller WE (2006) The stem cell concept in sponges (Porifera): Metazoan traits. Semin Cell Dev Biol 17: 481-491. https://doi.org/10.1016/j.semcdb.2006.05.006

Muscatine L (1980) Productivity of Zooxanthellae. In: Falkowski PG (ed) Primary Productivity in the Sea. Springer US, Boston, MA, pp 381-402

Muscatine L, R. McCloskey L, E. Marian R (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration1. Limnology and Oceanography 26: 601-611. https://doi.org/10.4319/lo.1981.26.4.0601

Nagelkerken I (2009) Evaluation of nursury function of mangroves and seagrass beds for tropical Decapods and reef fishes: Pattern and underlying mechanisms. In: Nagelkerken I (ed) Ecological Connectivity among Tropical Coastal Ecosystems. Springer Science and Business Media, Dordrecht, the Netherlands, pp 357-399

Nagelkerken I, van der Velde G, Gorissen MW, Meijer GJ, Van't Hof T, den Hartog C (2000) Importance of mangroves, seagrass beds and the shallow coral reef as a nursery for important coral reef fishes, using a visual census technique. Estuarine, Coastal and Shelf Science 51: 31-44. https://doi.org/10.1006/ecss.2000.0617

NCBI (2022) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 44: D7-D19. Accessed at https://doi.org/10.1093/nar/gkv1290 on 2022-04-06.

Nordlund L, Erlandsson J, de la Torre-Castro M, Jiddawi N (2011) Changes in an East African social-ecological seagrass system: invertebrate harvesting affecting species composition and local livelihood. Aquatic Living Resources 23: 399-416. https://doi.org/10.1051/alr/2011006

O'Brien KR, Waycott M, Maxwell P, Kendrick GA, Udy JW, Ferguson AJP, Kilminster K, Scanes P, McKenzie LJ, McMahon K, Adams MP, Samper-Villarreal J, Collier C, Lyons M, Mumby PJ, Radke L, Christianen MJA, Dennison WC (2018) Seagrass ecosystem trajectory depends on the relative timescales of resistance, recovery and disturbance. Marine Pollution Bulletin 134: 166-176. https://doi.org/10.1016/j.marpolbul.2017.09.006

Odum HT (1956) Primary production in flowing waters. Limnology and Oceanography 1: 102-117. https://doi.org/10.4319/lo.1956.1.2.0102

Ogawa H, Tanoue E (2003) Dissolved Organic Matter in Oceanic Waters. Journal of Oceanography 59: 129-147. https://doi.org/10.1023/A:1025528919771

Ogden JC, Ogden NB (1982) A preliminary study of two representative seagrass communities in Palau, Western Caroline Islands (Micronesia). Aquatic Botany 12: 229-244. https://doi.org/10.1016/0304-3770(82)90019-5

Ooi JLS, Kendrick GA, Van Niel KP, Affendi YA (2011) Knowledge gaps in tropical Southeast Asian seagrass systems. Estuarine, Coastal and Shelf Science 92: 118-131. https://doi.org/10.1016/j.ecss.2010.12.021

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, Fourqurean JW, Jr KLH, Hughes AR, Kendrick GA, Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL (2006) A global crisis for seagrass ecosystems. Bioscience 56: 987-996. https://doi.org/10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2

Palumbi SR (1984) Tactics of acclimation: Morphological changes of sponges in an unpredictable environment. Science 225: 1478-1480. https://doi.org/10.1126/science.225.4669.1478

Partensky F, Hess WR, Vaulot D (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiology and Molecular Biology Reviews 63: 106-127. https://doi.org/10.1128/MMBR.63.1.106-127.1999

Pawlik JR, McMurray SE, Henkel TP (2007) Abiotic factors control sponge ecology in Florida mangroves. Marine Ecology Progress Series 339: 93-98. https://doi.org/10.3354/meps339093

Perea-Blazquez A, Davy SK, Bell JJ (2012) Estimates of particulate organic carbon flowing from the pelagic environment to the benthos through sponge assemblages. PLoS One 7: e29569. https://doi.org/10.1371/journal.pone.0029569

Perea-Blázquez A, Davy SK, Magana-Rodríguez B, Bell JJ (2013) Temporal variation in food utilisation by three species of temperate demosponge. Marine Ecology Progress Series 485: 91-103. https://doi.org/10.3354/meps10316

Perea-Blázquez A, Price K, Davy SK, Bell JJ (2010) Diet composition of two temperate calcareous sponges: *Leucosolenia echinata* and *Luecetta* sp. from Wellington South Coast, New Zealand. The Open Marine Biology Journal 4: 65-73. http://dx.doi.org/10.2174/1874450801004010065

Pet-Soede L, Erdmann MV (2004) Rapid Ecological Assessment Wakatobi National Park. WWF/TNC Joint Publication, Denpasar, Bali

Peterson BJ, Chester CM, Jochem FJ, Fourqurean JW (2006) Potential role of sponge communities in controlling phytoplankton blooms in Florida Bay. Marine Ecology Progress Series 328: 93-103. https://doi.org/10.3354/meps328093

Pile AJ, Grant A, Hinde R, Borowitzka MA (2003) Heterotrophy on ultraplankton communities is an important source of nitrogen for a sponge-rhodophyte symbiosis. Journal of Experimental Biology 206: 4533-4538. https://doi.org/10.1242/jeb.00698

Pile AJ, Patterson MR, Witman JD (1996) In situ grazing on plankton <10 pm by the boreal sponge *Mycale lingua*. Marine Ecology Progress Series 141: 95-102. https://doi.org/10.3354/meps141095

Pineda MC, Duckworth A, Webster N (2016) Appearance matters: sedimentation effects on different sponge morphologies. Journal of the Marine Biological Association of the United Kingdom 96: 481-492. https://doi.org/10.1017/s0025315414001787

Pisani D, Pett W, Dohrmann M, Feuda R, Rota-Stabelli O, Philippe H, Lartillot N, Wörheide G (2015) Genomic data do not support comb jellies as the sister group to all other animals. Proceedings of the National Academy of Sciences 112: 15402-15407. https://doi.org/10.1073/pnas.1518127112

Pita L, Rix L, Slaby BM, Franke A, Hentschel U (2018) The sponge holobiont in a changing ocean: from microbes to ecosystems. Microbiome 6: 46. https://doi.org/10.1186/s40168-018-0428-1

PIX4D (2018) PIX4Dcapture. Free drone flight planning app for optimal 3D mapping and modeling. Accessed at https://www.pix4d.com/product/pix4dcapture on 2019-07-22.

Pomeroy LR (1974) The Ocean's Food Web, A Changing Paradigm. BioScience 24: 499-504. https://doi.org/10.2307/1296885

Powell A, Smith DJ, Hepburn LJ, Jones T, Berman J, Jompa J, Bell JJ (2014) Reduced diversity and high sponge abundance on a sedimented Indo-Pacific reef system: implications for future changes in environmental quality. PLoS One 9: e85253. https://doi.org/10.1371/journal.pone.0085253

Pronzato R, Bavestrello G, Cerrano C (1998) Morpho-functional adaptations of the three species of Spongia (Porifera, Demospongiae) from a Mediterranean vertical cliff. Bulletin of Marine Science 63: 317-328

QGIS (2002) QGIS 3.18. A Free and Open Source Geographic Information System. Accessed at https://www.qgis.org/en/site/index.html on 2020-02-14.

Rahmawati S, Irawan A, Supriyadi IH, Azkab MH (2014) Panduan Monitoring Padang Lamun. COREMAP CTI LIPI, Jakarta

Ramírez F, Afán I, Davis LS, Chiaradia A (2017) Climate impacts on global hot spots of marine biodiversity. Science Advances 3: e1601198. https://doi.org/10.1126/sciadv.1601198

Ramsby BD, Hoogenboom MO, Whalan S, Webster NS, Thompson A (2017) A decadal analysis of bioeroding sponge cover on the inshore Great Barrier Reef. Scientific Reports 7: 2706. https://doi.org/10.1038/s41598-017-02196-z

Redmond NE, McCormack GP (2009) Ribosomal internal transcribed spacer regions are not suitable for intra- or inter-specific phylogeny reconstruction in haplosclerid sponges (Porifera: Demospongiae). Journal of the Marine Biological Association of the United Kingdom 89: 1251-1256. https://doi.org/10.1017/S0025315409000411

Reiswig HM (1971a) In situ pumping activities of tropical Demospongiae. Marine Biology 9: 38-50. https://doi.org/10.1007/BF00348816

Reiswig HM (1971b) Particle feeding in natural populations of three marine demosponges. Biological Bulletin 141: 568-591. https://doi.org/10.2307/1540270

Reiswig HM (1974) Water transport, respiration and energetics of three tropical marine sponges. Journal of Experimental Marine Biology and Ecology 14: 231-249. https://doi.org/10.1016/0022-0981(74)90005-7

Reiswig HM (1975) Bacteria as food for temperate-water marine sponges. Canadian Journal of Zoology 53: 582-589. https://doi.org/10.1139/z75-072

Reiswig HM (1981) Partial carbon and energy budgets of the bacteriosponge *Verongia fistularis* (Porifera: Demospongiae) in Barbados. Marine Ecology 2: 273-293. https://doi.org/10.1111/j.1439-0485.1981.tb00271.x

Renard E, Gazave E, Fierro-Constain L, Schenkelaars Q, Ereskovsky A, Vacelet J, Borchiellini C (2013) Porifera (Sponges): Recent Knowledge and New Perspectives eLS

Reynolds PL, Duffy E, Knowlton N (2018) Seagrass and seagrass beds. Ocean Portal. Accessed at https://ocean.si.edu/ocean-life/plants-algae/seagrass-and-seagrass-beds on 2022-04-20.

Ribes M, Coma R, Atkinson MJ, III RAK (2005) Sponges and ascidians control removal of particulate organic nitrogen from coral reef water. Limnology and Oceanography 50: 1480-1489. https://doi.org/10.4319/lo.2005.50.5.1480

Ribes M, Coma R, Gili J-M (1999) Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology Progress Series 176: 179-190. https://doi.org/10.3354/meps176179

Ribes M, Jimenez E, Yahel G, Lopez-Sendino P, Diez B, Massana R, Sharp JH, Coma R (2012) Functional convergence of microbes associated with temperate marine sponges. Environmental Microbiology 14: 1224-1239. https://doi.org/10.1111/j.1462-2920.2012.02701.x

Rice EW, Baird RB, Eaton AD (2017) Standard Methods for the Examination of Water and Wastewater, 23rd Edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC.

Richter C, Wunsch M, Rasheed M, Kötter I, Badran MI (2001) Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. Nature 413: 726-730. https://doi.org/10.1038/35099547

Ritchie RJ (2008) Universal chlorophyll equations for estimating chlorophyll a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. Photosynthetica 46: 115-126. https://doi.org/10.1007/s11099-008-0019-7

Rix L, de Goeij JM, Mueller CE, Struck U, Middelburg JJ, van Duyl FC, Al-Horani FA, Wild C, Naumann MS, van Oevelen D (2016a) Coral mucus fuels the sponge loop in warm- and cold-water coral reef ecosystems. Scientific Reports 6: 18715. https://doi.org/10.1038/srep18715

Rix L, de Goeij J, Oevelen D, Struck U, Al-Horani FA, Wild C, Naumann MS (2018) Reef sponges facilitate the transfer of coral-derived organic matter to their associated fauna via the sponge loop. Marine Ecology Progress Series 589: 85-96. https://doi.org/10.3354/meps12443

Rix L, Goeij JM, Oevelen D, Struck U, Al-Horani FA, Wild C, Naumann MS, Power S (2016b) Differential recycling of coral and algal dissolved organic matter via the sponge loop. Functional Ecology 31: 778-789. https://doi.org/10.1111/1365-2435.12758

Roberts DE, Cummins SP, Davies AR, Pangway C (1999) Evidence for symbiotic algae in sponges from temperate coastal reefs in New South Wales, Australia. Memoirs of the Queensland Museum 44: 493-497

Robertson ML, Mills AL, Zieman JC (1982) Microbial synthesis of detritus-like particulates from dissolved organic carbon released by tropical seagrasses. Marine Ecology Progress Series 7: 279-285. https://doi.org/10.3354/meps007279

Romero J, Lee K-S, Mateo MPaMA, Alcoverro T (2006) Nutrient dynamics in seagrass ecosystems. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, ecology and conservation. Springer, Doordrecht, Netherlands, pp 227-254

Rosen BR (1988) Progress, problems and patterns in the biogeography of reef corals and other tropical marine organisms. Helgoländer Meeresuntersuchungen 42: 269-301. https://doi.org/10.1007/BF02366046

Rot C, Goldfarb I, Ilan M, Huchon D (2006) Putative cross-kingdom horizontal gene transfer in sponge (Porifera) mitochondria. BMC Evolutionary Biology 6: 71. https://doi.org/10.1186/1471-2148-6-71

Rovellini A, Dunn MR, Fulton EA, Webster NS, Smith DJ, Jompa J, Haris A, Berman J, Bell JJ (2019) Decadal variability in sponge abundance and biodiversity on an Indo-Pacific coral reef. Marine Ecology Progress Series 620: 63-76. https://doi.org/10.3354/meps12968

Rützler K (1975) The role of burrowing sponges in bioerosion. Oecologia 19: 203-216. https://doi.org/10.1007/BF00345306

Rützler K (1978) Sponges in coral reefs. In: Stoddart DR, Johannes RR (eds) Monographs on Oceanic Methodology. UNESCO, Paris, France, pp 299-313

Rützler K (1985) Associations between Caribbean Sponges and Photosynthetic Organisms. In: Rützler K (ed) New Perspective in Sponge Biology. Smithsonian Institution Press, Washington, D.C., pp 455-466

Rützler K, Duran S, Piantoni C (2007) Adaptation of reef and mangrove sponges to stress-evidence for ecological speciation exemplified by *Chondrilla caribensis* new species (Demospongiae, Chondrosida). Marine Ecology 28: 95-111. https://doi.org/10.1111/j.1439-0485.2007.00183.x

Sacristán-Soriano O, Turon X, Hill M (2020) Microbiome structure of ecologically important bioeroding sponges (family Clionaidae): the role of host phylogeny and environmental plasticity. Coral Reefs 39: 1285-1298. https://doi.org/10.1007/s00338-020-01962-2

Sala E, Knowlton N (2006) Global Marine Biodiversity Trends. Annual Review of Environment and Resources 31: 93-122. https://doi.org/10.1146/annurev.energy.31.020105.100235

Sarà M (1971) Ultrastructural aspects of the symbiosis between two species of the genus *Aphanocapsa* (Cyanophyceae) and *Ircinia variabilis* (Demospongiae). Marine Biology 11: 214-221. https://doi.org/10.1007/BF00401270

Saunders MI, Leon JX, Callaghan DP, Roelfsema CM, Hamylton S, Brown CJ, Baldock T, Golshani A, Phinn SR, Lovelock CE, Hoegh-Guldberg O, Woodroffe CD, Mumby PJ (2014) Interdependency of tropical marine ecosystems in response to climate change. Nature Climate Change 4: 724-729. https://doi.org/10.1038/nclimate2274

Schönberg CHL (2000) Bioeroding sponges common to the Central Australian Great Barrier Reef: Description of three new species, two new records, and additions to two previously described species. Senckenbergiana maritima 30: 161-221. https://doi.org/10.1007/BF03042965

Schönberg CHL (2021) No taxonomy needed: Sponge functional morphologies inform about environmental conditions. Ecological Indicators 129: 107806. https://doi.org/10.1016/j.ecolind.2021.107806

Schönberg CHL, Fang JK-H, Carballo JL (2017) Bioeroding sponges and the future of coral reefs. In: Carballo JL, Bell JJ (eds) Climate Change, Ocean Acidification and Sponges: Impacts Across Multiple Levels of Organization. Springer International Publishing, Cham, pp 179-372

Schönberg CHL, Fromont J (2011) Sponge gardens of Ningaloo Reef (Carnarvon Shelf, Western Australia) are biodiversity hotspots. Hydrobiologia 687: 143-161. https://doi.org/10.1007/s10750-011-0863-5

Schönberg CHL, Loh WKW (2005) Molecular identity of the unique symbiotic dinoflagellates found in the bioeroding demosponge *Cliona orientalis*. Marine Ecology Progress Series 299: 157-166. https://doi.org/10.3354/meps299157

Serôdio J, Silva R, Ezequiel J, Calado R (2010) Photobiology of the symbiotic acoel flatworm *Symsagittifera roscoffensis*: algal symbiont photoacclimation and host photobehaviour. Journal of the Marine Biological Association of the United Kingdom 91: 163-171. https://doi.org/10.1017/s0025315410001001

Setiawan E, Chodiantoro MR, Insany GF, Subagio IB, Dewi NK, Muzaki FK (2021) Short Communication: Diversity of sponges in seagrass meadows at coastal area of Pacitan District, East Java, Indonesia. Biodiversitas 22: 3105-3112. https://doi.org/10.13057/biodiv/d220803

Sheldon RW, Rassoulzadegan F, Azam F, Berman T, Bezanson DS, Bianchi M, Bonin D, Hagström Å, Laval-Peuto M, Neveux J, Raimbault P, Rivier A, Sherr B, Sherr E, Van Wambeke F, Wikner J, Wood AM, Yentsch CM (1992) Nano- and picoplankton growth and production in the Bay of Villefranche sur Mer (N.W. Mediterranean). Hydrobiologia 241: 91-106. https://doi.org/10.1007/BF00008262

Short F, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: A bioregional model. Journal of Experimental Marine Biology and Ecology 350: 3-20. https://doi.org/10.1016/j.jembe.2007.06.012

Short FT, Coles RG, Pergent-Martini C (2001) Global seagrass distribution. In: Short FT, Coles RG (eds) Global seagrass research methods. Elsevier, Amsterdam, Netherlands

Short FT, Coles RG, Short CA (2015) SeagrassNet Manual for Scientific Monitoring of Seagrass Habitat, Worldwide Habitat. University of New Hampshire Publication. 73 pp.

Sieburth JM, Lenz VSJ (1978) Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnology and Oceanography 23: 1256-1263. https://doi.org/10.4319/lo.1978.23.6.1256

Simon JC, Marchesi JR, Mougel C, Selosse MA (2019) Host-microbiota interactions: from holobiont theory to analysis. Microbiome 7: 5. https://doi.org/10.1186/s40168-019-0619-4

Simpson TL, Gilbert JJ (1973) Gemmulation, gemmule hatching, and sexual reproduction in fresh-water sponges I. The life cycle of *Spongilla lacustris* and *Tubella pennsylvanica*. Transactions of the American Microscopical Society 92: 422-433. https://doi.org/10.2307/3225246

Sjafrie NDM, Hernawan UE, Prayudha B, Supriyadi IH, Iswari MY, Rahmat, Anggraini K, Rahmawati S, Suyarso (2018) Status Padang Lamun Indonesia 2018 Ver.02. Puslit Oseanografi - LIPI, Jakarta

Smith JHC, French CS (1963) The major and accessory pigments in photosynthesis. Annual Review of Plant Physiology 14: 181-224. https://doi.org/10.1146/annurev.pp.14.060163.001145

Smith TM, Smith RL (2015) Elements of ecology. Pearson, Boston

Southwell MW, Weisz JB, Martens CS, Lindquist N (2008) In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnology and Oceanography 53: 986-996. https://doi.org/10.4319/lo.2008.53.3.0986

Spalding M, Taylor M, Ravilious C, Short F, Green E (2003) Global Overview: The distribution and status of seagrasses. In: Green EP, Short FT (eds) World Atlas of Seagrasses. UNEP World Conservation Monitoring Centre by the University of California Press, Berkeley, USA.

Starmans A, Gutt J, Arntz WE (1999) Mega-epibenthic communities in Arctic and Antarctic shelf areas. Marine Biology 135: 269-280. https://doi.org/10.1007/s002270050624

Stat M, Carter D, Hoeghguldberg O (2006) The evolutionary history of Symbiodinium and scleractinian hosts—Symbiosis, diversity, and the effect of climate change. Perspectives in Plant Ecology, Evolution and Systematics 8: 23-43. https://doi.org/10.1016/j.ppees.2006.04.001

Steindler L, Beer S, Ilan M (2002) Photosymbiosis in intertidal and subtidal tropical sponges. Symbiosis 33: 263-273

Stephens DW, Krebs JR (1986) Foraging Theory. Princeton University Press

Stockner JG, Antia NJ (1986) Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Canadian Journal of Fisheries and Aquatic Sciences 43. https://doi.org/10.1139/f86-307

Subhan B, Arafat D, Santoso P, Madduppa H, Prabowo B, Yugiswara RS, Hakim MR, Susilo SB, Khairudi D, Awak DSHLMK, Khair M (2018) Drone dan Biodiversitas Kelautan. IPB Press, Bogor

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. https://doi.org/10.1128/MMBR.00040-06

Thingstad T (2008) Control of bacterial growth in idealized food webs. In: Kirchman DL, Mitchell R (eds) Microbial Ecology of the Oceans. John Wiley & Sons, Incorporated, pp 229-260

Tittensor DP, Mora C, Jetz W, Lotze HK, Ricard D, Berghe EV, Worm B (2010) Global patterns and predictors of marine biodiversity across taxa. Nature 466: 1098-1101. https://doi.org/10.1038/nature09329

Tortell PD, Maldonado MT, Granger J, Price NM (1999) Marine bacteria and biogeochemical cycling of iron in the oceans. FEMS Microbiology Ecology 29: 1-11. https://doi.org/10.1111/j.1574-6941.1999.tb00593.x

Trench RK, Wethey DS, Porter JW (1981) Observations on the symbiosis with zooxanthellae among the Tridacnidae (Molusca, Bivalvia). Biology Bulletin 161: 180-198. https://doi.org/10.2307/1541117

Unsworth RKF, Ambo-Rappe R, Jones BL, La Nafie YA, Irawan A, Hernawan UE, Moore AM, Cullen-Unsworth LC (2018) Indonesia's globally significant seagrass meadows are under widespread threat. Science of The Total Environment 634: 279-286. https://doi.org/10.1016/j.scitotenv.2018.03.315

Unsworth RKF, Collier CJ, Waycott M, McKenzie LJ, Cullen-Unsworth LC (2015) A framework for the resilience of seagrass ecosystems. Marine Pollution Bulletin 100: 34-46. https://doi.org/10.1016/j.marpolbul.2015.08.016

Unsworth RKF, De León PS, Garrard SL, Jompa J, Smith DJ, Bell JJ (2008) High connectivity of Indo-Pacific seagrass fish assemblages with mangrove and coral reef habitats. Marine Ecology Progress Series 353: 213-224. https://doi.org/10.3354/meps07199

Unsworth RKF, Hinder SL, Bodger OG, Cullen-Unsworth LC (2014) Food supply depends on seagrass meadows in the coral triangle. Environmental Research Letters 9: 094005. https://doi.org/10.1088/1748-9326/9/9/094005

Unsworth RKF, Nordlund LM, Cullen-Unsworth LC (2019) Seagrass meadows support global fisheries production. Conservation Letters 12: e12566. https://doi.org/10.1111/conl.12566

Unsworth RKF, Wylie E, Smith DJ, Bell JJ (2007) Diel trophic structuring of seagrass bed fish assemblages in the Wakatobi Marine National Park, Indonesia. Estuarine, Coastal and Shelf Science 72: 81-88. https://doi.org/10.1016/j.ecss.2006.10.006

Usher KM (2008) The ecology and phylogeny of cyanobacterial symbionts in sponges. Marine Ecology 29: 178-192. https://doi.org/10.1111/j.1439-0485.2008.00245.x

Utami RT, Zamani NP, Madduppa HH (2018) Molecular identification, abundance and distribution of the coral-killing sponge *Terpios hoshinota* in Bengkulu and Seribu Islands, Indonesia. Biodiversitas Journal of Biological Diversity 19: 2238-2246. https://doi.org/10.13057/biodiv/d190632

Vacelet J (1996) Deep-sea sponges in a Mediterranean cave. In: Uiblein F, Ott J, Statchowitsch M (eds) Deep-sea and extreme shallow-water habitats: affinities and adaptations

Vacelet J, Boury-Esnault N (1995) Carnivorous sponges. Nature 373: 333-335. https://doi.org/10.1038/373333a0

Vacelet J, Boury-Esnault N (1996) A new species of carnivorous sponge (Demospongiae: Cladorhizidae) from a Mediterranean cave. Bulletin de l'Institut Royal des Sciences Naturelles de Belgique (Biologie) 66: 109-115

Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. Journal of Experimental Marine Biology and Ecology 30: 301-314. https://doi.org/10.1016/0022-0981(77)90038-7

Valiela I, Bowen JL, York JK (2001) Mangrove Forests: One of the World's Threatened Major Tropical Environments: At least 35% of the area of mangrove forests has been lost in the past two decades, losses that exceed those for tropical rain forests and coral reefs, two other well-known threatened environments. BioScience 51: 807-815. https://doi.org/10.1641/0006-3568(2001)051[0807:MFOOTW]2.0.CO;2

Van Soest RW, Boury-Esnault N, Vacelet J, Dohrmann M, Erpenbeck D, De Voogd NJ, Santodomingo N, Vanhoorne B, Kelly M, Hooper JN (2012) Global diversity of sponges (Porifera). PLoS One 7: e35105. https://doi.org/10.1371/journal.pone.0035105

Van Soest RWM (1989) The Indonesian sponge fauna: A status report. Netherland Journal of Sea Research 23: 223-230. https://doi.org/10.1016/0077-7579(89)90016-1

Vargas S, Schuster A, Sacher K, Büttner G, Schätzle S, Läuchli B, Hall K, Hooper JNA, Erpenbeck D, Wörheide G (2012) Barcoding Sponges: An Overview Based on Comprehensive Sampling. PLOS ONE 7: e39345. https://doi.org/10.1371/journal.pone.0039345

Vaulot D, Eikrem W, Viprey M, Moreau H (2008) The diversity of small eukaryotic phytoplankton (≤ 3 μm) in marine ecosystems. FEMS Microbiology Reviews 32: 795-820. https://doi.org/10.1111/j.1574-6976.2008.00121.x

Vaulot D, Marie D (1999) Diel variability of photosynthetic picoplankton in the equatorial Pacific. Journal of Geophysical Research: Oceans 104: 3297-3310. https://doi.org/10.1029/98JC01333

Veron JEN, Devantier LM, Turak E, Green AL, Kininmonth S, Stafford-Smith M, Peterson N (2009) Delineating the Coral Triangle. Galaxea, Journal of Coral Reef studies 11: 91-100. https://doi.org/10.3755/galaxea.11.91

Veron JEN, Stafford-Smith MG, Turak E, DeVantier LM (2016) Corals of the World. Accessed 10 Apr 2022. http://wwwcoralsoftheworldorg/page/home/

Vicente VP (1990) Response of sponges with autotrophic endosymbionts during the coral-bleaching episode in Puerto Rico. Coral Reefs 8: 199-202. https://doi.org/10.1007/BF00265011

Vicente VP, Rutzler K, Carballeira NM (1991) Comparative Morphology, Ecology, and Fatty Acid Composition of West Indian Spheciospongia (Demospongea). Marine Ecology 12: 211-226. https://doi.org/10.1111/j.1439-0485.1991.tb00254.x

Vinod K, George RM, Manisseri MK (2009) Preliminary studies on the growth in captivity of Spirastrella inconstans (Dendy) collected from the intertidal region of Palk Bay, south-east

coast of India. Marine Fisheries Information Service - Technical and Extension Series 202: 4-6

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of earth's ecosystems. Science 277: 494-499. https://doi.org/10.1126/science.277.5325.494

Voigt O, Eichmann V, Wörheide G (2012) First evaluation of mitochondrial DNA as a marker for phylogeographic studies of Calcarea: a case study from Leucetta chagosensis Ancient Animals, New Challenges. Springer, pp 101-106

Vosmaer G (1880) The sponges of the Leyden Museum. I. The family of the Desmacidinae. Notes from the Leyden Museum 2: 99-159

Wahyudi AaJ, Rahmawati S, Irawan A, Hadiyanto H, Prayudha B, Hafizt M, Afdal A, Adi NS, Rustam A, Hernawan UE, Rahayu YP, Iswari MY, Supriyadi IH, Solihudin T, Ati RNA, Kepel TL, Kusumaningtyas MA, Daulat A, Salim HL, Sudirman N, Suryono DD, Kiswara W (2020) Assessing Carbon Stock and Sequestration of the Tropical Seagrass Meadows in Indonesia. Ocean Science Journal 55: 85-97. https://doi.org/10.1007/s12601-020-0003-0

Wakatobi R (2019) Kondisi geografis daerah. www.wakatobikab.go.id. Accessed in 8 January 2019. Regency of Wakatobi

Warren CR (2008) Rapid Measurement of Chlorophylls with a Microplate Reader. Journal of Plant Nutrition 31: 1321-1332. https://doi.org/10.1080/01904160802135092

Waterbury JB, Watson SW, Guillard RRL, Brand LE (1979) Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature 277: 293-294. https://doi.org/10.1038/277293a0

Waycott M, Duarte CM, Carruthers TJ, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck KL, Jr., Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106: 12377-12381. https://doi.org/10.1073/pnas.0905620106

Waycott M, McKenzie LJ, Mellors JE, Ellison JC, Sheaves MT, Collier C, Schwarz A-m, Webb A, Johnson JE, Payri CE (2011) Vulnerability of mangroves, seagrasses and intertidal flats in the tropical Pacific to climate change. In: Bell JD, Johnson JE, Hobday AJ (eds) Vulnerability of Tropical Pacific Fisheries and Aquaculture to Climate Change Secretariat of the Pacific Community. WorldFish, Noumea, New Caledonia., pp 297-368

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

Webster NS, Thomas T (2016) The Sponge Hologenome. mBio 7: e00135-00116. https://doi.org/10.1128/mBio.00135-16

Weinbauer MG (2004) Ecology of prokaryotic viruses. FEMS Microbiology Reviews 28: 127-181. https://doi.org/10.1016/j.femsre.2003.08.001

Weisz JB, Lindquist N, Martens CS (2008) Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? Oecologia 155: 367-376. https://doi.org/10.1007/s00442-007-0910-0

Weisz JB, Massaro AJ, Ramsby BD, Hill MS (2010) Zooxanthellar symbionts shape host sponge trophic status through translocation of carbon. Biological Bulletin 219: 189-197. https://doi.org/10.1086/bblv219n3p189

Wetz MS, Wheeler PA (2007) Release of dissolved organic matter by coastal diatoms. Limnology and Oceanography 52: 798-807. https://doi.org/10.4319/lo.2007.52.2.0798

White JR, Patel J, Ottesen A, Arce G, Blackwelder P, Lopez JV (2012) Pyrosequencing of Bacterial Symbionts within Axinella corrugata Sponges: Diversity and Seasonal Variability. PLOS ONE 7: e38204. https://doi.org/10.1371/journal.pone.0038204

White TCR (1978) The importance of a relative shortage of food in animal ecology. Oecologia 33: 71-86. https://doi.org/10.1007/BF00376997

Wilkinson C (Ed) (2008) Status of coral reefs of the world: 2008. Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, Townsville, Australia.

Wilkinson CR (1978) Microbial Associations in Sponges. I. Ecology, Physiology and Microbial Populations of Coral Reef Sponges. Marine Biology 49: 161-167. https://doi.org/10.1007/BF00387115

Wilkinson CR (1980) Nutrient translocation from green algal symbionts to the freshwater sponge *Ephydatia fluviatilis*. Hydrobiologia 75: 241-250. https://doi.org/10.1007/BF00006488

Wilkinson CR (1983) Net primary productivity in coral reefs sponges. Science 219: 410-412. https://doi.org/10.1126/science.219.4583.410

Wilkinson CR (1987a) Interocean differences in size and nutrition of coral reef sponge populations. Science 236: 1654-1657. https://doi.org/10.1126/science.236.4809.1654

Wilkinson CR (1987b) Productivity and abundance of large sponge populations on Flinders Reef Flats, Coral Sea. Coral Reefs 5: 183-188. https://doi.org/10.1007/BF00300961

Wilkinson CR (1988) Foliose Dictyoceratida of the Australian Great Barrier Reef. Marine Ecology 9: 321-327. https://doi.org/10.1111/j.1439-0485.1988.tb00210.x

Wilkinson CR, Cheshire AC (1990) Comparison of sponge populations accross the Great Barrier Reefs of Australia and Belize: evidence for higher productivity in the Caribbean. Marine Ecology Progress Series 67: 285-294. https://doi.org/10.3354/meps067285

Wilkinson CR, Fay P (1979) Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. Nature 279: 527-529. https://doi.org/10.1038/279527a0

Wilkinson CR, Trott LA (1985) Light as a factor determining the distribution of sponges across the Central Great Barrier Reef. In: Vivien MH, Salvat B (eds) 5th International Coral Reef Symposium Tahiti, pp 125-130

Willenz P, Hartman WD (1989) Micromorphology and ultrastructure of Caribbean sclerosponges. Marine Biology 103: 387-401. https://doi.org/10.1007/BF00397274

Wisshak M, Schönberg CHL, Form A, Freiwald A (2013) Effects of ocean acidification and global warming on reef bioerosion—lessons from a clionaid sponge. Aquatic Biology 19: 111-127. https://doi.org/10.3354/ab00527

Wisshak M, Schönberg CHL, Form A, Freiwald A (2014) Sponge bioerosion accelerated by ocean acidification across species and latitudes? Helgoland Marine Research 68: 253-262. https://doi.org/10.1007/s10152-014-0385-4

Wooster MK, McMurray SE, Pawlik JR, Morán XAG, Berumen ML (2019) Feeding and respiration by giant barrel sponges across a gradient of food abundance in the Red Sea. Limnology and Oceanography 64: 1790-1801. https://doi.org/10.1002/lno.11151

Wörheide G (2006) Low variation in partial cytochrome oxidase subunit I (COI) mitochondrial sequences in the coralline demosponge Astrosclera willeyana across the Indo-Pacific. Marine Biology 148: 907-912. https://doi.org/10.1007/s00227-005-0134-y

Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Lotze HK, 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. https://doi.org/10.1126/science.1132294

Wulff JL (1984) Sponge-mediated coral reef growth and rejuvenation. Coral Reefs 3: 157-163. https://doi.org/10.1007/BF00301960

Wulff JL (1991) Asexual fragmentation, genotype success, and population dynamics of erect branching sponges. Journal of Experimental Marine Biology and Ecology 149: 227-247. https://doi.org/10.1016/0022-0981(91)90047-Z

Wulff JL (1995) Sponge-feeding by the Caribbean starfish *Omaster reticulatus*. Marine Biology 123: 313-325. https://doi.org/10.1007/BF00353623

Wulff JL (2006) Ecological interaction of marine sponges. Canadian Journal of Zoology 84: 146-166. https://doi.org/10.1139/z06-019

Wulff JL (2008) Collaboration among sponge species increases sponge diversity and abundance in a seagrass meadow. Marine Ecology 29: 193-204. https://doi.org/10.1111/j.1439-0485.2008.00224.x

Yahel G (2003) Feeding on ultraplankton and dissolved organic carbon in coral reefs: from the individual grazers to the community. Doctorate Thesis. Department of Ecology, Systematics and Evolution

Yahel G, Eerkes-Medrano DI, Leys SP (2006) Size independent selective filtration of ultraplankton by hexactinellid glass sponges. Aquatic Microbial Ecology 45: 181-194. https://doi.org/10.3354/ame045181

Yahel G, Marie D, Genin A (2005) InEx - a direct in situ method to measure filtration rates, nutrition, and metabolism of active suspension feeders. Limnology and Oceanography: Methods 3: 46-58. https://doi.org/10.4319/lom.2005.3.46

Yahel G, Sharp JH, Marie D, Häse 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. https://doi.org/10.4319/lo.2003.48.1.0141

Yang Q, Franco CM, Sorokin SJ, Zhang W (2017) Development of a multilocus-based approach for sponge (phylum Porifera) identification: refinement and limitations. Scientific Reports 7: 41422. https://doi.org/10.1038/srep41422

Yarbro LA, Carlson PR (2008) Community Oxygen and Nutrient Fluxes in Seagrass Beds of Florida Bay, USA. Estuaries and Coasts 31: 877-897. https://doi.org/10.1007/s12237-008-9071-6

Appendix A – Supplementary data for Chapter 2, Contrasting drivers of sponge and seagrass assemblage composition in an Indo-Pacific seagrass meadow

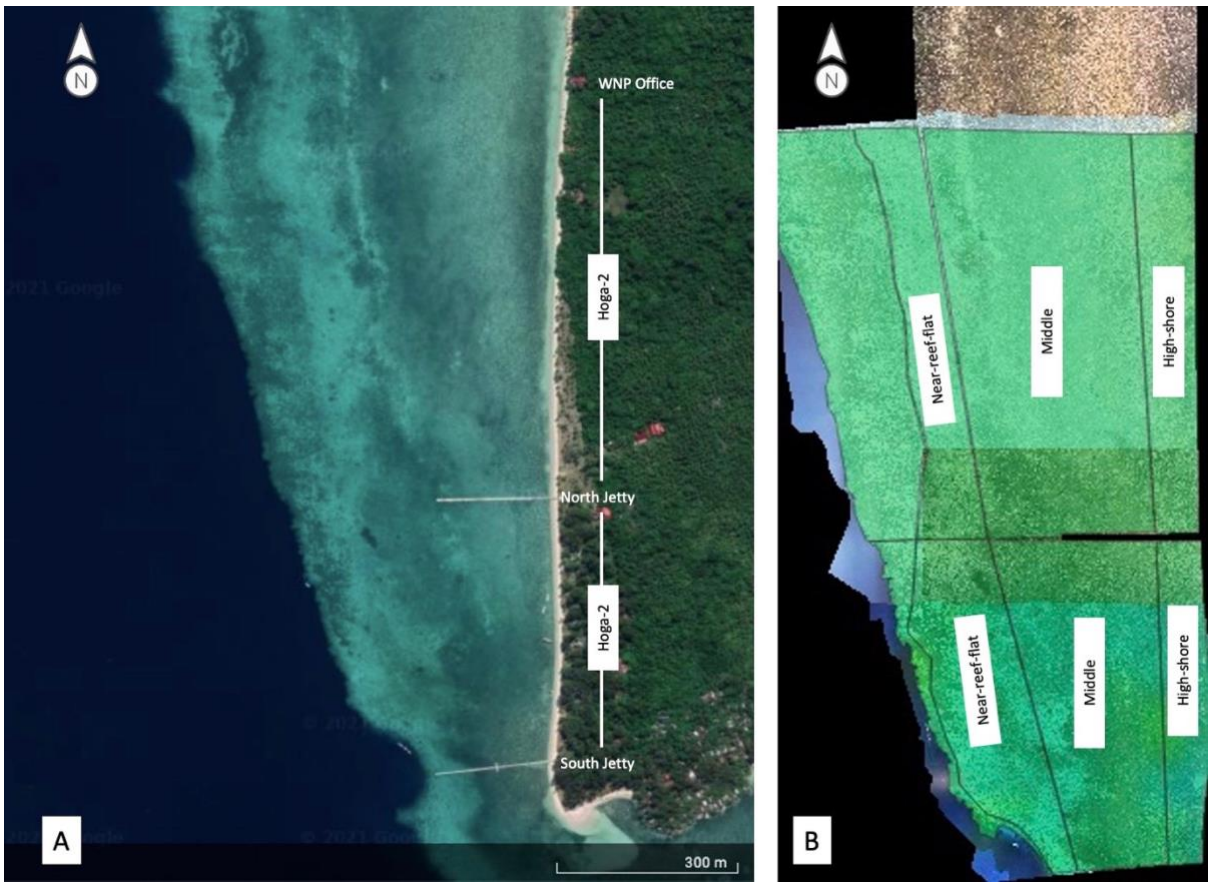

Figure S.2.1. (A) Google Earth image of the studied seagrass meadow, with the human-built structures used to be the boundaries of the site separation (i.e. Hoga-1 and Hoga-2). (B) Orthophoto that build from aerial pictures, which used to draw the boundaries of the zones and calculated the area coverage.

Table S.2.1. Summary of BLAST results and phylogenetic analysis for every DNA markers. Red colour denotes that the results was under the threshold limit or not a Porifera member.

Sponge	Sponge Code	BLAST result				Phylogenetic analysis - Maximum Likelihood				Phylogenetic analysis - Neighbor-Joining			
		18S Identity (Bit Score, Query covers, % similarity)	28S Identity (Bit Score, Query covers, % similarity)	ITS Identity (Bit Score, Query covers, % similarity)	COX-1 Identity (Bit Score, Query covers, % similarity)	18S locus	28S locus	ITS locus	COX-1 locus	18S locus	28S locus	ITS locus	COX-1 locus
SPONGE A	HOGA A1	*Petrosaspongia nigra* ; *Spongia zimocca* (1366, 100%, 100%)	*Hippospongia ammata* ; *Spongia zimocca* (673, 100%, 99.5%)	*Hippospongia ammata* (1190, 87.50%, 98.9%)	na	*Petrosaspongia nigra* ; *Spongia zimocca*	*Hippospongia ammata*	*Hippospongia ammata*	na	*Petrosaspongia nigra* ; *Spongia zimocca*	*Hippospongia ammata*	*Hippospongia ammata*	na
	HOGA A2	*Petrosaspongia nigra* ; *Spongia zimocca* (1246, 100%, 100%)	*Hippospongia ammata* ; *Spongia zimocca* (662, 100%, 98.9%)	*Hippospongia ammata* (1043, 86.8%, 94.7%)	na	*Spongia zimocca*	*Spongia zimocca*	*Hippospongia ammata*	na	*Spongia zimocca*	*Spongia zimocca*	*Hippospongia ammata*	na
	HOGA A3	*Petrosaspongia nigra* ; *Spongia zimocca* (1364, 100%, 99.9%)	*Hippospongia ammata* ; *Spongia zimocca* (659, 100%, 98.7%)	*Hippospongia ammata* (1159, 82.12%, 98%)	na	*Petrosaspongia nigra* ; *Spongia zimocca*	*Hippospongia ammata*	*Hippospongia ammata*	na	*Spongia zimocca*	*Hippospongia ammata*	*Hippospongia ammata*	na
SPONGE B	HOGA B1	*Spheciospongia vesparium* ; *Spheciospongia* sp. (1303, 100%, 99.9%)	*Spheciospongia inconstans* (581, 76.92%, 93.6%)	*Spheciospongia solida* (693, 100%, 85.4%)	na	*Spheciospongia vesparium* ; *Spheciospongia* sp.	*Spheciospongia inconstans*	*Cliona orientalis*	na	*Spheciospongia vesparium* ; *Spheciospongia* sp.	*Spheciospongia inconstans*	*Spheciospongia solida*	na
	HOGA B2	*Spheciospongia vesparium* (1394, 100%, 99.9%)	*Spheciospongia inconstans* (630, 90.74%, 94.8%)	*Spheciospongia solida* (367, 76.15%, 78.9%)	na	*Spheciospongia vesparium*	*Spheciospongia inconstans*	Uncultured Ascomycota	na	*Spheciospongia vesparium*	*Spheciospongia inconstans*	Uncultured Ascomycota	na
	HOGA B3	*Cliona delitrix* ; *Cliona viridis* ; *Spheciospongia* sp.; *Cliona* sp. (1011, 100%, 100%)	Cf. Spheciospongia (563, 100%, 93.2%)	*Spheciospongia solida* (265, 74.61%, 75.7%)	na	*Cliona delitrix* ; *Cliona viridis* ; *Spheciospongia* sp.; *Cliona* sp.	*Spheciospongia inconstans* ; Cf. Spheciospongia	Uncultured Ascomycota	na	*Cliona delitrix* ; *Cliona viridis* ; *Spheciospongia* sp.; *Cliona* sp.	*Spheciospongia inconstans*	Uncultured Ascomycota	na
SPONGE C	HOGA C1	*Strepsichordaia lendenfeldi* (1322, 100%, 98.8%)	*Carteriospongia foliascens* (644, 100%, 99.2%)	*Carteriospongia flabellifera* (1142, 86.49%, 98.9%)	na	*Strepsichordaia lendenfeldi*	*Carteriospongia foliascens*	*Carteriospongia flabellifera*		*Strepsichordaia lendenfeldi*	*Carteriospongia foliascens*	*Carteriospongia flabellifera*	
	HOGA C2	*Strepsichordaia lendenfeldi* (1362, 100%, 100%) *Phyllospongia papyracea* (1357, 100%, 99.9%) *Phyllospongia lamellosa* (1340, 100%, 99.9%)	*Carteriospongia foliascens* (681, 100%, 99.5%)	*Carteriospongia flabellifera* (1157, 87.07%, 99.2%) *Carteriospongia foliascens* (1141, 87.02%, 98.8%)	na	*Strepsichordaia lendenfeldi*	*Carteriospongia foliascens*	*Carteriospongia flabellifera*	na	*Strepsichordaia lendenfeldi*	*Carteriospongia foliascens*	*Carteriospongia flabellifera*	na
	HOGA C3	*Strepsichordaia lendenfeldi* (1129, 100%, 100%) *Phyllospongia papyracea* (1124, 100%, 99.8%) *Phyllospongia lamellosa* (1113, 100%, 99.5%)	*Carteriospongia foliascens* (664, 100%, 98.9%)	*Carteriospongia flabellifera* (1157, 81.14%, 99.2%) *Carteriospongia foliascens* (1141, 81.14%, 98.8%)	na	*Strepsichordaia lendenfeldi*	*Carteriospongia foliascens*	*Carteriospongia foliascens*	na	*Strepsichordaia lendenfeldi*	*Carteriospongia foliascens*	*Carteriospongia foliascens*	na

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SPONGE D	HOGA D1	*Callyspongia* sp. (1480, 100%, 99.3%)	Demospongiae (588, 92.94%, 94.5%)	*Haliclona* sp. (302, 28.07%, 98.8%)	Haplosclerida (949, 100%, 99.4%)	*Callyspongia* sp.	Haplosclerida	*Haliclona* aff.	*Haliclona koremella*	*Callyspongia* sp.	Demospongiae	*Haliclona* aff.	*Callyspongia* sp.
	HOGA D2	*Callyspongia* sp. (1551, 100%, 99.4%)	Demospongiae (598, 94.06%, 95%)	*Haliclona* sp. (297, 24.14%, 98.2%)	Haplosclerida (943, 100%, 99.8%) *Callyspongia* sp. (891, 95.13%, 99.6%) *Haliclona koremella* (888, 93.57%, 100%)	*Callyspongia* sp.	Haplosclerida	*Haliclona* aff.	*Haliclona koremella*	*Callyspongia* sp.	Demospongiae	*Haliclona* aff.	*Haliclona koremella*
	HOGA D3	*Callyspongia* sp. (1373, 100%, 99.3%)	Demospongiae (581, 94.4%, 94.9%)	*Haliclona* sp. (302, 23.9%, 98.8%)	Haplosclerida (952, 99.42%, 99.8%) *Callyspongia* sp. (899, 94.43%, 99.6%) *Haliclona koremella* (895, 92.90%, 100%)	*Callyspongia* sp.	Haplosclerida	*Haliclona* aff.	*Haliclona koremella*	*Callyspongia* sp.	Haplosclerida	*Haliclona* aff.	*Haliclona koremella*
SPONGE E	HOGA E1	*Amphimedon queenslandica* (1430, 100%, 99.6%)	*Amphimedon queenslandica* (561, 50.97%%, 89.3%)	na	*Amphimedon queenslandica* (974, 100%%, 99.3%)	*Amphimedon queenslandica*	only one hit	na	*Amphimedon queenslandica*	*Amphimedon queenslandica*	only one hit	na	*Amphimedon queenslandica*
	HOGA E2	*Amphimedon queenslandica* (1258, 100%, 99.5%)	*Amphimedon queenslandica* (686, 100%, 98%)	na	*Amphimedon queenslandica* (1008, 58.22%, 98.3%)	*Amphimedon queenslandica*	*Amphimedon queenslandica*	na	*Amphimedon queenslandica*	*Amphimedon queenslandica*	*Amphimedon queenslandica*	na	*Amphimedon queenslandica*
	HOGA E3	*Amphimedon queenslandica* (1419, 100%, 99.6%)	*Amphimedon queenslandica* (641, 100%, 95.5%)	*Amphimedon queenslandica* (463, 60.53%, 85%)	*Amphimedon queenslandica* (875, 100%, 99.4%)	*Amphimedon queenslandica*	*Amphimedon queenslandica*	*Amphimedon queenslandica*	*Amphimedon queenslandica*	*Amphimedon queenslandica*	*Amphimedon queenslandica*	*Amphimedon queenslandica*	*Amphimedon queenslandica*
SPONGE F	HOGA F1	*Dactylospongia elegans* (1342, 100%, 99.9%)	*Dactylospongia elegans* (526, 100%, 95.0%)	*Dactylospongia elegans* (1183, 85.73%, 99.2%)	*Ircinia campana* (683, 98.63%, 95.2%)	*Dactylospongia elegans*	*Dactylospongia elegans*	*Dactylospongia elegans*	*Hippospongia lachne*	*Dactylospongia elegans*	*Dactylospongia elegans*	*Dactylospongia elegans*	*Hippospongia lachne*
	HOGA F2	*Dactylospongia elegans* (1371, 100%, 99.9%)	*Dactylospongia elegans* (677, 100%, 99.5%)	*Dactylospongia elegans* (1209, 83.42%, 99.3%)	*Spongia nitens* ; *Hippospongia lachne* (712, 99.78%, 94.4%)	*Dactylospongia elegans*	*Dactylospongia elegans*	*Dactylospongia elegans*	*Vacelitia* sp.	*Dactylospongia elegans*	*Dactylospongia elegans*	*Dactylospongia elegans*	*Vacelitia* sp.
	HOGA F3	*Dactylospongia elegans* (1362, 100%, 100%)	*Dactylospongia elegans* (714, 100%, 99.0%)	*Dactylospongia elegans* (950, 73.71%, 92.8%)	*Ircinia strobilina* (790, 99.61%, 94.4%)	*Dactylospongia elegans*	*Dactylospongia elegans*	*Dactylospongia elegans*	*Vaceletia* sp.	*Dactylospongia elegans*	*Dactylospongia elegans*	*Lendenfeldia chondrodes*	*Vaceletia* sp.
SPONNGE G	HOGA G1	*Axinella verrucosa* ; *Axinella corrugata* (1364, 100%, 99.9%)	*Stylissa carteri* ; Halichondriid; *Axinella* sp.; *Timea lowchoyi* (696, 100%, 98.0%)	*Axinella corrugata* (1294, 100%, 97.5%)	*Axinella corrugata* (956, 100%, 99.1%)	*Axinella* sp.	*Stylissa carteri* ; Halichondriid; *Axinella* sp.; *Timea lowchoyi*	Axinellid	*Stylissa carteri* ; *Stylissa massa*	*Axinella* sp.	*Stylissa carteri* ; Halichondriid; *Axinella* sp.; *Timea lowchoyi*	Axinellid	*Stylissa carteri*
	HOGA G2	*Axinella* sp. (1349, 100%, 99.9%) *Axinella verrucosa* & *Axinella corrugata* (1347, 100%, 99.9%)	*Stylissa carteri* ; Halichondriid; *Axinella* sp. (677, 100%, 99.7%)	*Axinella corrugata* (1368, 99.62%, 97.9%)	*Axinella corrugata* (874, 100%, 98.6%)	*Axinella* sp.	*Stylissa carteri* ; Halichondriid; *Axinella* sp.	Axinellid	*Stylissa carteri* ; Stylissa massa	*Axinella* sp.	*Stylissa carteri* ; Halichondriid; *Axinella* sp.	Axinellid	*Stylissa carteri*
	HOGA G3	*Axinella verrucosa* ; *Axinella corrugata* (1357, 100%, 99.3%)	*Stylissa carteri* ; Halichondriid; *Axinella* sp.; *Timea lowchoyi* (714, 99.25%, 99.2%)	*Axinella corrugata* (1370, 99.5%, 97.9%)	*Axinella corrugata* (904, 100%, 99.0%)	*Axinella verrucosa* ; *Axinella corrugata*	*Stylissa carteri* ; Halichondriid; *Axinella* sp.; *Timea lowchoyi*	Axinellid	*Stylissa carteri*	*Axinella verrucosa* ; *Axinella corrugata*	*Stylissa carteri*	Axinellid	*Stylissa carteri*

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SPONGE H	HOGA H1	*Clathria reinwardti* (1196, 100%, 96.9%)	*Clathria reinwardti* (577, 88.58%, 90.3%)	na	*Clathria toxitenuis* (718, 99.44%, 91.0%)	*Clathria reinwardti*	*Clathria reinwardti*	na	*Clathria toxitenuis*	*Clathria reinwardti*	*Clathria reinwardti*	na	*Clathria toxitenuis*
	HOGA H2	na	na	*Mycale fibrexilis* (295, 22.88%, 97.1%)	*Clathria toxitenuis* (631, 99.79%, 90.79%)	na	na	*Batzella aurantiaca*	*Clathria toxitenuis*	na	na	*Batzella aurantiaca*	*Clathria toxitenuis*
SPONGE I	HOGA I1	*Petrosaspongia nigra* ; *Spongia zimocca* (1373, 100%, 99.9%)	*Hippospongia ammata* ; *Spongia zimocca* (692, 100%, 98.7%)	*Hippospongia ammata* (1028, 83.42%, 94.5%)	na	*Petrosaspongia nigra* ; *Spongia zimocca*	*Hippospongia ammata* ; *Spongia zimocca*	*Hippospongia ammata*	na	Petrosaspongia nigra	*Spongia zimocca*	*Hippospongia ammata*	na
	HOGA I2	*Axinyssa topsenti* (1320, 100%, 99.7%)	Bubarida (415, 91.27%, 83.8%)	na	na	*Axinyssa topsenti*	Bubarida	na	na	*Axinyssa topsenti*	Bubarida	na	na
	HOGA I3	*Rhopaloeides* sp. (1375, 100%, 100%) *Smenospongia* sp. (1363, 100%, 99.7%)	Demospongiae (655, 100%, 98.4%)	*Dactylospongia elegans* (1153, 82.28%, 98.0%)	*Ircinia strobilina* (795, 100%, 94.7%)	*Rhopaloeides* sp.	*Dactylospongia elegans*	*Dactylospongia elegans*	*Vaceletia* sp.	*Rhopaloeides* sp.	Demospongiae	*Dactylospongia elegans*	*Vaceletia* sp.
SPONGE J	HOGA J1	Sphaerocorynidae (1272, 100%, 99.6%)	*Siphonodictyon mucosa* (708, 100%, 97.4%)	*Siphonodictyon mucosa* (1216, 81.44%, 99.6%)	na	Sphaerocorynidae	*Siphonodictyon mucosa*	*Siphonodictyon mucosa*	na	Sphaerocorynidae	*Siphonodictyon mucosa*	*Siphonodictyon mucosa*	na

Table S.2.2. Two-way PERMANOVA testing differences in overall seagrass and sponge assemblage composition in the studied seagrass meadow, between sites (Si), zones (Zo), and their interactions. Significant p-values are given in bold.

Seagrass assemblage						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique Perms**
Si	1	1440.2	1440.2	1.3181	0.301	999
Zo	2	3961.4	1980.7	1.8127	0.127	999
Si x Zo	2	1171	585.5	0.53584	0.746	999
Res	12	13112	1092.7			
Total	17	19685				
Habitat resilience parameters						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique Perms**
Si	1	90.993	90.993	1.1767	0.351	999
Zo	2	227.52	113.76	1.4711	0.255	997
Si x Zo	2	54.745	27.373	0.35397	0.863	999
Res	12	927.97	77.331			
Total	17	1301.2				
Sponge assemblage						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique Perms**
Si	1	3032.4	3032.4	2.7285	**0.042**	999
Zo	2	11642	5820.9	5.2376	**0.001**	999
Si x Zo	2	7560.5	3780.2	3.4014	**0.003**	999
Res	12	133336	1111.4			
Total	17	35571				

Table S.2.3. Estimated range of the total sponge abundance (in volume) at each zone in each site. Values are in litre.

Sponge	Hoga-1			Hoga-2		
	High-shore	Middle	Near-reef-flat	High-shore	Middle	Near-reef-flat
Spongia sp.	267 – 291	207 – 649	0	78 – 91	759 – 1229	28 – 150
Spheciospongia sp.	79 – 584	0 – 8	1 – 158	62 – 159	796 – 2976	162 – 510
Phyllospongia foliascens	0	136 – 334	77 – 282	0	0 – 127	109 – 191
Haliclona koremella	0	0 – 16	0	0	0 – 186	9 – 112
Amphimedon sp.	0 – 19	93 – 152	3 – 10	0	0 – 874	45 – 99
Dactylospongia elegans	0	0 – 221	15 – 47	0	0 – 415	0
Axinella sp.	0	0 – 61	0	0	0	9 – 64
Clathria reinwardti	0	0 – 12	0 – 5	0	38 – 235	0
Siphonodictyon mucosum	0	0	19 – 68	21 – 74	35 – 81	0 – 32
Rhopaloeides sp.	0 – 1	0 – 5	8 – 16	0 – 1	0 – 58	0 – 17
Total	346 – 894	436 – 1458	123 - 586	161 – 325	1628 – 6181	362 – 1175

Table S.2.4. List of the inhabitant sponge species with its growth form, based on morphological classification by Schönberg (2021).

Sponge species	Growth form	
	Basic form	Sub-category
Spongia sp.	Massive	Fistular, endopsamnic
Spheciospongia sp.	Massive	Fistular, endopsamnic
Phyllospongia foliascens	Erect	mostly erect-laminar, but can form functionally intermediate forms towards cup-like morphologies
Haliclona koremella	Erect	Branching in 3D
Amphimedon sp.	Cup-like	Amphoras, sack-like sponges
Dactylospongia elegans	Massive	Often composite massive
Axinella sp.	Massive	Often composite massive
Clathria reinwardti	Crust-like	Creeping
Siphonodictyon mucosum	Massive	Fistular, endopsamnic
Rhopaloeides sp.	Massive	Simple massive

Table S.2.5. SIMPER analysis on sponge assemblage. Species key: A= *Spongia sp.*; B= *Spheciospongia* sp.; C= *Phyllospongia foliascens*; D= *Haliclona koremella*; E= *Amphimedon sp.*; F= *Dactylospongia elegans*; G= *Axinella sp.*; H= *Clathria reinwardti*; I= Rhopaloeides sp.; J= *Siphonodictyon mucosum*.

Examines Site groups
(across all Zone groups)
Group Hoga-1
Average similarity: 55.42

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
A	1.38	24.41	1.02	44.04	44.04
C	0.85	10.65	0.86	19.22	63.26
I	0.52	6.91	0.66	12.47	75.73

Group Hoga-2
Average similarity: 56.01

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
A	1.39	19.56	1.46	34.93	34.93
B	1.81	19.16	1.62	34.22	69.15
C	0.61	5.70	0.65	10.18	79.33

Groups Hoga-1 & Hoga-2
Average dissimilarity = 60.35

	Group Hoga-1	Group Hoga-2				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
B	0.83	1.81	15.83	1.97	26.23	26.23
A	1.38	1.39	10.54	1.41	17.46	43.69
E	0.44	0.59	6.36	1.42	10.54	54.23
I	0.52	0.48	5.49	1.07	9.09	63.32
J	0.18	0.44	5.24	1.17	8.68	72.00

Examines Zone groups
(across all Site groups)

Group High Shore
Average similarity: 69.48

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
A	1.82	43.16	3.58	62.11	62.11
B	1.55	19.92	1.26	28.67	90.79

Group Middle
Average similarity: 51.12

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%

A	1.86	20.29	2.98	39.70	39.70
B	1.22	9.88	0.88	19.32	59.02
E	0.86	8.46	0.72	16.56	75.58

Group Reef Flat
Average similarity: 46.55

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
C	1.42	16.95	1.67	36.42	36.42
I	0.92	10.37	0.90	22.28	58.70
B	1.18	5.20	0.42	11.18	69.88
E	0.55	4.98	0.91	10.69	80.57

Groups High Shore & Middle
Average dissimilarity = 57.08

	Group High Shore	Group Middle				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
B	1.55	1.22	13.65	1.21	23.91	23.91
A	1.82	1.86	8.79	1.29	15.41	39.32
C	0.00	0.77	7.55	1.00	13.22	52.54
E	0.13	0.86	6.96	0.97	12.19	64.73
F	0.00	0.57	4.43	0.68	7.76	72.49

Groups High Shore & Reef Flat
Average dissimilarity = 76.98

	Group High Shore	Group Reef Flat				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
A	1.82	0.47	17.34	1.44	22.53	22.53
B	1.55	1.18	15.03	1.51	19.53	42.05
C	0.00	1.42	14.67	2.21	19.05	61.11
I	0.12	0.92	8.68	1.24	11.28	72.38

Groups Middle & Reef Flat
Average dissimilarity = 64.69

	Group Middle	Group Reef Flat				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
A	1.86	0.47	12.19	1.55	18.85	18.85
C	0.77	1.42	8.22	1.45	12.71	31.56
E	0.86	0.55	8.22	2.06	12.70	44.26
I	0.45	0.92	8.11	1.18	12.54	56.80
B	1.22	1.18	7.41	0.95	11.46	68.26
F	0.57	0.22	5.18	1.03	8.01	76.2

Appendix B – Supplementary data for Chapter 3, Variation in autotrophic and heterotrophic sponge abundance in a shallow water seagrass system

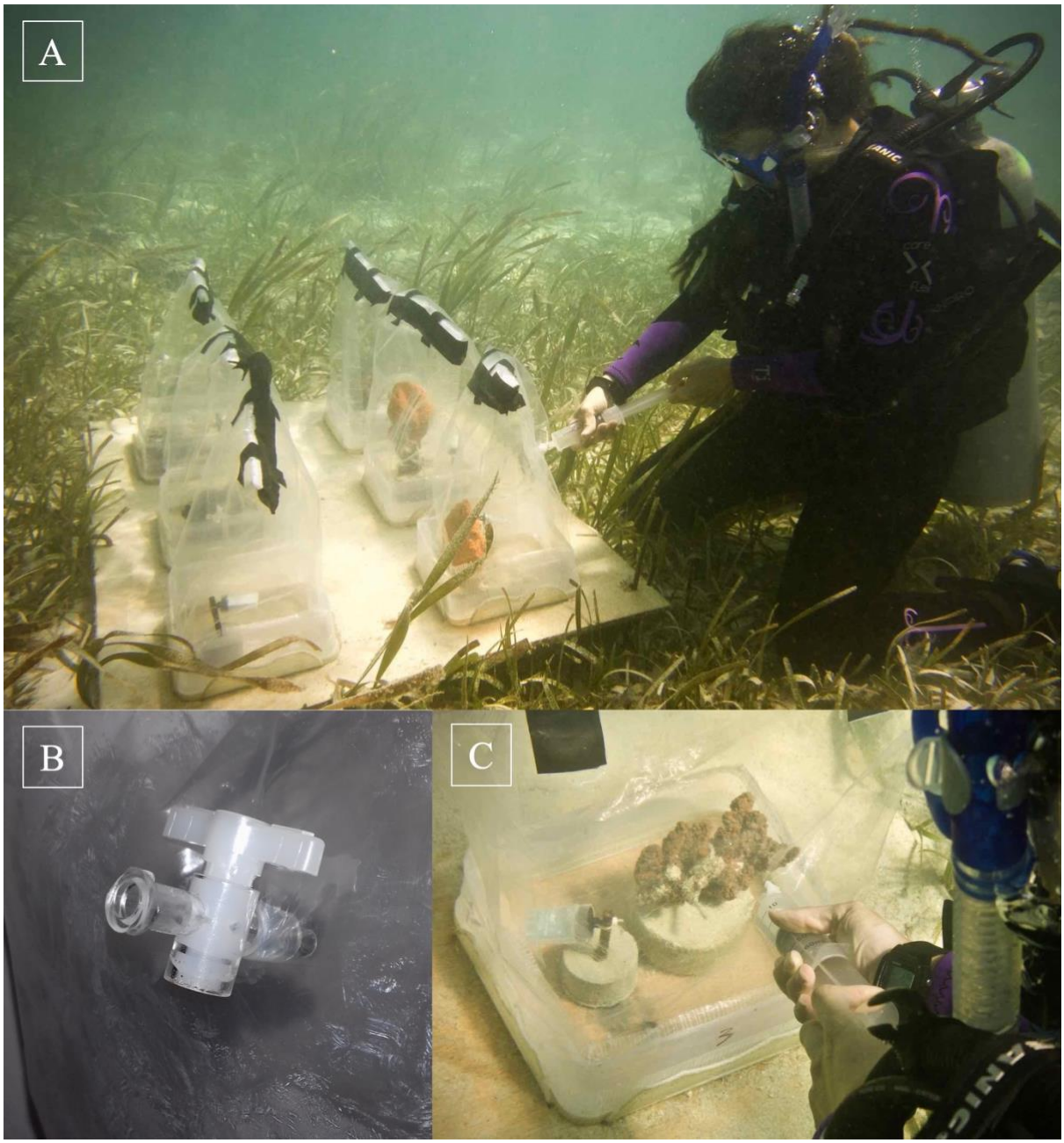


Figure S.3.1. Incubation bag set-up on the floor of the seagrass bed (A), the three-way valve on the chamber (B). and how the water sample was withdrawn with a syringe through the valve (C).

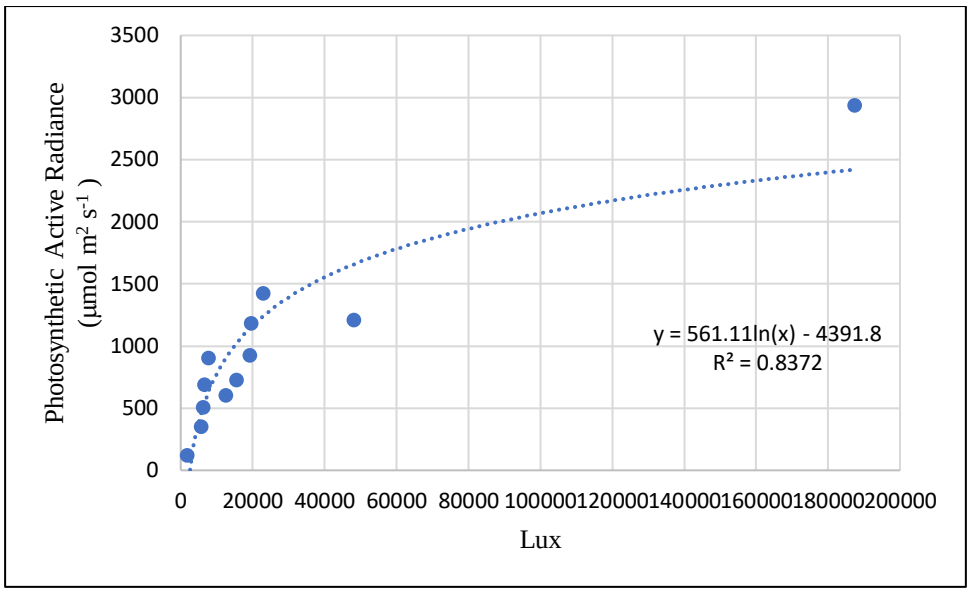


Figure S.3.2. Lux to PAR fitting function light conversion on the seafloor of the seagrass meadow (d = 1.2 m).

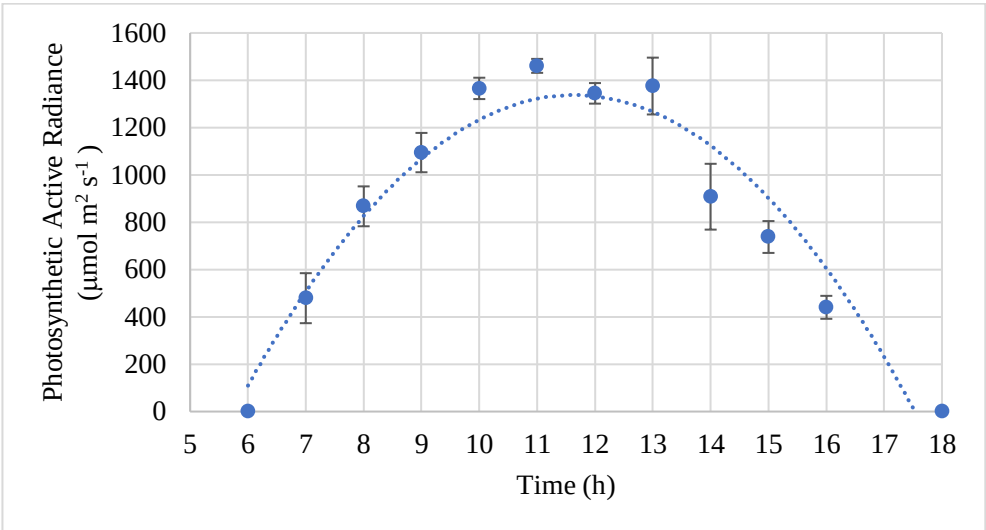


Figure S.3.3. Light intensity (mean ± SE, n = 12) through time at the bottom of the seagrass meadow. The values plotted for each hour were the average of the readings during that hour. The light intensity is measured as photosynthetic active radiance (PAR); µmol m^2 s^{-1}.

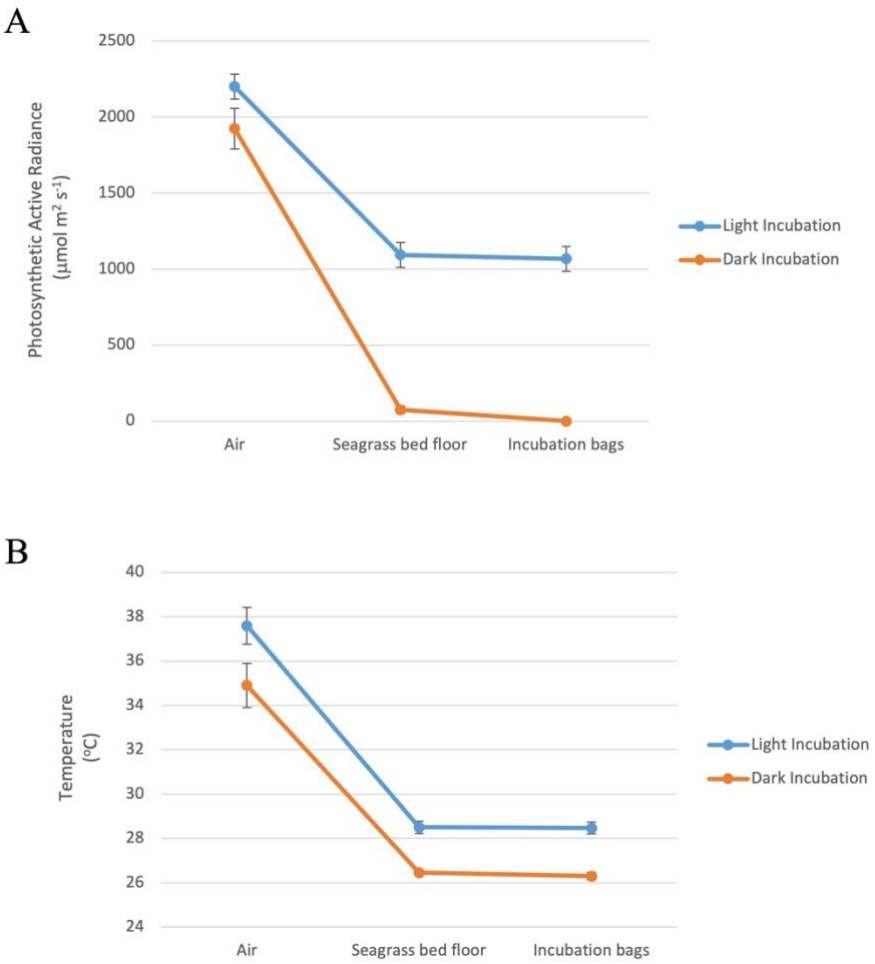


Figure S.3.4. The average light intensity (A) and temperature (B) above the surface (air), seagrass bed floor, and inside the incubation bags during light and dark incubations.

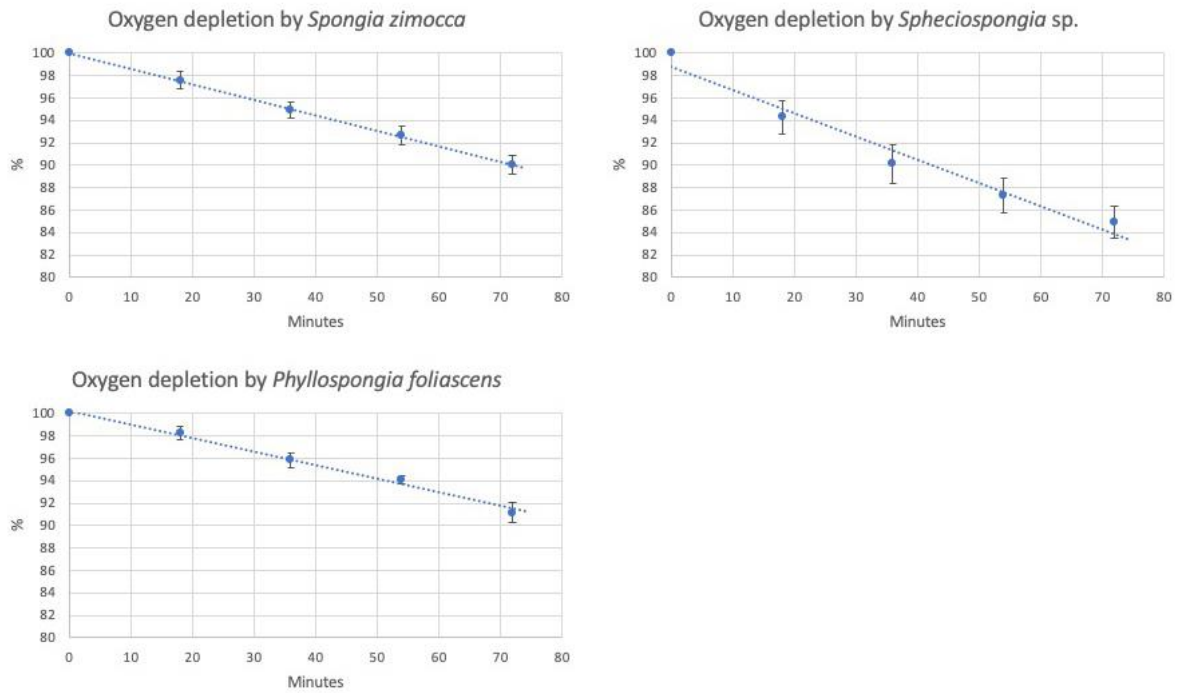


Figure S.3.5. Oxygen depletion (in percentage) in dark incubations every 18 minutes for *Spongia* sp., *Spheciospongia* sp., and *Phyllospongia foliascens*. Values are expressed in mean ± SE (n = 5).

Table S.3.1. The average light intensity and temperature during light and dark incubations from all Hobo loggers inside the incubation bags (n = 6). The incubation for each sponge species were conducted over three days to capture the inter-day variability. The values are expressed in mean ± SE, and the sponge number being measured under the light intensity and temperature are in bracket under the values. Light intensity was measured as photosynthetic active radiance (PAR) and the unit was μmol m^2 s^{-1}; and temperature was in ℃.

Sponge	Incubations	Light Intensity			Temperature		
		I	II	III	I	II	III
Spongia sp.	Light	1067 ± 56 (n = 1)	713 ± 47 (n = 2)	968 ± 23 (n = 2)	28.2 ± 0.08 (n = 1)	27.4 ± 0.04 (n = 2)	28.8 ± 0.10 (n = 2)
	Dark	0	0	0	26.6 ± 0.03 (n = 1)	26.6 ± 0.03 (n = 2)	26.8 ± 0.01 (n = 2)
Spheciospongia sp.	Light	1067 ± 56 (n = 2)	713 ± 47 (n = 1)	968 ± 23 (n = 2)	28.2 ± 0.08 (n = 2)	27.4 ± 0.04 (n = 1)	28.8 ± 0.10 (n = 2)
	Dark	0	0	0	26.6 ± 0.03 (n = 2)	26.6 ± 0.03 (n = 1)	26.8 ± 0.01 (n = 2)
Phyllospongia foliascens	Light	1067 ± 56 (n = 2)	713 ± 47 (n = 2)	968 ± 23 (n = 1)	28.2 ± 0.08 (n = 2)	27.4 ± 0.04 (n = 2)	28.8 ± 0.10 (n = 1)
	Dark	0	0	0	26.6 ± 0.03 (n = 2)	26.6 ± 0.03 (n = 2)	26.8 ± 0.01 (n = 1)
Haliclona koremella	Light	1376 ± 42 (n = 2)	1359 ± 71 (n = 1)	1502 ± 75 (n = 2)	30.3 ± 0.14 (n = 2)	28.7 ± 0.10 (n = 1)	27.9 ± 0.16 (n = 2)
	Dark	0	0	0	26.6 ± 0.04 (n = 2)	26.4 ± 0.03 (n = 1)	26.3 ± 0.04 (n = 2)
Amphimedon sp.	Light	1376 ± 42 (n = 2)	1359 ± 71 (n = 2)	1502 ± 75 (n = 1)	30.3 ± 0.14 (n = 2)	28.7 ± 0.10 (n = 2)	27.9 ± 0.16 (n = 1)
	Dark	0	0	0	26.6 ± 0.04 (n = 2)	26.4 ± 0.03 (n = 2)	26.3 ± 0.04 (n = 1)

Dactylospongia elegans	Light	1376 ± 42 (n = 1)	1359 ± 71 (n = 2)	1502 ± 75 (n = 2)	30.3 ± 0.14 (n = 1)	28.7 ± 0.10 (n = 2)	27.9 ± 0.16 (n = 2)
	Dark	0	0	0	26.6 ± 0.04 (n = 1)	26.4 ± 0.03 (n = 2)	26.3 ± 0.04 (n = 2)
Axinella sp.	Light	1337 ± 38 (n = 2)	1291 ± 50 (n = 2)	1324 ± 59 (n = 1)	28.3 ± 0.10 (n = 2)	28.0 ± 0.07 (n = 2)	28.7 ± 0.07 (n = 1)
	Dark	0	0	0	26.0 ± 0.04 (n = 2)	25.3 ± 0.04 (n = 2)	26.2 ± 0.04 (n = 1)
Clathria reinwardti	Light	1337 ± 38 (n = 2)	1291 ± 50 (n = 1)	1324 ± 59 (n = 2)	28.3 ± 0.10 (n = 2)	28.0 ± 0.07 (n = 1)	28.7 ± 0.07 (n = 2)
	Dark	0	0	0	26.0 ± 0.04 (n = 2)	25.3 ± 0.04 (n = 1)	26.2 ± 0.04 (n = 2)

Table S.3.2. Net primary production (P_N), gross primary production (P_G), and dark respiration (R_D) of the studied sponges based on the sponge volume (mol mL^{-1} h^{-1}), wet weight (mol g_{WW}^{-1} h^{-1}), dry weight (mol g_{DW}^{-1} h^{-1}), and ash-free dry-weight (mol g_{AFDW}^{-1} h^{-1}). Values are expressed in mean ± SE, n = 5.

Sponge	Net Primary Production				Gross Primary Production				Respiration			
	Based on sponge volume	**Based on sponge wet weight**	**Based on sponge dry weight**	**Based on sponge AFDW**	**Based on sponge volume**	**Based on sponge wet weight**	**Based on sponge dry weight**	**Based on sponge AFDW**	**Based on sponge volume**	**Based on sponge wet weight**	**Based on sponge dry weight**	**Based on sponge AFDW**
Spongia sp.	-0.0002 ± 0.0001	-0.0008 ± 0.0003	-0.0009 ± 0.0003	-0.0056 ± 0.0018	0.0003 ± 0.0003	0.0016 ± 0.0012	0.0020 ± 0.0015	0.0127 ± 0.0097	-0.0007 ± 0.0002	-0.0047 ± 0.0021	-0.0080 ± 0.0051	-0.0538 ± 0.0348
Spheciospongia sp.	0.0010 ± 0.0003	0.0026 ± 0.0009	0.0032 ± 0.0012	0.0285 ± 0.0109	0.0013 ± 0.0008	0.0018 ± 0.0006	0.0046 ± 0.0031	0.0363 ± 0.0255	-0.0006 ± 0.0002	-0.0016 ± 0.0004	-0.0019 ± 0.0006	-0.0158 ± 0.0061
Phyllospongia foliascens	0.0054 ± 0.0012	0.0063 ± 0.0015	0.0324 ± 0.0064	0.0518 ± 0.0122	0.0078 ± 0.0014	0.0092 ± 0.0018	0.0472 ± 0.0074	0.0751 ± 0.0149	-0.0025 ± 0.0002	-0.0029 ± 0.0003	-0.0149 ± 0.0011	-0.0232 ± 0.0024
Haliclona koremella	0.0117 ± 0.0007	0.0149 ± 0.0007	0.1980 ± 0.0176	0.3039 ± 0.0273	0.0152 ± 0.0008	0.0194 ± 0.0013	0.2548 ± 0.0165	0.3929 ± 0.0302	-0.0035 ± 0.0007	-0.0045 ± 0.0011	-0.0568 ± 0.0095	-0.0890 ± 0.0180
Amphimedon sp.	-0.0016 ± 0.0003	-0.0051 ± 0.0025	-0.0176 ± 0.0056	-0.0275 ± 0.0102	0.0004 ± 0.0006	0.0005 ± 0.0013	0.0069 ± 0.0063	0.0089 ±0.0090	-0.0020 ± 0.0003	-0.0050 ± 0.0015	-0.0251 ± 0.0058	-0.0373 ± 0.0082
Dactylospongia elegans	0.0023 ± 0.0007	0.0042 ± 0.0009	0.0220 ± 0.0099	0.0293 ± 0.0118	0.0035 ± 0.0010	0.0067 ± 0.0014	0.0331 ± 0.0134	0.0442 ± 0.0157	-0.0012 ± 0.0003	-0.0025 ± 0.0007	-0.0111 ± 0.0037	-0.0149 ± 0.0043
Axinella sp.	0.0009 ± 0.0003	0.0024 ± 0.0011	0.0088 ± 0.0020	0.0134 ± 0.0036	0.0014 ± 0.0004	0.0041 ± 0.0021	0.0137 ± 0.0028	0.0205 ± 0.0044	-0.0005 ± 0.0001	-0.0021 ± 0.0009	-0.0049 ± 0.0014	-0.0066 ± 0.0018
Clathria reinwardti	0.0013 ± 0.0001	0.0024 ± 0.0006	0.0169 ± 0.0041	0.0278 ± 0.0067	0.0031 ± 0.0005	0.0054 ± 0.0011	0.0375 ± 0.0076	0.0609 ± 0.0122	-0.0015 ± 0.0004	-0.0026 ± 0.0007	-0.0182 ± 0.0044	-0.0289 ± 0.0068

Table S.3.3. (A) Results of two-factor permutational analysis of variance (PERMANOVA) testing the effect of light intensity (Li), temperature (Te), and the interaction of light intensity and temperature (LixTe) on differences in net primary production (P_N); and (B) results of one-factor PERMANOVAs testing the effect of temperature (Te) in the difference in dark respiration (R_D).

(A)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Li	1	1.101	1.101	1.0064	0.321	995
Te	3	0.39519	0.13173	0.12041	0.956	998
LixTe**	1	0.22521	0.22521	0.20586	0.65	999
Res	28	30.632	1.094			
Total	33	33				

(B)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Te	2	2.9165E-2	1.4583E-2	2.2357	0.106	998
Res	38	0.24786	6.5227E-3			
Total	40	0.27703				

Table S.3.4. (A) Full results of PERMANOVA based on sponge abundance (g_{AFDW}) of the autotrophic and heterotrophic sponges; and (B) based on the proportion of autotrophic and heterotrophic sponge (%). Zo denotes tidal zones, and Si for sites.

(A)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Zo	2	7169.8	3584.9	3.9008	0.012	999
Si	1	280.74	280.74	0.30548	0.734	999
ZoxSi	2	7330.7	3665.3	3.9883	0.013	998
Res	12	11028	919.02			
Total	17	25809				

(B)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Zo	2	5019.7	2509.9	4.6266	0.032	999
Si	1	46.985	46.985	8.6611E-2	0.746	995
ZoxSi	2	888.21	444.1	0.81864	0.475	998
Res	12	6509.9	542.49			
Total	17	12465				

Table S.3.5 Multiple comparisons of means between sponge species' P_{net} : R_{dark} ratio, from one-way ANOVA. A = *Spongia* sp; B = *Spheciospongia* sp.; C = *Phyllospongia foliascens*; D = *Haliclona koremella*; E = *Amphimedon* sp.; F = *Dactylospongia elegans*; G = *Axinella* sp.; H = *Clathria reinwardti*.

(I) Sponge	(J) Sponge	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-2.43332	.82302	.128	-5.2541	.3874
	C	-2.70856*	.78079	.046	-5.3845	-.0326
	D	-4.44006*	.75431	<.001	-7.0253	-1.8548
	E	.38360	.78079	1.000	-2.2924	3.0596
	F	-2.55423	.78079	.069	-5.2302	.1218
	G	-2.43813	.82302	.127	-5.2589	.3826
	H	-1.45416	.82302	.702	-4.2749	1.3666
	I	.66131	.82302	.996	-2.1594	3.4820
B	A	2.43332	.82302	.128	-.3874	5.2541
	C	-.27524	.68859	1.000	-2.6352	2.0848
	D	-2.00673	.65842	.108	-4.2633	.2499
	E	2.81692*	.68859	.012	.4569	5.1769
	F	-.12090	.68859	1.000	-2.4809	2.2391
	G	-.00481	.73613	1.000	-2.5278	2.5181
	H	.97916	.73613	.911	-1.5438	3.5021
	I	3.09463*	.73613	.009	.5717	5.6176
C	A	2.70856*	.78079	.046	.0326	5.3845
	B	.27524	.68859	1.000	-2.0848	2.6352
	D	-1.73149	.60480	.153	-3.8043	.3413
	E	3.09216*	.63751	.002	.9072	5.2771
	F	.15434	.63751	1.000	-2.0306	2.3393
	G	.27043	.68859	1.000	-2.0896	2.6304
	H	1.25440	.68859	.669	-1.1056	3.6144
	I	3.36987*	.68859	.002	1.0099	5.7299
D	A	4.44006*	.75431	<.001	1.8548	7.0253
	B	2.00673	.65842	.108	-.2499	4.2633
	C	1.73149	.60480	.153	-.3413	3.8043
	E	4.82366*	.60480	<.001	2.7508	6.8965
	F	1.88583	.60480	.094	-.1870	3.9586
	G	2.00192	.65842	.109	-.2547	4.2585
	H	2.98590*	.65842	.004	.7293	5.2425
	I	5.10136*	.65842	<.001	2.8448	7.3580
E	A	-.38360	.78079	1.000	-3.0596	2.2924
	B	-2.81692*	.68859	.012	-5.1769	-.4569
	C	-3.09216*	.63751	.002	-5.2771	-.9072
	D	-4.82366*	.60480	<.001	-6.8965	-2.7508
	F	-2.93783*	.63751	.004	-5.1228	-.7529
	G	-2.82173*	.68859	.011	-5.1817	-.4617
	H	-1.83776	.68859	.215	-4.1978	.5222
	I	.27771	.68859	1.000	-2.0823	2.6377

F	A	2.55423	.78079	.069	-.1218	5.2302
	B	.12090	.68859	1.000	-2.2391	2.4809
	C	-.15434	.63751	1.000	-2.3393	2.0306
	D	-1.88583	.60480	.094	-3.9586	.1870
	E	2.93783*	.63751	.004	.7529	5.1228
	G	.11609	.68859	1.000	-2.2439	2.4761
	H	1.10007	.68859	.796	-1.2599	3.4601
	I	3.21553*	.68859	.003	.8555	5.5755
G	A	2.43813	.82302	.127	-.3826	5.2589
	B	.00481	.73613	1.000	-2.5181	2.5278
	C	-.27043	.68859	1.000	-2.6304	2.0896
	D	-2.00192	.65842	.109	-4.2585	.2547
	E	2.82173*	.68859	.011	.4617	5.1817
	F	-.11609	.68859	1.000	-2.4761	2.2439
	H	.98397	.73613	.909	-1.5390	3.5069
	I	3.09944*	.73613	.009	.5765	5.6224
H	A	1.45416	.82302	.702	-1.3666	4.2749
	B	-.97916	.73613	.911	-3.5021	1.5438
	C	-1.25440	.68859	.669	-3.6144	1.1056
	D	-2.98590*	.65842	.004	-5.2425	-.7293
	E	1.83776	.68859	.215	-.5222	4.1978
	F	-1.10007	.68859	.796	-3.4601	1.2599
	G	-.98397	.73613	.909	-3.5069	1.5390
	I	2.11547	.73613	.150	-.4075	4.6384
I	A	-.66131	.82302	.996	-3.4820	2.1594
	B	-3.09463*	.73613	.009	-5.6176	-.5717
	C	-3.36987*	.68859	.002	-5.7299	-1.0099
	D	-5.10136*	.65842	<.001	-7.3580	-2.8448
	E	-.27771	.68859	1.000	-2.6377	2.0823
	F	-3.21553*	.68859	.003	-5.5755	-.8555
	G	-3.09944*	.73613	.009	-5.6224	-.5765
	H	-2.11547	.73613	.150	-4.6384	.4075

*. The mean difference is significant at the 0.05 level.

Appendix C – Supplementary data for Chapter 5, Autotrophic vs heterotrophic sponge-mediated picoplankton flux in an Indo-Pacific seagrass meadow

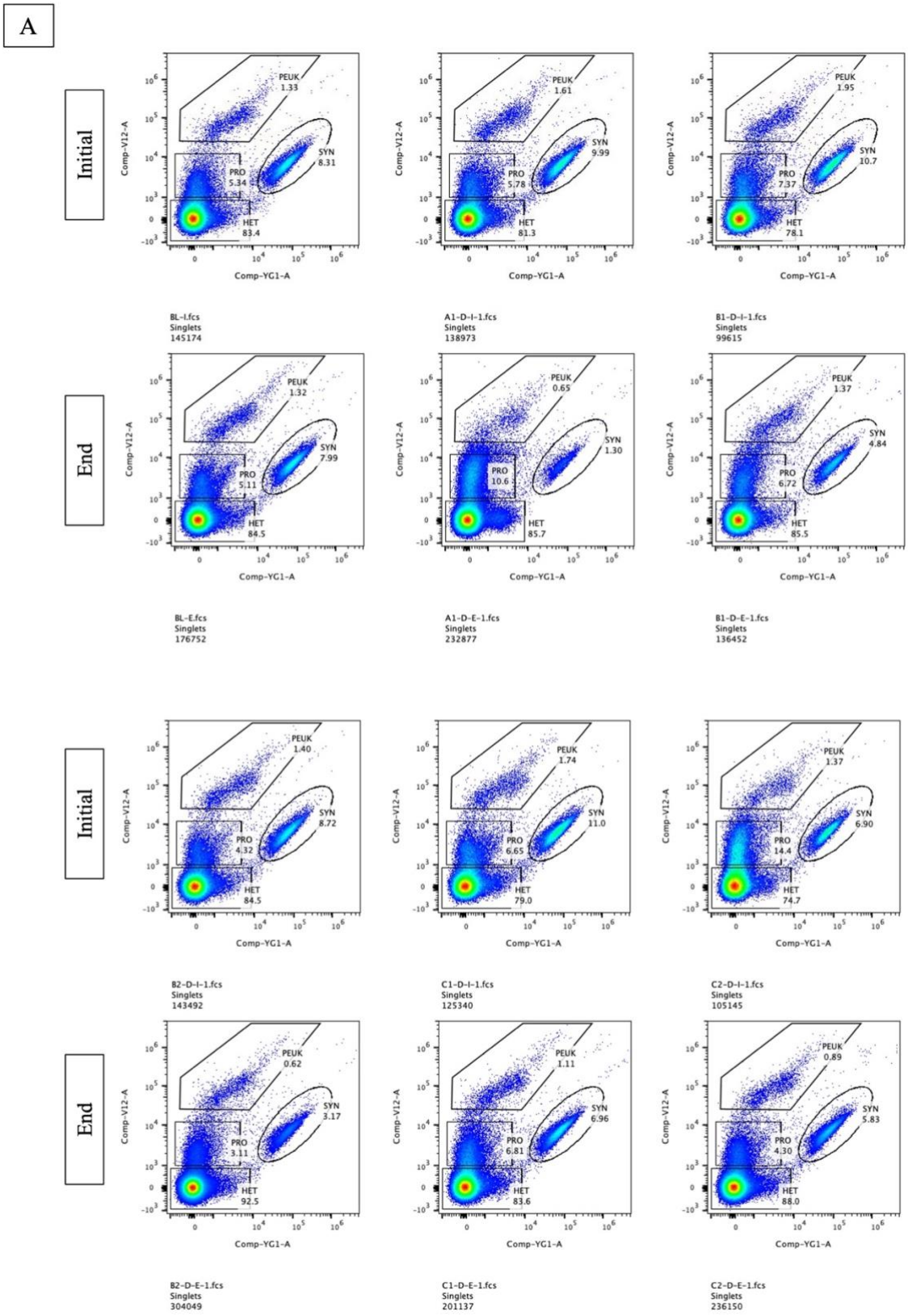
Table S.5.1. Summary of t-test results of the picoplankton removal rate against zero removal by the seagrass sponges in the Wakatobi National Park, Southeast Sulawesi – Indonesia. The values showed are the P-values between picoplankton removal efficiency and zero removal. P-value larger than 0.1 ($p > 0.1$) indicates for not significant different; lower or equal with 0.1 ($p \leq 0.1$) indicates marginally significant different; and lower or equal with 0.05 ($p \leq 0.05$) indicates highly significant different with zero removal.

Sponge	**Dark**			**Light**		
	HET	**SYN**	**PEUK**	**HET**	**SYN**	**PEUK**
Heterotrophic sponge						
Spongia sp.	0.1802	0.0004	0.0005	0.0369	0.0039	0.3426
Amphimedon sp.	0.3422	0.0024	0.2921	0.0257	0.0003	0.4471
Autotrophic sponge						
Spheciospongia sp.	0.0027	0.0687	0.0836	0.1024	0.0251	0.4699
Phyllospongia foliascens	0.0000	0.0020	0.1240	0.2815	0.0388	0.4048
Haliclona koremella	0.4743	0.0006	0.1236	0.0582	0.0233	0.4502
Dactylospongia elegans	0.0824	0.0509	0.0001	0.4775	0.0013	0.4866

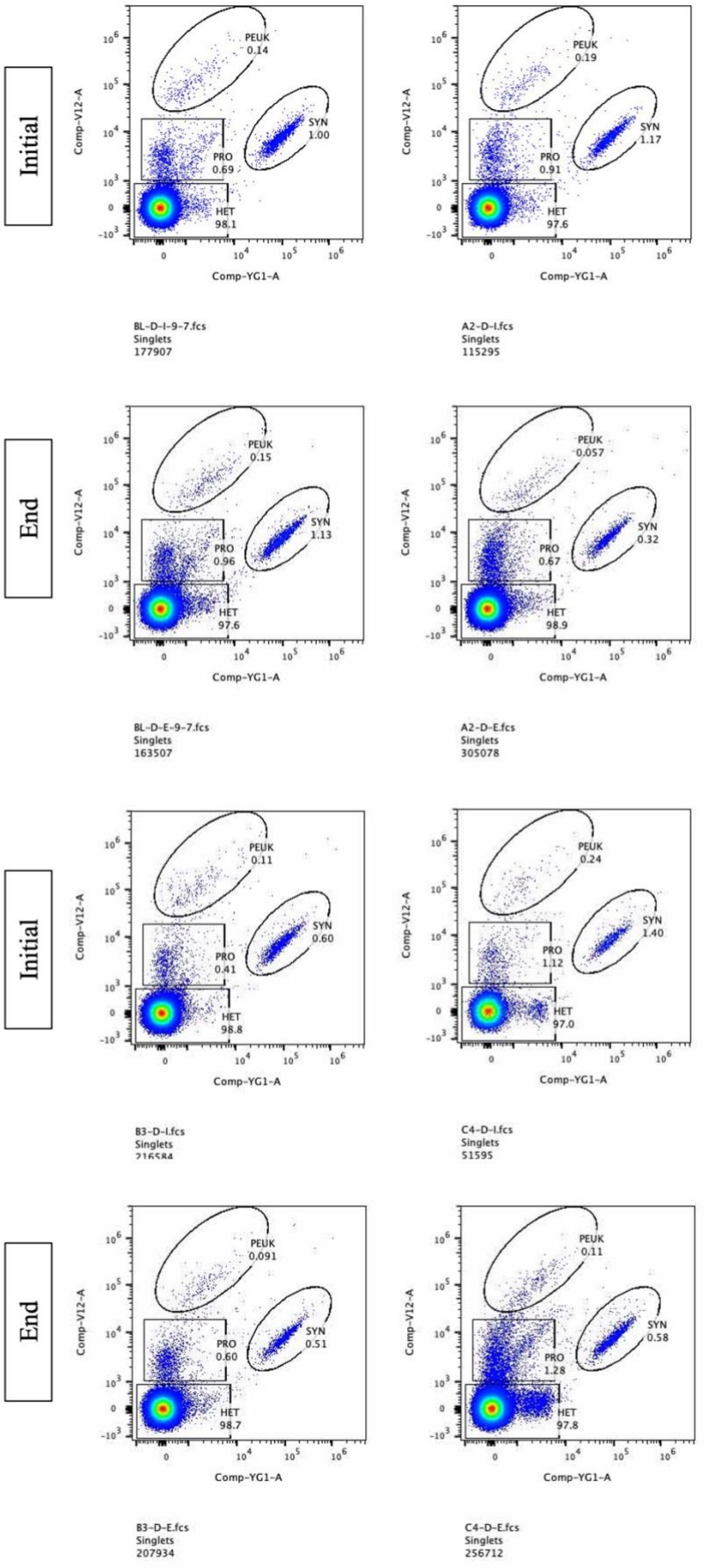
Table S.5.2. Full results of the two-way PERMANOVA test for differences between nutritional mode (i.e. auto- and heterotrophic sponges; "Nu"), between light and dark incubations (representing day and night-time; "Da"), and interaction between the sponge's nutritional mode and diel cycle (day & night).

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Nutritional mode (Nu)	1	5205.3	5205.3	4.4512	0.007	998
Day period (Da)	1	3497.9	3497.9	2.9912	0.048	998
Nu x Da	1	622.99	622.99	0.53275	0.652	998
Res	34	39760	1169.4			
Total	37	48715				

Figure S.5.1. The enumeration gating of picoplankton groups from the dark incubations (seven batches, A – G).



B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SYN 1.00
PRO 0.69
HET 98.1
Comp-V12-A
Comp-YG1-A
BL-D-I-9-7.fcs
Singlets
177907
PEUK 0.19
SYN 1.17
PRO 0.91
HET 97.6
A2-D-I.fcs
Singlets
115295
End
PEUK 0.15
SYN 1.13
PRO 0.96
HET 97.6
BL-D-E-9-7.fcs
Singlets
163507
PEUK 0.057
SYN 0.32
PRO 0.67
HET 98.9
A2-D-E.fcs
Singlets
305078
Initial
PEUK 0.11
SYN 0.60
PRO 0.41
HET 98.8
B3-D-I.fcs
Singlets
PEUK 0.24
SYN 1.40
PRO 1.12
HET 97.0
C4-D-I.fcs
Singlets
51595
End
PEUK 0.091
SYN 0.51
PRO 0.60
HET 98.7
B3-D-E.fcs
Singlets
207934
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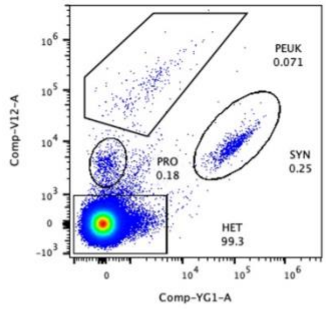
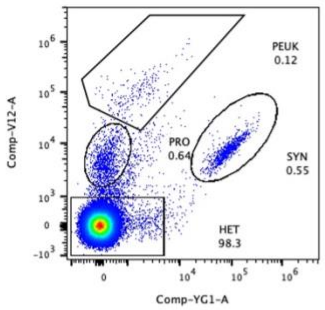
SYN 0.58
PRO 1.28
HET 97.8
C4-D-E.fcs
Singlets
256712

C

Initial



BL-S-D-I-11-7.fcs
Singlets
329038



A3-D-I.fcs
Singlets
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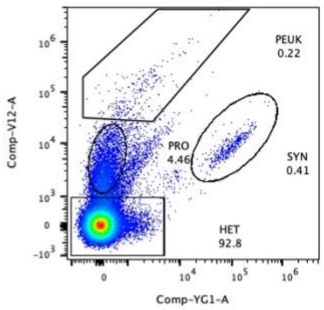
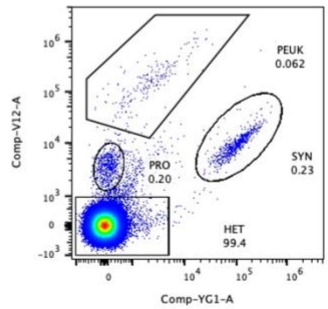


C3-D-I.fcs
Singlets
116100

End



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Singlets
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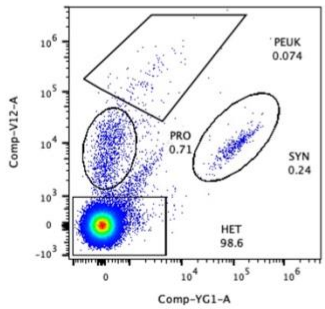
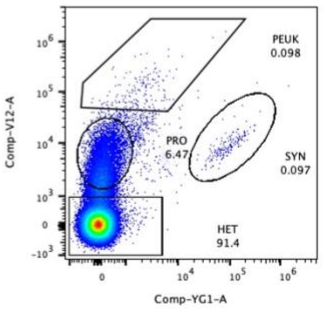
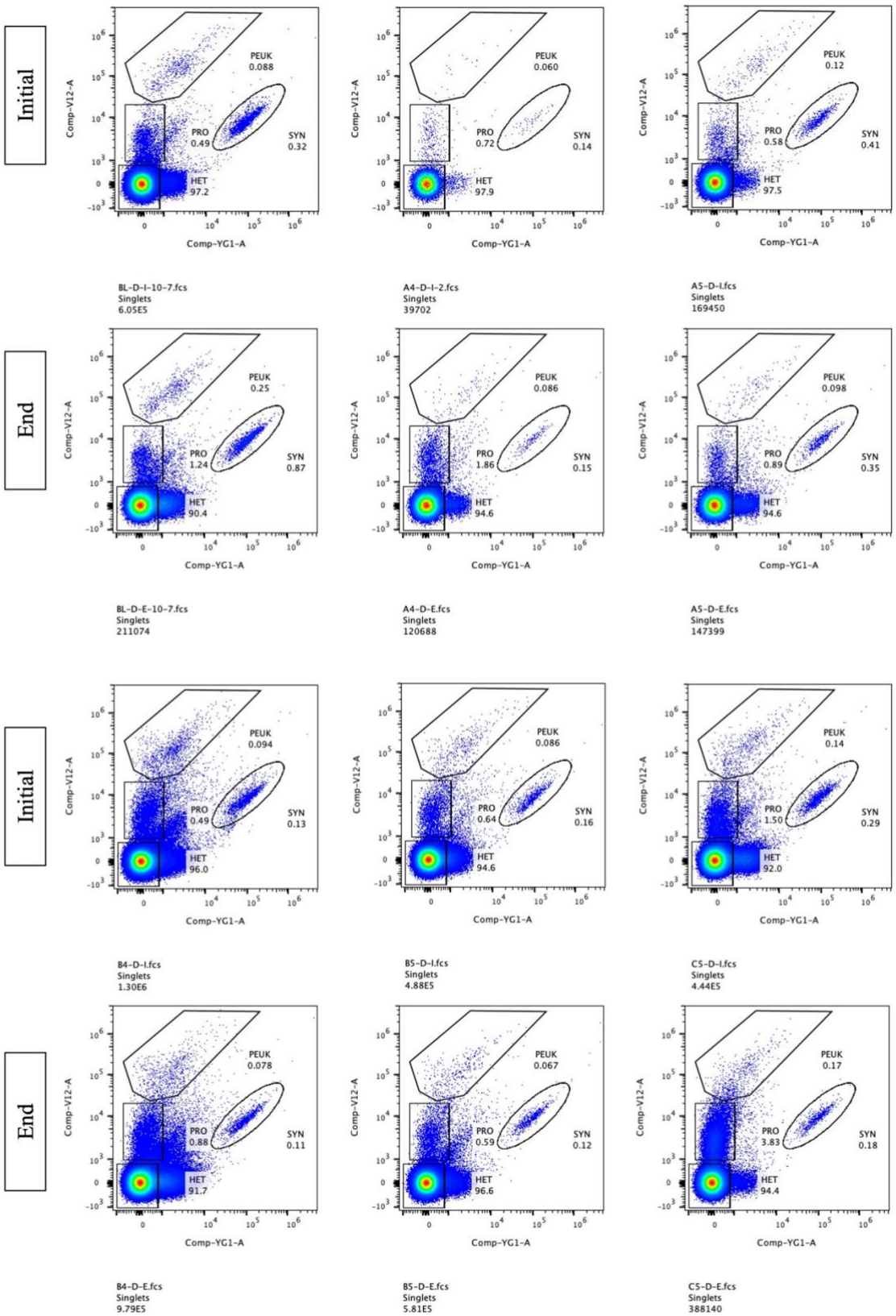


A3-D-E.fcs
Singlets
154618



C3-D-E.fcs
Singlets
182512

D


Initial
End
Initial
End
Comp-V12-A
Comp-YG1-A
PEUK
PRO
SYN
HET
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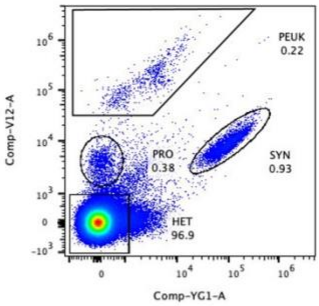
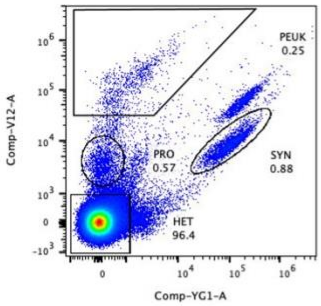
0.49
0.32
97.2
BL-D-I-10-7.fcs
Singlets
6.05E5
0.060
0.72
0.14
97.9
A4-D-I-2.fcs
Singlets
39702
0.12
0.58
0.41
97.5
A5-D-I.fcs
Singlets
169450
0.25
1.24
0.87
90.4
BL-D-E-10-7.fcs
Singlets
211074
0.086
1.86
0.15
94.6
A4-D-E.fcs
Singlets
120688
0.098
0.89
0.35
94.6
A5-D-E.fcs
Singlets
147399
0.094
0.49
0.13
96.0
B4-D-I.fcs
Singlets
1.30E6
0.086
0.64
0.16
94.6
B5-D-I.fcs
Singlets
4.88E5
0.14
1.50
0.29
92.0
C5-D-I.fcs
Singlets
4.44E5
0.078
0.88
0.11
91.7
B4-D-E.fcs
Singlets
9.79E5
0.067
0.59
0.12
96.6
B5-D-E.fcs
Singlets
5.81E5
0.17
3.83
0.18
94.4
C5-D-E.fcs
Singlets
388140

E

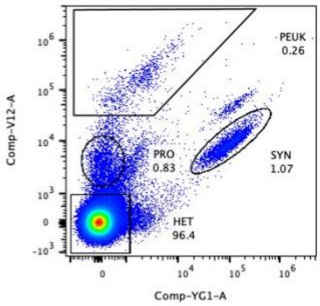
Initial



BL-D-I-12-7.fcs
Singlets
347188

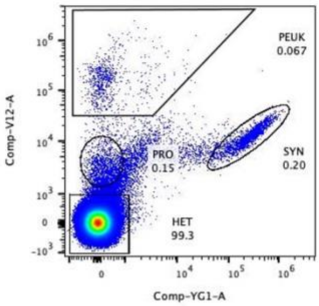


D1-D-I.fcs
Singlets
364654

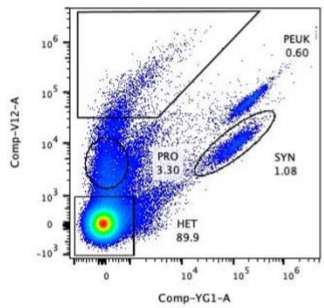


D2-D-I.fcs
Singlets
304077

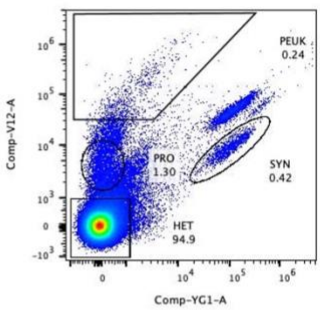
End



BL-D-E-12-7.fcs
Singlets
1.17E6

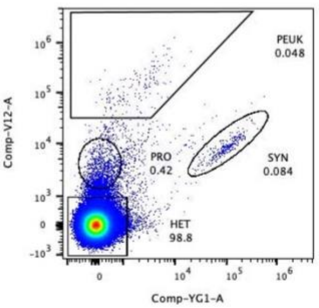


D1-D-E.fcs
Singlets
247092

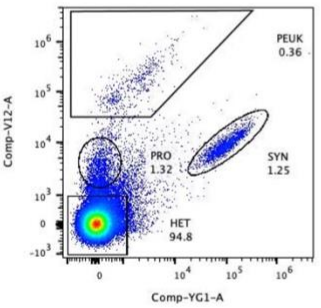


D2-D-E.fcs
Singlets
357539

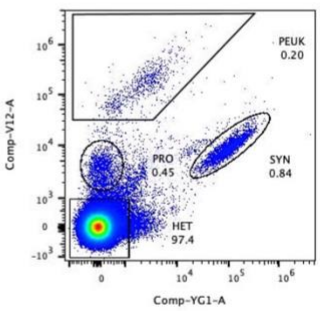
Initial



E1-D-I.fcs
Singlets
333310

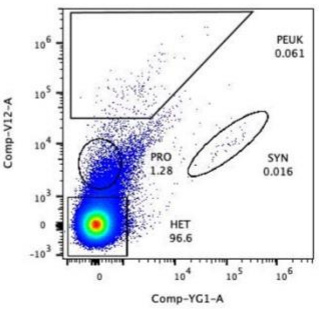


E2-D-I.fcs
Singlets
126645

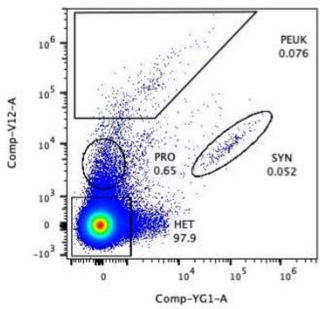


F1-D-I.fcs
Singlets
363601

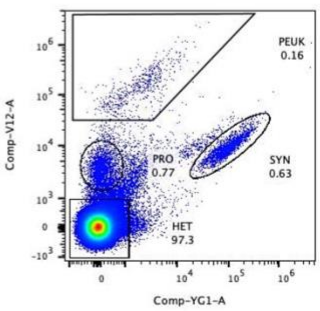
End



E1-D-E.fcs
Singlets
247066

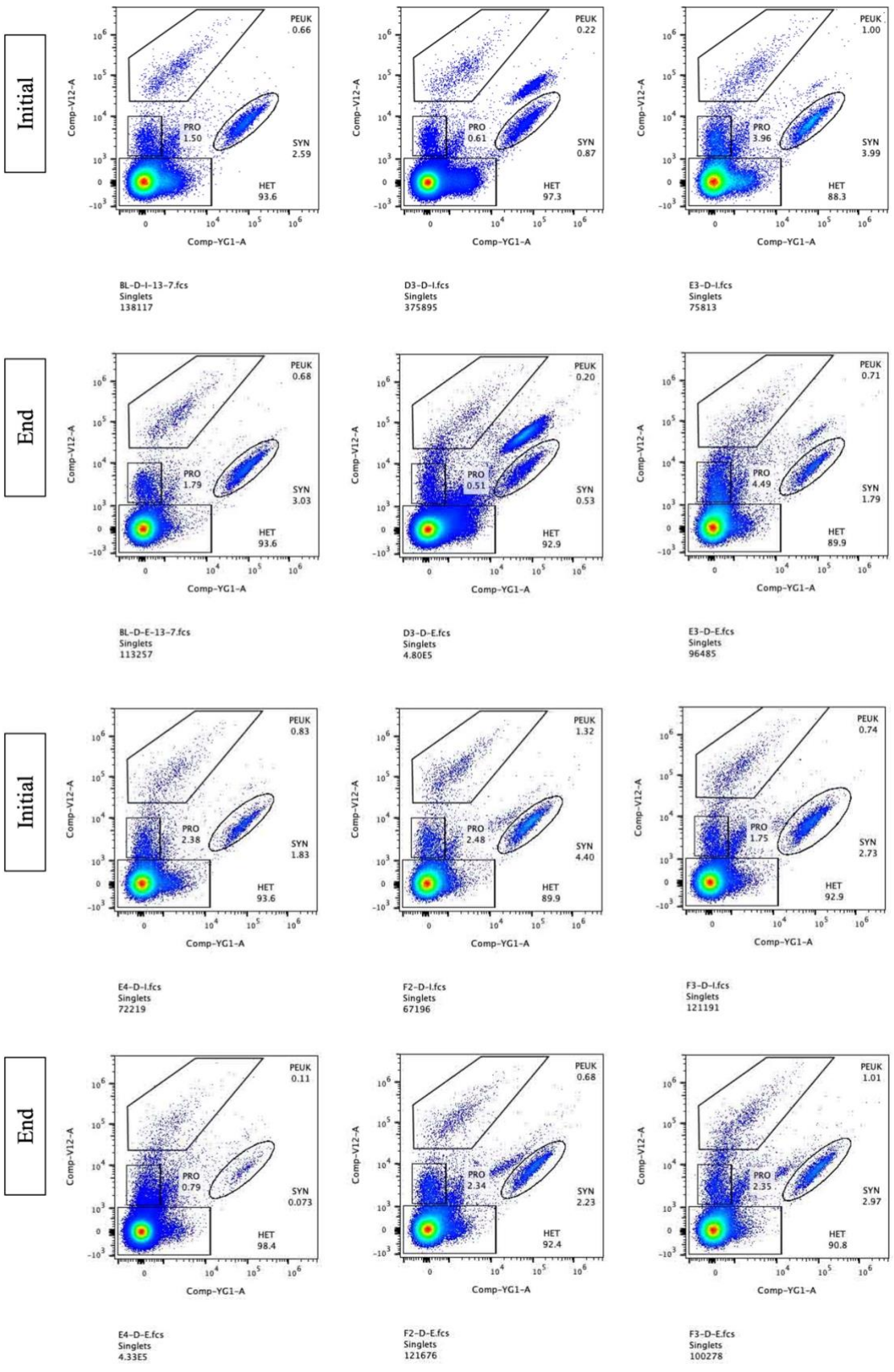


E2-D-E.fcs
Singlets
341235



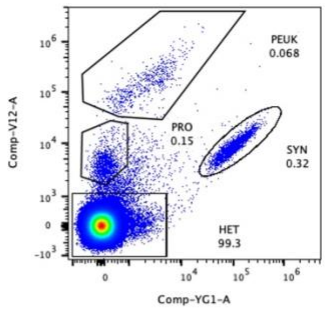
F1-D-E.fcs
Singlets
346208

F

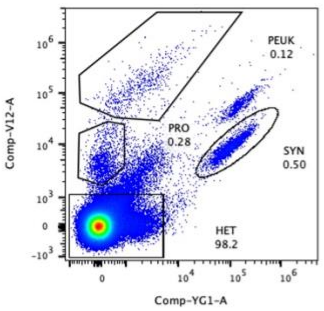

Initial
PEUK 0.66
PRO 1.50
SYN 2.59
HET 93.6
Comp-V12-A
Comp-YG1-A
BL-D-I-13-7.fcs
Singlets
138117
PEUK 0.22
PRO 0.61
SYN 0.87
HET 97.3
D3-D-I.fcs
Singlets
375895
PEUK 1.00
PRO 3.96
SYN 3.99
HET 88.3
E3-D-I.fcs
Singlets
75813
End
PEUK 0.68
PRO 1.79
SYN 3.03
HET 93.6
BL-D-E-13-7.fcs
Singlets
113257
PEUK 0.20
PRO 0.51
SYN 0.53
HET 92.9
D3-D-E.fcs
Singlets
4.80E5
PEUK 0.71
PRO 4.49
SYN 1.79
HET 89.9
E3-D-E.fcs
Singlets
96485
Initial
PEUK 0.83
PRO 2.38
SYN 1.83
HET 93.6
E4-D-I.fcs
Singlets
72219
PEUK 1.32
PRO 2.48
SYN 4.40
HET 89.9
F2-D-I.fcs
Singlets
67196
PEUK 0.74
PRO 1.75
SYN 2.73
HET 92.9
F3-D-I.fcs
Singlets
121191
End
PEUK 0.11
PRO 0.79
SYN 0.073
HET 98.4
E4-D-E.fcs
Singlets
4.33E5
PEUK 0.68
PRO 2.34
SYN 2.23
HET 92.4
F2-D-E.fcs
Singlets
121676
PEUK 1.01
PRO 2.35
SYN 2.97
HET 90.8
F3-D-E.fcs
Singlets
100278

G

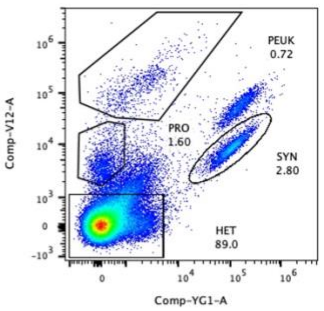
Initial



BL-D-I-14-7.fcs
Singlets
9.04E5

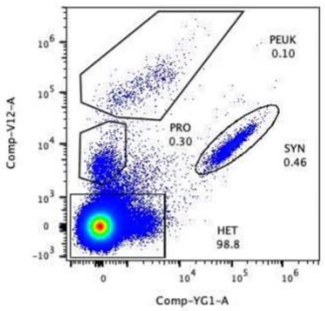


D4-D-I.fcs
Singlets
5.17E5

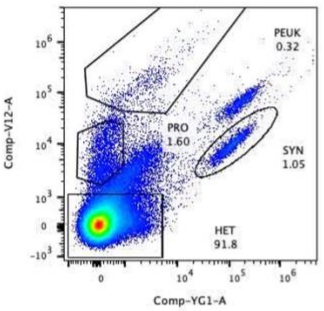


D5-D-I.fcs
Singlets
102470

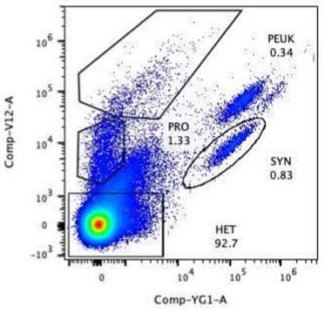
End



BL-D-E-14-7.fcs
Singlets
6.19E5

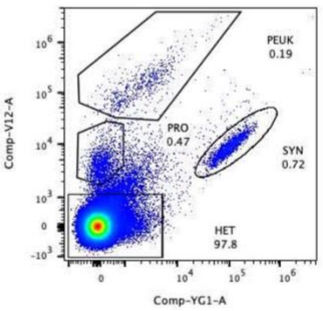


D4-D-E.fcs
Singlets
198953

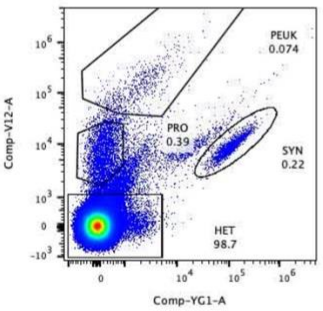


D5-D-E.fcs
Singlets
270911

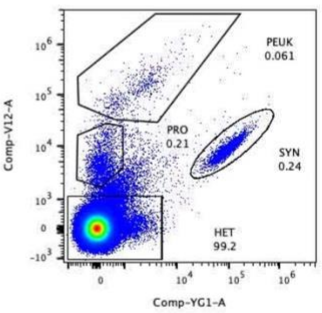
Initial



E5-D-I.fcs
Singlets
361059

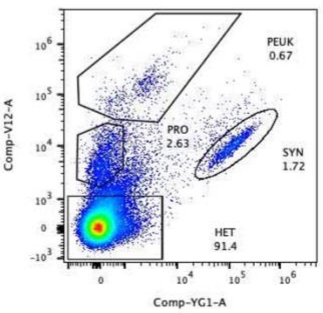


F4-D-I.fcs
Singlets
8.44E5

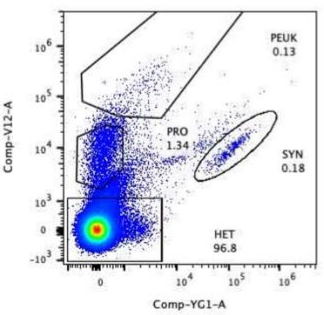


F5-D-I.fcs
Singlets
9.56E5

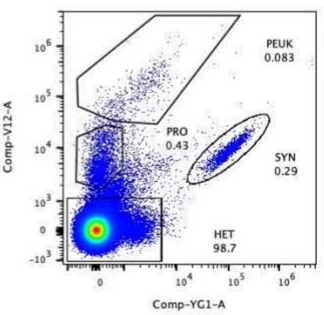
End



E5-D-E.fcs
Singlets
89291

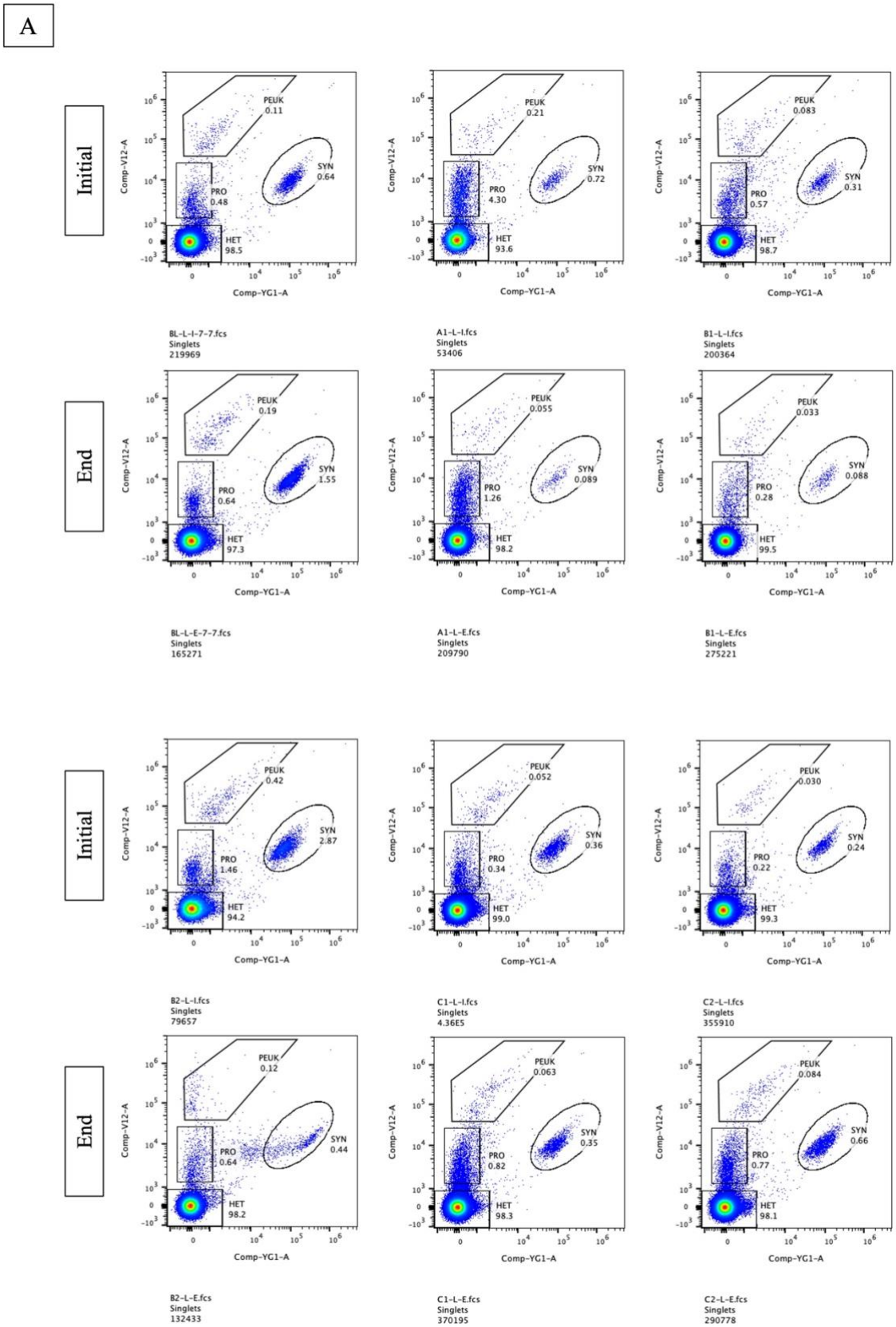


F4-D-E.fcs
Singlets
266586

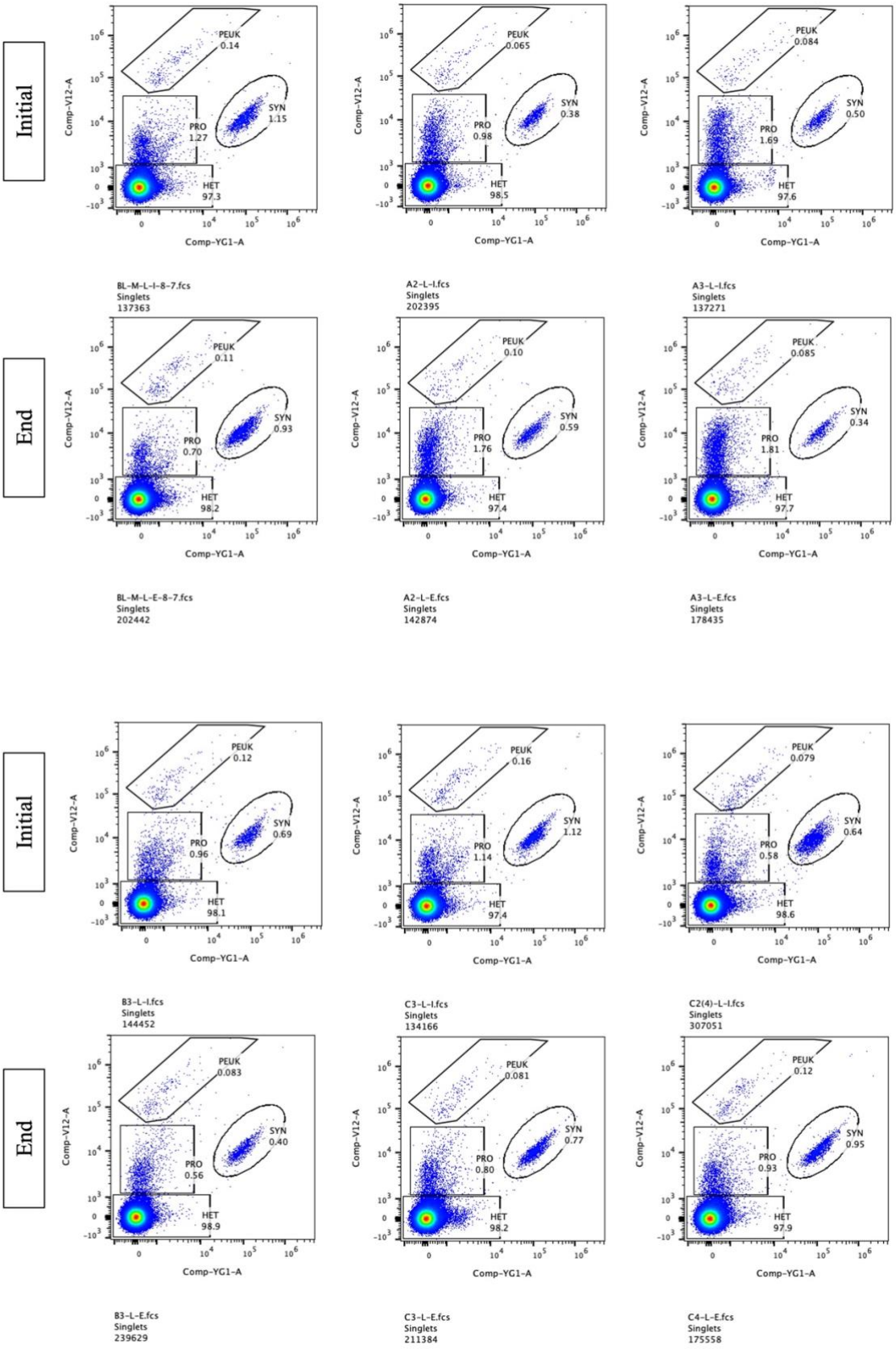


F5-D-E.fcs
Singlets
6.10E5

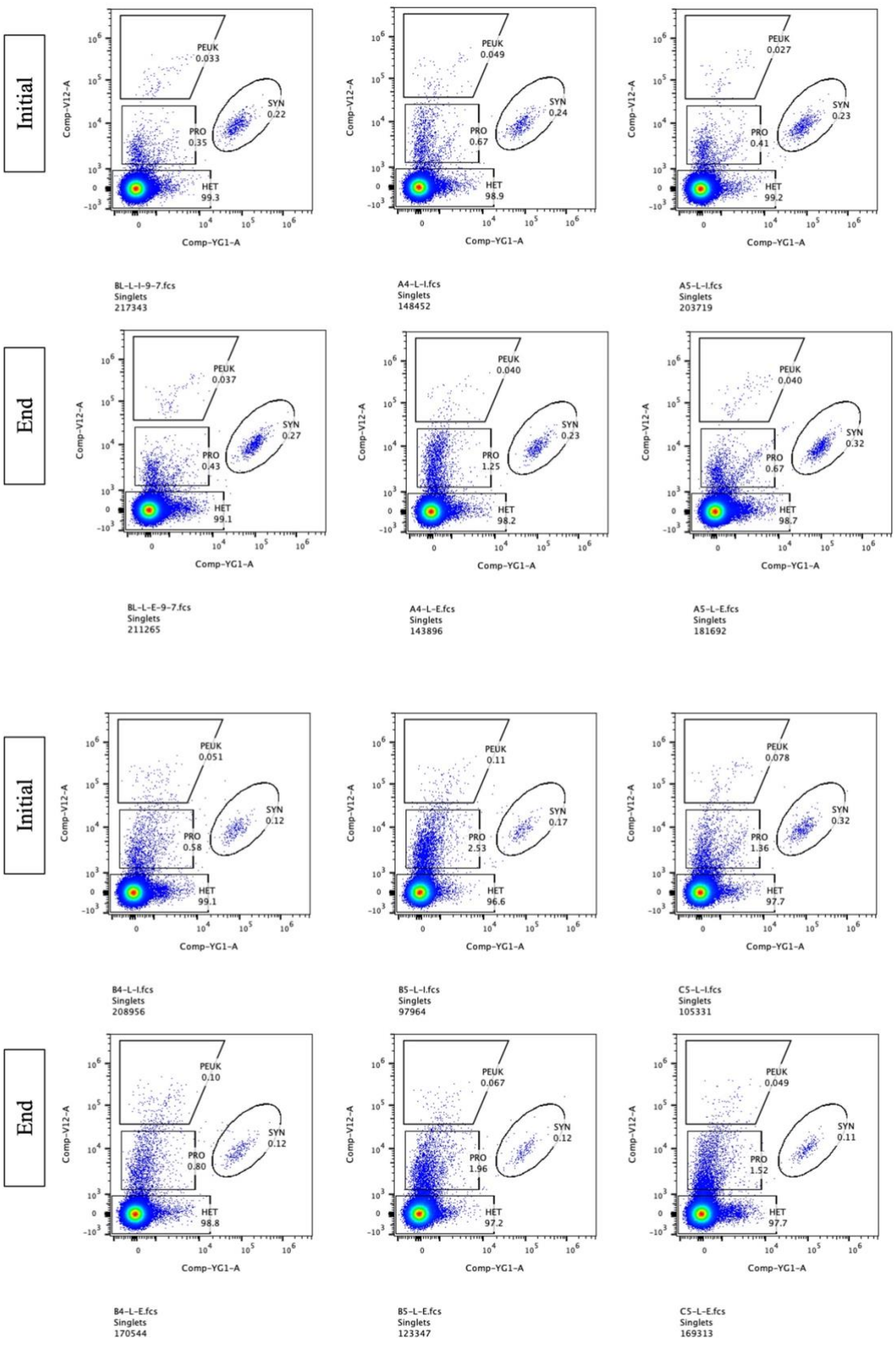
Figure S.5.2. The enumeration gating of picoplankton groups from the light incubations (six measurement batches, A – F).



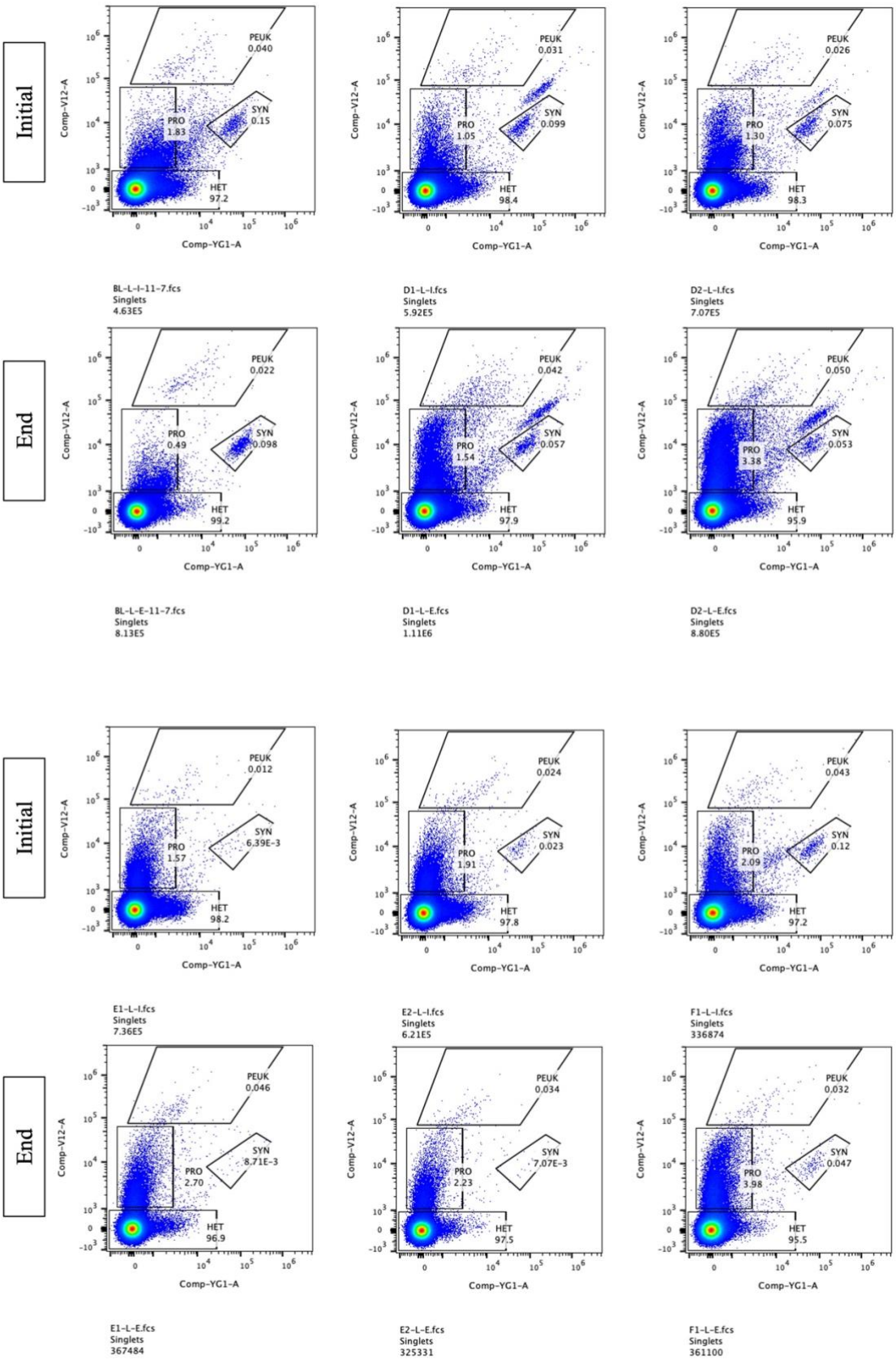
B


Initial
End
Initial
End
Comp-V12-A
Comp-YG1-A
PEUK
PRO
SYN
HET
0.14
1.27
1.15
97.3
0.065
0.98
0.38
98.5
0.084
1.69
0.50
97.6
BL-M-L-I-8-7.fcs
Singlets
137363
0.11
0.70
0.93
98.2
A2-L-I.fcs
Singlets
202395
0.10
1.76
0.59
97.4
A3-L-I.fcs
Singlets
137271
0.085
1.81
0.34
97.7
BL-M-L-E-8-7.fcs
Singlets
202442
A2-L-E.fcs
Singlets
142874
A3-L-E.fcs
Singlets
178435
0.12
0.96
0.69
98.1
B3-L-I.fcs
Singlets
144452
0.16
1.14
1.12
97.4
C3-L-I.fcs
Singlets
134166
0.079
0.58
0.64
98.6
C2(4)-L-I.fcs
Singlets
307051
0.083
0.56
0.40
98.9
B3-L-E.fcs
Singlets
239629
0.081
0.80
0.77
98.2
C3-L-E.fcs
Singlets
211384
0.12
0.93
0.95
97.9
C4-L-E.fcs
Singlets
175558

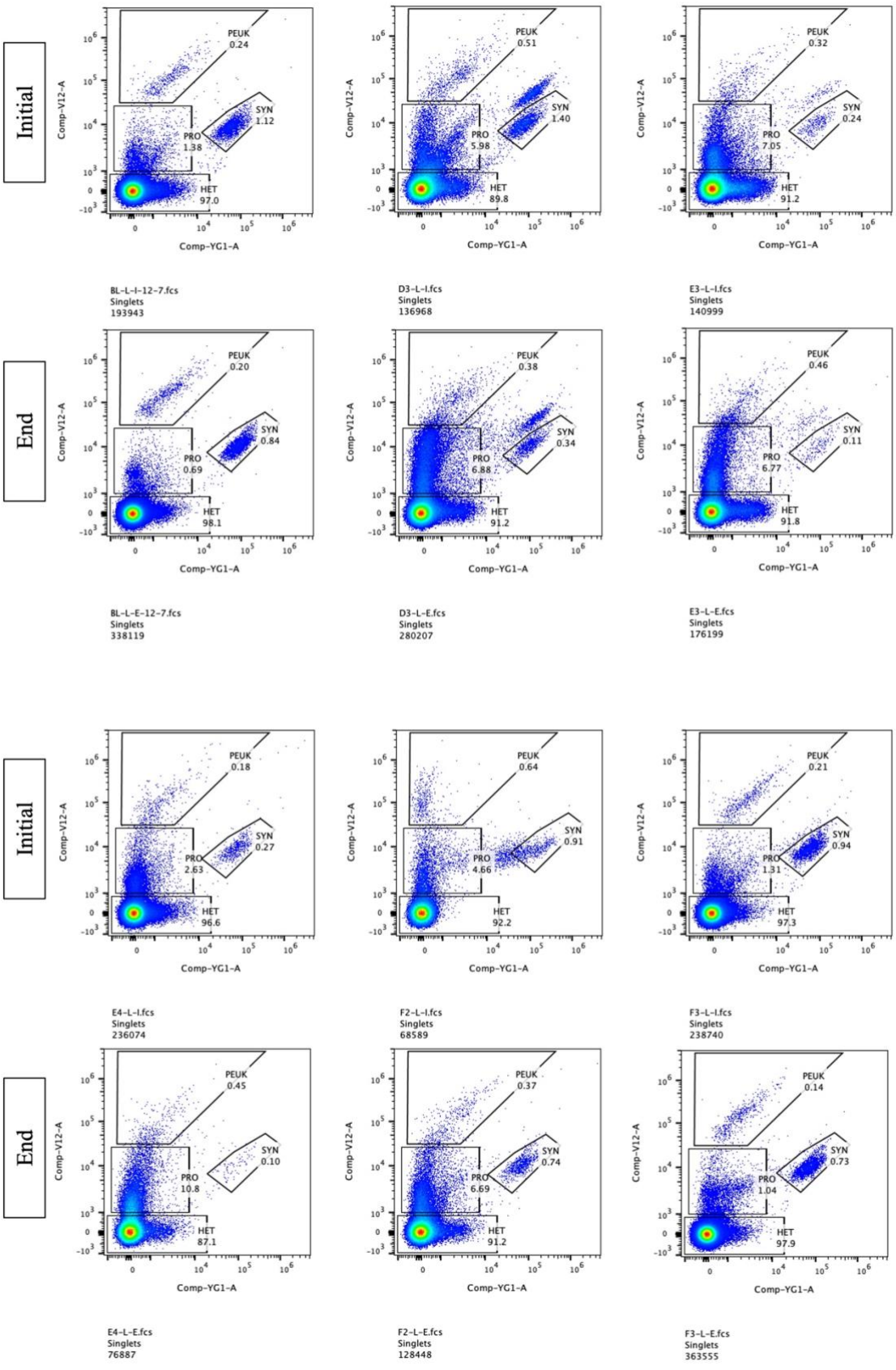
C


Initial
PEUK 0.033
SYN 0.22
PRO 0.35
HET 99.3
Comp-V12-A
Comp-YG1-A
BL-L-I-9-7.fcs
Singlets
217343
PEUK 0.049
SYN 0.24
PRO 0.67
HET 98.9
A4-L-I.fcs
Singlets
148452
PEUK 0.027
SYN 0.23
PRO 0.41
HET 99.2
A5-L-I.fcs
Singlets
203719
End
PEUK 0.037
SYN 0.27
PRO 0.43
HET 99.1
BL-L-E-9-7.fcs
Singlets
211265
PEUK 0.040
SYN 0.23
PRO 1.25
HET 98.2
A4-L-E.fcs
Singlets
143896
PEUK 0.040
SYN 0.32
PRO 0.67
HET 98.7
A5-L-E.fcs
Singlets
181692
Initial
PEUK 0.051
SYN 0.12
PRO 0.58
HET 99.1
B4-L-I.fcs
Singlets
208956
PEUK 0.11
SYN 0.17
PRO 2.53
HET 96.6
B5-L-I.fcs
Singlets
97964
PEUK 0.078
SYN 0.32
PRO 1.36
HET 97.7
C5-L-I.fcs
Singlets
105331
End
PEUK 0.10
SYN 0.12
PRO 0.80
HET 98.8
B4-L-E.fcs
Singlets
170544
PEUK 0.067
SYN 0.12
PRO 1.96
HET 97.2
B5-L-E.fcs
Singlets
123347
PEUK 0.049
SYN 0.11
PRO 1.52
HET 97.7
C5-L-E.fcs
Singlets
169313

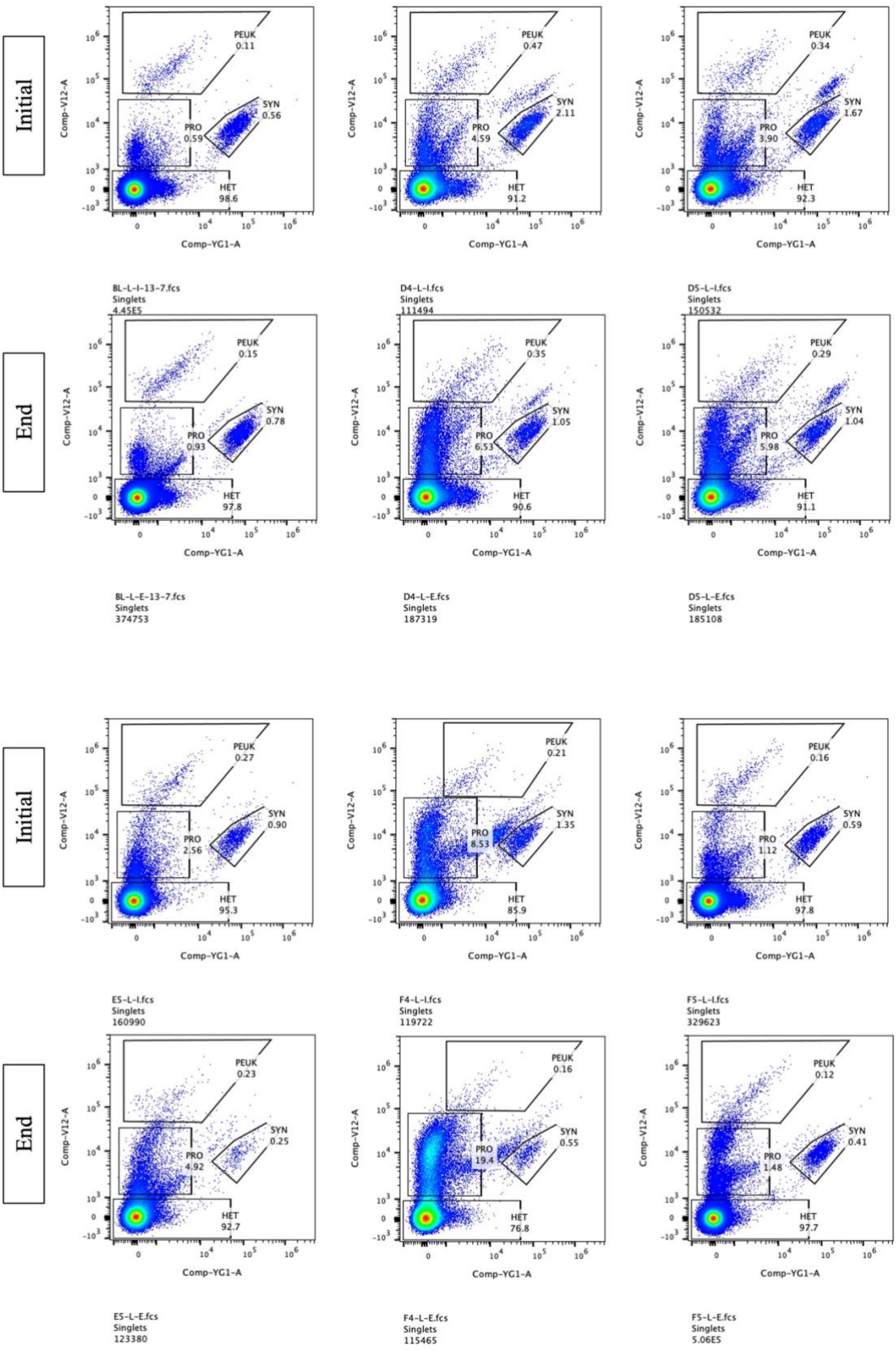
D


Initial
End
Initial
End
Comp-V12-A
Comp-YG1-A
PEUK
PRO
SYN
HET
0.040
1.83
0.15
97.2
BL-L-I-11-7.fcs
Singlets
4.63E5
0.031
1.05
0.099
98.4
D1-L-I.fcs
Singlets
5.92E5
0.026
1.30
0.075
98.3
D2-L-I.fcs
Singlets
7.07E5
0.022
0.49
0.098
99.2
BL-L-E-11-7.fcs
Singlets
8.13E5
0.042
1.54
0.057
97.9
D1-L-E.fcs
Singlets
1.11E6
0.050
3.38
0.053
95.9
D2-L-E.fcs
Singlets
8.80E5
0.012
1.57
6.39E-3
98.2
E1-L-I.fcs
Singlets
7.36E5
0.024
1.91
0.023
97.8
E2-L-I.fcs
Singlets
6.21E5
0.043
2.09
0.12
97.2
F1-L-I.fcs
Singlets
336874
0.046
2.70
8.71E-3
96.9
E1-L-E.fcs
Singlets
367484
0.034
2.23
7.07E-3
97.5
E2-L-E.fcs
Singlets
325331
0.032
3.98
0.047
95.5
F1-L-E.fcs
Singlets
361100

E


Initial
End
Initial
End
Comp-V12-A
Comp-YG1-A
PEUK
SYN
PRO
HET
0.24
1.12
1.38
97.0
BL-L-I-12-7.fcs
Singlets
193943
0.51
1.40
5.98
89.8
D3-L-I.fcs
Singlets
136968
0.32
0.24
7.05
91.2
E3-L-I.fcs
Singlets
140999
0.20
0.84
0.69
98.1
BL-L-E-12-7.fcs
Singlets
338119
0.38
0.34
6.88
91.2
D3-L-E.fcs
Singlets
280207
0.46
0.11
6.77
91.8
E3-L-E.fcs
Singlets
176199
0.18
0.27
2.63
96.6
E4-L-I.fcs
Singlets
236074
0.64
0.91
4.66
92.2
F2-L-I.fcs
Singlets
68589
0.21
0.94
1.31
97.3
F3-L-I.fcs
Singlets
238740
0.45
0.10
10.8
87.1
E4-L-E.fcs
Singlets
76887
0.37
0.74
6.69
91.2
F2-L-E.fcs
Singlets
128448
0.14
0.73
1.04
97.9
F3-L-E.fcs
Singlets
363555

F


Initial
End
Initial
End
Comp-V12-A
Comp-YG1-A
PEUK
PRO
SYN
HET
0.11
0.59
0.56
98.6
0.47
4.59
2.11
91.2
0.34
3.90
1.67
92.3
BL-L-I-13-7.fcs
Singlets
4.45E5
0.15
0.93
0.78
97.8
D4-L-I.fcs
Singlets
111494
0.35
6.53
1.05
90.6
D5-L-I.fcs
Singlets
150532
0.29
5.98
1.04
91.1
BL-L-E-13-7.fcs
Singlets
374753
D4-L-E.fcs
Singlets
187319
D5-L-E.fcs
Singlets
185108
0.27
2.56
0.90
95.3
0.21
8.53
1.35
85.9
0.16
1.12
0.59
97.8
E5-L-I.fcs
Singlets
160990
F4-L-I.fcs
Singlets
119722
F5-L-I.fcs
Singlets
329623
0.23
4.92
0.25
92.7
0.16
19.4
0.55
76.8
0.12
1.48
0.41
97.7
E5-L-E.fcs
Singlets
123380
F4-L-E.fcs
Singlets
115465
F5-L-E.fcs
Singlets
5.06E5

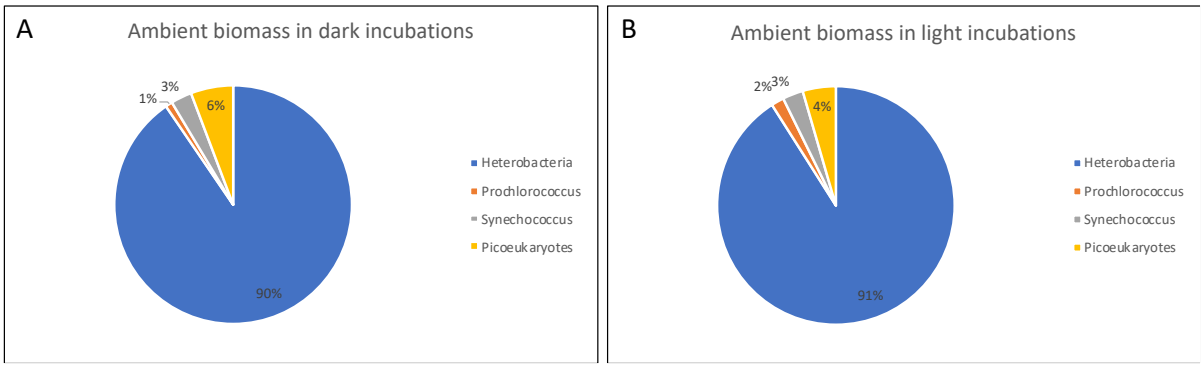


Figure S.5.3. The composition of picoplankton biomass in dark (A) and light (B) incubations.